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Original Research

HPV and Systemic Response in Cervical Intraepithelial Neoplasia

HPV e Resposta Sistêmica na Neoplasia Intraepitelial Cervical

VPH y Respuesta Sistémica en la Neoplasia Intraepitelial Cervical

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Abstract

Objectives: to verify whether there is an association of low and high-risk HPV with blood count parameters in patients with cervical intraepithelial neoplasia. Methods: We prospectively evaluated 48 patients with Pap smear showing low or high-grade intraepithelial lesion, submitted to collection of material to perform PCR for high-risk and low-risk HPV. The following blood count parameters were evaluated: hemoglobin, red blood cells, hematocrit, absolute number of leukocytes, neutrophils, lymphocytes, eosinophils, basophils, monocytes and platelets, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and Red Cell Distribution Width (RDW). Receiver operating characteristic (ROC) curve was used to obtain the area under the curve (AUC) relating the positivity or not of HPV with the blood count parameters evaluated. Mann-Whitney test and multivariate analysis were performed, with level of significance less than 0.05. Results: Relating the positivity or not of HPV with the blood count parameters (Roc curves), there was statistical significance only between high-risk HPV and the platelet count (sensitivity=75%, specificity=66.7%, AUC=0.674, p=0.039). Platelet count ≤ 177,000/mm^3 is associated with high-risk HPV. Subsequently, the multivariate analysis was performed, and the absolute value of platelets was considered an independent variable in this association with high-risk oncogenic HPV. Conclusion: A systemic response is found with the presence of high-risk HPV in CIN. Thus, platelet count ≤ 177,000/mm^3 is associated with high-risk HPV.

Descriptors: Blood Cell Count; Cervical Intraepithelial Neoplasia; Papillomaviridae; Platelet Count.

Resumo

Objetivos: verificar se existe associação do HPV de baixo e alto risco com parâmetros de hemograma em pacientes com neoplasia intraepitelial cervical. Métodos: Foram avaliadas prospectivamente 48 pacientes com exame de Papanicolaou apresentando lesão intraepitelial de baixo ou alto grau, submetidas à coleta de material para realização de PCR para HPV de alto e baixo risco. Foram avaliados os seguintes parâmetros de hemograma: hemoglobina, hemácias, hematócrito, número absoluto de leucócitos, neutrófilos, linfócitos, eosinófilos, basófilos, monócitos e plaquetas, relação neutrófilo-linfócito (NLR), razão plaqueta-linfócito (PLR) e amplitude de distribuio dos eritrócitos distribuição celular (RDW). A curva Receiver Operating Characteristic (ROC) foi utilizada para obter a área sob a curva (AUC) relacionando a positividade ou não do HPV com os parâmetros do hemograma avaliados. Foram realizados teste de Mann-Whitney e análise multivariada, com nível de significância inferior a 0,05. Resultados: Relacionando a positividade ou não do HPV com os parâmetros do hemograma (curvas Roc), houve significância estatística apenas entre o HPV de alto risco oncognico na NIC e a contagem de plaquetas (sensibilidade=75%, especificidade=66,7%, AUC=0,674, p= 0,039). A contagem de plaquetas ≤ 177.000/mm3 está associada ao HPV de alto risco. Posteriormente, foi realizada a análise multivariada, sendo o valor absoluto das plaquetas considerado variável independente nesta associação com o HPV oncogênico de alto risco. Conclusão: É encontrada resposta sistêmica com a presença de HPV de alto risco na NIC. Assim, a contagem de plaquetas ≤ 177.000/mm3 está associada ao HPV de alto risco.

Descritores: Hemograma; Neoplasia Intraepitelial Cervical; Papillomaviridae; Contagem de Plaquetas.

Resumen

Objetivos: verificar si existe asociación del VPH de bajo y alto riesgo con los parámetros hemogramas en pacientes con neoplasia intraepitelial cervical. Métodos: Se evaluaron prospectivamente 48 pacientes con prueba de Papanicolaou que mostraron lesión intraepitelial de bajo o alto grado, sometidas a recolección de material para realizar PCR para VPH de alto y bajo riesgo. Se evaluaron los siguientes parámetros del hemograma: hemoglobina, glóbulos rojos, hematocrito, número absoluto de leucocitos, neutrófilos, linfocitos, eosinófilos, basófilos, monocitos y plaquetas, índice neutrófilo-linfocito (NLR), índice plaqueta-linfocito (PLR) y rojo. Ancho de distribución de celdas (RDW). Se utilizó la curva Receiver Operating Characteristic (ROC) para obtener el área bajo la curva (AUC) que relaciona la positividad o no del VPH con los parámetros del hemograma evaluados. Se realizó prueba de Mann-Whitney y análisis multivariado, con nivel de significación menor a 0,05. Resultados: Al relacionar la positividad o no del VPH con los parámetros del hemograma (curvas Roc), hubo significación estadística sólo entre el VPH de alto riesgo y el recuento de plaquetas (sensibilidad=75%, especificidad=66,7%, AUC=0,674, p= 0,039). Un recuento de plaquetas ≤ 177 000/mm3 se asocia con VPH de alto riesgo. Posteriormente se realizó el análisis multivariado y se consideró el valor absoluto de plaquetas como variable independiente en esta asociación con el VPH oncogénico de alto riesgo. Conclusión: Se encuentra respuesta sistémica con la presencia de VPH de alto riesgo en NIC. Por tanto, un recuento de plaquetas ≤ 177 000/mm3 se asocia con VPH de alto riesgo.

Descriptores: Recuento de Células Sanguíneas; Neoplasia Intraepitelial Cervical; Papillomaviridae; Recuento de Plaquetas.

INTRODUCTION

Cervical cancer accounts for about 15% of all types of cancer in women, and is currently the second most common cancer among women in the world.[1] It is in the uterine cervix epithelium, and results from an uncontrolled cell replication,[2] going

through detectable pre-clinical phases with high healing potential, ending in an invasive process. The annual incidence of new cases of cervical cancer expected for Brazil between 2020-2022 will be 16,590, estimating 15.43 cases per 100,000 women.[3]

The main risk factor for the development of cervical cancer is infection by the HPV virus (human papillomavirus) and its oncogenic subtypes. HPV DNA is found in about 97% of cervical tumors and subtypes 16, 18, 31, 35, 39, 45, 51, 52, 56, and 58 cause the majority of invasive tumors.[4] HPV has been suggested as the first identifiable and necessary cause of cancer in humans, that is, cervical neoplasia does not develop in the absence of persistent HPV DNA.[5] Other factors related to the appearance of cervical cancer include low educational level, early onset of sexual activity, greater number of children, high number of sexual partners throughout life, history of sexually transmitted diseases, use of hormonal contraceptives for more than 4 years and smoking.[6]

High-grade intraepithelial lesion (HSIL) corresponds to cervical intraepithelial neoplasia grades 2 and 3 (CIN 2 and 3), which represents the precursor lesion of cervical cancer. In this lesion, HPV causes cytoarchitectural changes in at least two thirds of the epithelial thickness of the cervix.[7]

Although HPV and cervical cancer precursor lesions are initially local alterