Comparison of the Quantity of Langerhans Cells in Cervical Intraepithelial Neoplasia and Chronic Cervicitis

doi: https://doi.org/10.32635/2176-9745.RBC.2019v65n2.65

Comparação da Quantidade de Células de Langerhans em Neoplasias Intraepiteliais Cervicais e Cervicites Crônicas

Comparación de la Cantidad de Células de Langerhans en Neoplasias Intraepiteliales Cervicales y Cervicites Crónicas

Júlia Tavares Lopes[1]; Marcella Castro[2]; Luiz Martins Collaço[3]

Abstract

Introduction: Cervical cancer is attributed to human papillomavirus (HPV), whose infection mostly undergoes spontaneous regression. The smaller part of cases that evolve to low and high-grade lesions or invasive lesions may be related to failure of Langerhans cell activity to eliminate the virus. **Objective:** To determine if there is reduction of Langerhans' cells in cervix uterus affected by cervical intraepithelial neoplasms (CIN) grades I and III compared to control group (chronic cervicitis) by immunohistochemistry, granting the correlation of the immune system action with the development of these lesions. **Method:** It were analyzed 40 cases of chronic cervicitis, CIN I and III with anatomopathological diagnosis between January 2014 and December 2015, attempting to compare the amount of positively labeled Langerhans cells nuclei by S-100 protein by immunohistochemistry, quantifying them in standard areas. **Results:** Of the 40 evaluated cases, 17 were chronic cervicitis, 13 CIN I and 10 CIN III. The comparative analysis of the number of cells in each group showed that the mean, standard deviation and median number of Langerhans cells per area were higher in the chronic cervicitis group and lower in the CIN III group. The p value found in the variation of the Langerhans cells number among the groups was significant (p=0.0442). However, when comparing the CIN groups directly with the control group, only the CIN III group had a significant variation (p=0.0209). **Conclusion:** There is a significant decrease in the number of marked Langerhans cell nuclei in CIN III type lesions compared to chronic cervicitis.
Key words: Cervical Intraepithelial Neoplasia; Langerhans Cells; Papillomaviridae; Carcinoma in Situ; Immunohistochemistry.

Resumo

Introdução: O câncer cervical é atribuído ao papilomavírus humano (HPV) cuja infecção, na maioria das vezes, sofre regressão espontânea. A menor porção de casos que evoluem para lesão precursora de baixo e alto graus e invasora pode ter relação com uma falha na atividade das células de Langerhans em eliminar o vírus. **Objetivo:** Determinar se há redução do número de células de Langerhans em colos uterinos acometidos por neoplasias intraepiteliais cervicais (NIC), graus I e III, comparado ao grupo controle (cervicites crônicas), por imuno-histoquímica, possibilitando correlacionar a ação do sistema imune com o desenvolvimento dessas lesões. **Método:** Foram analisados 40 casos de cervicite crônica, NIC I e III, com diagnóstico anatomopatológico entre janeiro de 2014 e dezembro de 2015, buscando-se comparar a quantidade de núcleos marcados positivamente como célula de Langerhans pela proteína S-100 por imuno--histoquímica, quantificando-os em áreas padronizadas. **Resultados:** Dos 40 casos avaliados, 17 foram cervicite crônica, 13 NIC I e 10 NIC III. Na análise comparativa do número de células em cada grupo a média, desvio-padrão e mediana foram maiores no grupo cervicite crônica e menores no grupo NIC III. O valor de p encontrado para a variação do número de células de Langerhans, entre os grupos, foi significativo (p=0,0442); mas, ao comparar os grupos de NIC com o controle, só o grupo NIC III teve variação significativa (p=0,0209). **Conclusão:** Há diminuição significativa do número de núcleos de células de Langerhans marcados em lesões do tipo NIC III em comparação a cervicites crônicas.
Palavras-chaves: Neoplasia Intraepitelial Cervical; Células de Langerhans; Papillomaviridae; Carcinoma in Situ; Imuno-Histoquímica.

Resumen

Introducción: El cáncer cervical puede atribuirse al virus del papiloma humano (VPH) cuya infección a menudo sufre regresión espontánea. El menor número de casos que evolucionan a lesiones precursoras de bajo y alto grado o invasivas puede estar relacionado con una falla en la actividad de las células de Langerhans para eliminar el virus. **Objetivo:** Determinar si hay reducción del número de células de Langerhans en colos uterinos acometidos por neoplasias intraepiteliales cervicales (NIC) grados I y III comparado al grupo control (cervicitis crónicas), por medio de inmunohistoquímica, posibilitando correlacionar la acción del sistema inmune con estas lesiones. **Método:** Se analizaron 40 casos de cervicitis crónica, NIC I y III, con diagnóstico anatomopatológico entre enero de 2014 y diciembre de 2015, comparando la cantidad de núcleos marcados positivamente como célula de Langerhans por la proteína S-100 por inmuno-histoquímica, cuantificándolos. **Resultados:** De 40 casos, 17 fueron cervicitis crónica, 13 NIC I y 10 NIC III. En el análisis comparativo del número de células en cada grupo la media, desviación estándar y mediana fueron mayores en el grupo cervicite crónica y menores en el NIC III. El valor de p encontrado para la variación del número de células de Langerhans entre los grupos fue significativo (p=0,0442), pero al comparar los grupos de NIC con el control sólo el grupo NIC III tuvo variación significativa (p=0,0209). **Conclusión:** Hay disminución significativa del número de núcleos marcados de células de Langerhans en lesiones de tipo CIN III en comparación con cervicitis crónica.
Palabras clave: Neoplasia Intraepitelial Cervical, Células de Langerhans; Papillomaviridae; Carcinoma in Situ; Inmunohistoquímica.

[1] Faculdade Evangélica Mackenzie of Paraná (Fempar). Curitiba (PR), Brazil. Orcid iD: https://orcid.org/0000-0002-3215-1650
[2] Fempar. Curitiba (PR), Brazil. Orcid iD: https://orcid.org/0000-0002-8454-0447
[3] Fempar. Curitiba (PR), Brazil. Orcid iD: https://orcid.org/0000-0002-3215-1650
Address for Correspondence: Luiz Martins Collaço. Rua Padre Anchieta, 2770 – Bigorrilho. Curitiba (PR), Brazil. CEP 80730-000. E-mail: lmcollaco@uol.com.br

INTRODUCTION

Cervical cancer is an extremely prevalent and damaging disease, it is a health public problem. It is the third most frequent cancer in the female population and the fourth cause of death of women by cancer in Brazil[1]. This neoplasm is considered preventable and presents an already known natural history[2].

Initially, there are pre-cancer lesions, the cervical intraepithelial neoplasia (CIN) that may evolve to invasive carcinoma[3]. The CIN can be classified according to the grade of epithelial involvement, CIN I, compromising the baseline layers; CIN II, until three quarts of the epithelium and CIN III, all its layers[4].

Cervical cancer is the only one recognized by the World Health Organization (WHO) as totally attributed to an infectious agent, the human papillomavirus (HPV)[5], a DNA virus of mostly sexually transmitted. The bigger part of the HIV infections is eliminated with the activation of the immune system, but some do not recede and evolve to a cervical intraepithelial lesion or even to cancer[6]. The high rate of spontaneous receding together with a small percent of cases that end up evolving to neoplasm indicate that the infection alone is not enough[7], a failure in the elimination of the virus by the immune system is necessary[6].

The Langerhans are antigen-presenters dendritic cells and mucosa[8] that also act in the mechanisms of defense against neo-antigens. In the cervical lesions provoked by HPV, the reduction of the Langerhans cells appear to be connected to its grade of atypia[9]. The identification and determination of the number of Langerhans cells can be done by the utilization of antibodies directed to the surface molecules by immunohistochemistry. The most used antibodies for this end are the anti-S-100, anti-Cd1a, anti-Cd83 and anti-Langherin[5].

This study has the objective of determining in the cervical mucosa whether there is reduction of Langerhans cells by immunochemistry markers with protein S-100 in epithelium affected by chronic cervicitis (control group), low grade lesion (CIN I) and high grade lesion (CIN III), making possible to correlate the action of the immune system with the development or nor of neoplasm.

METHOD

Observational, descriptive cross-sectional study. The material was obtained in the Laboratory of Pathological Anatomy of "Hospital Universitário Evangélico Mackenzie" of Curitiba, from January 2014 to December 2015. It were selected the cases of patients submitted to hysterectomy or conization with high frequency surgery (HFS) in this period.

Based in the histological blades examined, it were selected and marked the regions containing the relevant lesions for the study, the marked blades were utilized from the same region of the paraffin block.

From the paraffin blocks, it were prepared multi-sample blocks A (TMA), punching the donor blocks of the sampled area, obtaining a tissue cylinder, which was implanted in the receptor block. Each sample fragment removed from the donor block was placed in the receptor block according to a map type "Cartesian plan", totaling 16 samples of tissue in each TMA block.

From the TMA blocks, it were prepared blades and in them, protein S-100 was used to mark by immunohistochemistry. Further, the blades were scanned in Scanner Axio Scan.Z1 and conducted the quantitative evaluation of the cells only in the epithelial layer of the positively marked nuclei with morphology for Langerhans cell, utilizing the program Zen 2.3 Lite®.

In the evaluation made with the program, the positively marked nuclei was counted in the cervical epithelium, always in the region of more intense mark within a circular area of 37,507.089 µm. Because of the discrepancy of the cervical epithelial tissue in each blade, it was selected the standard area mentioned (Figures 1 and 2).

Further on, it were tabulated data about the lesion of the cervix and analysis of the number of positively marked nuclei by S-100 in the area of the lesion evaluated.

The data were reviewed by parametric statistical tests (mean, median and standard-deviation) and non-parametric (tests of Kruskal-Wallis) and to test the significance of the differences observed in the proportion of the categorical variables, it was considered statistically significant the value of $p<0.05$.

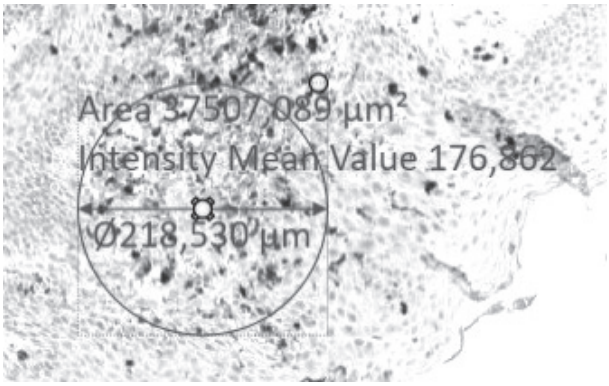

Figure 1. Langerhans cells positively marked by S-100

Note: Langerhans cells positively marked by S-100 inside the circular area limited. Detail of the positivity of S-100 in nuclei of Langerhans cells demonstrating strong nuclear marking (brown). Increase of 50% in the program Zen 2.3 Lite®.

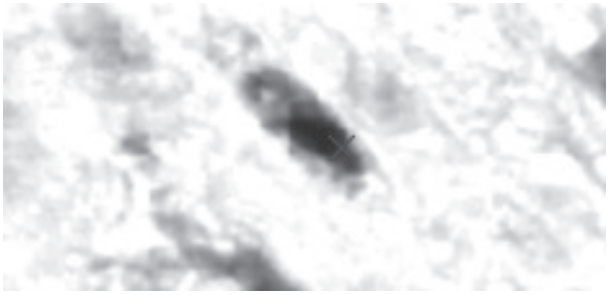

Figure 2. Cells considered as positive for dyed counting by S-100

Note: Cell considered positively marked for the study.

Among the values of exclusion, it were considered age lower than 18 years old, pieces unmatched to the original report of the biopsy after the review of the diagnosis, pieces where the paraffin block was unavailable and those that after preparation of the blades, it was not encountered cervical epithelial tissue in the analysis.

The entire material was under the responsibility of the investigators themselves who ensured the secrecy and confidentiality. The Institutional Review Board of "Sociedade Evangélica Beneficente" (CEP/SEB) approved the study, report number 1.852.973.

RESULTS

It were selected 119 cases through analysis of the reports in the system of the Pathological Laboratory Analysis of "Hospital Universitário Evangélico Mackenzie" of Curitiba. The final sample consisted of 40 cases of biopsies of the cervix obtained through conization by HFS (high frequency surgery) or hysterectomy with diagnosis of chronic cervicitis (17 cases), CIN I (13 cases) and III (10 cases).

In relation to ages, the bigger variation was of the control group (chronic cervicitis). The ages of the patients' group of chronic cervicitis varied from 24 to 78 years, mean of 48.82 years, standard-deviation of 13.28 years and median of 45 years. The patients with CIN I varied from 20 to 66 years, with mean of 39 years, standard deviation of 14.06 years and median of 33 years. Finally, the CIN III group with ages varying from 23 to 60 years old, median of 35.3 years, standard deviation of 10.49 years and median of 34.5 years.

The value of p obtained by the test of Kruskal-Wallis for the variation of age among the groups was 0.0027, showing a statistically significant difference of age among the three groups.

The mean, standard-deviation, and the median of the number of cells per area were bigger in the group of chronic cervicitis in relation to the comparative analysis. The lower values of these parameters were encountered in the group of CIN III (Table 1).

Table 1. Comparison between the number of marked nuclei

	Chronic Cervicitis	CIN I	CIN III
Mean	30.82	20.38	15
Standard Deviation	19.68	9.76	7.37
Median	23	20	14

Caption: CIN = Cervical Intraepithelial Neoplasia

There was a reduction of the number of positively marked nuclei in the comparison of the CIN groups in relation to the control group (Table 2).

Table 2. Comparison between the mean of the nuclei marked in each group and its reduction compared to the control group

	Mean	Reduction in comparison with control group
Chronic Cervicitis	30.82	
CIN I	20.38	- 44%
CIN III	15	- 51.33%

Caption: CIN = Cervical Intraepithelial Neoplasia

Also in relation to the number of positively marked cells, three groups were compared utilizing the non-parametric test of Kruskal-Wallis and it was obtained a significant value of p (p=0.0442).

Only in the comparison between the CIN I and the control group, in relation to the test of Kruskal-Wallis it was not encountered statistically significant difference (p=0.1805). For the analysis of the difference between the CIN III group with the control group, the significant p value was p=0.0209.

DISCUSSION

In the present study, 40 cases of chronic cervicitis, CIN I and CIN III, were evaluated attempting to quantify the number of Langerhans cells in each one of these lesions through immunohistochemistry.

To increase the reliability of the results and avoid distortions, it were taken several careful procedures and standardize the counting of Langerhans, respecting the exclusion criteria.

It was observed a more pronounced reduction of the number of Langerhans cells in patients with CIN III and less pronounced in patients with CIN I, utilizing patients with chronic cervicitis as group control.

Theories developed in studies revised by Southern and Herington suggest that the reduction of Langerhans cells would be a deffect in the local immunity of the cervix caused by alterations of the immunologic

surveillance added to the epithelial aggression by HPV [10]. HPV itself would be able to provoke an acquired immunodefficiency that would favor its persistence in the tissue. In other words, the persistence of HPV would depend of its capacity of reducing Langerhans cells [11,12]. The result encountered of the reduction of quantity of Langerhans cells, as the cervical intraepithelial neoplasia appears (reduction in patients with CIN I and III) appears and progresses (more pronounced reduction in CIN III), is favorable to the theory of the HPV-caused immunodefficiency which allows its neoplasic action.

However, other authors report an increase of the Langerhans cells in patients affected by CIN, which could be a result of one of the specific immune response aimed against cells in neoplasic transformation; the Langerhans cells would be an immune response of the organism to the phenomenon of carcinogenesis[11]. Our data diverge from the data encountered by Welkovic[11] that shows bigger number of Langerhans cells in women with high grade lesion when compared to low grade lesion, utilizing also S-100. The author reports that, with CIN present, there was a progressive increase of the density of the Langerhans cells according to the severity of the lesion, but without statistically significant results. In the present study, it was not observed increase of the quantity of Langerhans cells counted in patients with CIN I and III and neither other signs of possible immune response as, for instance, important lymphocitary infiltrate in the area affected.

This discrepancy of results in the literature can occur because of the difficulty in selecting precisely the site of the cervix biopsy, in addition to the diversity of stages presented by one infection by HPV, use of different markers among the studies and use of diverse methodologies[5]. It can be wondered about the reduction of the quantity of Langerhans cells as one of the initial conditions of HPV for the development of its oncogenic effects. After the installation of CIN, it may have a stimulation to the production of more Langerhans cells by migration, proliferation and retention[13]. Therefore, the phase of the progress of CIN and HPV infection where biopsy is collected may also be a factor that caused the discrepancies.

In its analysis, Jimenez-flores et al.[5] utilized the technique of sheet, which consists in separating the epithelium from the stroma per enzimatic action to better evaluate the epithelial dendritic cells. Therefore, the author also finds a reduction of approximately 50% in the number of Langerhans cells marked by Langerin in the samples of cervical epithelium with CIN I induced by HPV when compared to HPV negative samples. This indicates that a more accurate technique to analyze the Langerhans cells of the epithelium matches the theory of the reduction of these cells in CIN.

The choice of the marker can be a determining factor of the result: a study by Camargos et al.[14] showed that the number of positive Langerhans cells was bigger than the number of positive Langerhans cells per Langerin in normal epithelium when compared to the epithelium affected by CIN I. When S-100 is used, the number of Langerhans cells was bigger in the epithelium affected by CIN I than in the normal epithelium. In the same study, with both markers, it was observed a reduction of the number of Langerhans cells in epithelium with CIN III when compared to CIN II, indicating that the factor severity of the neoplasias would be determining on the reduction of these cells. This is consistent with our findings.

Connor et al.[15] suggest that studies that used S=100 as marker of Langerhans cells showed reduction of the amount, while other studies that utilized other markers as CD1 did not show this correlation, affirming that the reduction encountered in the study with S-100 can be result of a flaw in the expression of this protein in these cells.

Still, the correlation between the immune system and carcinogenesis is capable of changing the perspective of the oncologic treatment, since the action of the immune system, new therapies as the immune therapy, stimulators of the local immunity and prophylactic vaccination become relevant[14]. Consequently, it justifies the importance of the theory relating the local immunity with the development of a possible neoplasia, because new treatment can be developed.

CONCLUSION

In relation to the number of marked nuclei, it was concluded that there is a significant difference of the quantity of Langerhans cells among the groups (p=0.0442), therefore, it was noticed a reduction of the Langerhans cells as much as the grade of cervical intraepithelial increased.

The quantity of Langerhans cells is reduced in type CIN III in comparison with chronic cervicitis (p=0.0209) when quantified by the marker S-100. New studies with different markers could enrich the discussion.

However, there was no significant reduction of the cells in comparison between CIN I and chronic cervicitis (p=0.1805), indicating that the reduction of the Langerhans cells is related to the progression to high grade injuries alone.

CONTRIBUTIONS

Luiz Martins Collaço contributed substantially to the conception and design, critical review of the intellectual

content and final approval of the version to be published. Júlia Tavares Lopes and Marcella Castro contributed substantially to collect the data, analysis and interpretation of the data, wording of the manuscript and final approval of the version to be published.

ACKNOWLEDGMENTS

To "Faculdade Evangélica Mackenzie" of Paraná, to its faculty, employees and collaborators for providing the structure, support courseware, access to the important laboratories for the development of the study and for the institutional scholarship of incentive to research. To "Hospital Universitário Evangélico Mackenzie" of Curitiba, especially to the service of Pathological Anatomy and its employees for providing material to the study and support to collect the data. To "Centro de Patologia" of Curitiba for manufacturing the blades, of immunohistochemical and for receiving us. And to "Laboratório de Patologia Experimental" of "Pontifícia Universidade Católica" of Paraná (PUC-PR), for lending the device Axio Scan.Z1 and the software Zen 2.3 Lite® and assistance to analyze the blades.

DECLARATION OF CONFLICT OF INTERESTS

There are no conflict of interests to declare.

FUNDING SOURCES

Institutional scholarship granted by "Faculdade Evangélica Mackenzie" of Paraná.

REFERENCES

1. Boulet GA, Horvath CA, Berghmans S, et al. Human papillomavirus in cervical cancer screening: important role as biomarker. Cancer Epidemiol Biomarkers Prev. 2008;17(4):810-7. doi: http://dx.doi.org/10.1158/1055-9965.EPI-07-2865
2. Sousa AMV, Teixeira CCA, Medeiros SS, et al. Mortalidade por câncer do colo do útero no estado do Rio Grande do Norte, Brasil, 1996-2010: tendência e projeções até 2030. Epidemiol Serv Saúde. 2016;25(2):311-22. doi: http://dx.doi.org/10.5123/s1679-49742016000200010
3. Hernández-Ramirez LF, Cardona-Arias JA. Lesiones intraepiteliales, inflamación y atipias escamosas cérvico-uterinas en mujeres de un municipio de Antioquia, Colombia, 2014. Médicas UIS. 2016;29(1):29-36. doi: http://dx.doi.org/10.18273/revmed.v29n1-2016003
4. Teles CCGD. Estudo epidemiológico de mulheres com lesões precursoras para câncer do colo uterino na região Sudoeste de Mato Grosso. [dissertação na Internet]. Brasília, DF: Universidade de Brasília; 2010. [acesso 2017 set. 25]. Disponível em: http://repositorio.unb.br/handle/10482/7731
5. Jimenez-Flores R, Mendez-Cruz R, Ojeda-Ortiz J, et al. High–risk human papilloma virus infection decreases the frequency of dendritic Langerhans' cells in the human female genital tract. Immunology. 2006;117(2):220-228. doi: http://dx.doi.org/10.1111/j.1365-2567.2005.02282.x
6. Alves DB, Tozetti IA, Gatto FA, et al. Linfócitos CD4, CD8 e células NK no estroma da cérvice uterina de mulheres infectadas pelo papilomavírus humano. Rev Soc Bras Med Trop. 2010;43(4):425-29. doi: http://dx.doi.org/10.1590/S0037-86822010000400018
7. Silva C, Almeida ECS, Côbo EC, et al. A retrospective study on cervical intraepithelial lesions of low-grade and undetermined significance: evolution, associated factors and cytohistological correlation. São Paulo Med J. 2014;132(2):92-96. doi: http://dx.doi.org/10.1590/1516-3180.2014.1322579
8. Kalinski P, Giermasz A, Nakamura Y, et al. Helper role of NK cells during the induction of anticancer responses by dendritic cells. Mol Immunol. 2005;42(4):535-539. doi: http://dx.doi.org/10.1016/j.molimm.2004.07.038
9. Nadal SR, Calore EE, Cruz SHA, et al. Comparação das contagens das células de Langerhans de tecidos contendo carcinoma anal em doentes com e sem infecção pelo HIV. Rev Bras Coloproctol. 2006;26(3):269-274. doi: http://dx.doi.org/10.1590/S0101-98802006000300006
10. Southern SA, Herrington, CS. Molecular events in uterine cervical cancer. Sex Transm Infect. 1998;74(2);101-9. doi: http://dx.doi.org/10.1136/sti.74.2.101
11. Welkovic S. Células de Langerhans no colo uterino de mulheres hiv soropositivas com neoplasia intraepitelial cervical. [tese na Internet] Recife, PE: Universidade Federal de Pernanbuco; 2007. [acesso 2017 set. 28]. Disponível em: https://repositorio.ufpe.br/handle/123456789/7333
12. Carvalho MOO. Identificação da presença de células de Langerhans e a ativação de linfócitos T nos diferentes estágios das lesões do colo uterino associada à infecção causada pelo vírus do papiloma humano. [tese na Internet]. Rio de Janeiro: Instituto Nacional de Infectologia Evandro Chagas, Fundação Oswaldo Cruz; 2016. [acesso 2017 out. 2]. Disponível em: https://www.arca.fiocruz.br/handle/icict/26515
13. Caorsi I, Figueroa CD. Langerhans' cell density in the normal exocervical epithelium and in the cervical intraepithelial neoplasia. Br J Obst Gynaecol. 1986;93(9):993-8. doi: http://dx.doi.org/10.1111/j.1471-0528.1986.tb08022.x
14. Camargos DS, Tafuri A, Fernandes PA, et al. Langerhans cells ascertaining in cervical tissues obtained from women with cervical intraepithelial neoplasia. Reprod

Syst Sex Disord. 2017;6(1):203. doi: http://dx.doi.org/10.4172/2161-038X.1000203

15. Connor JP, Ferrer K, Kane JP, et al. Evaluation of Langerhans' cells in the cervical epithelium of women with cervical intraepithelial neoplasia. Gynecol Oncol. 1999;75(1):130-5. doi: http://dx.doi.org/10.1006/gyno.1999.5559

Recebido em 28/2/2019
Aprovado em 11/10/2019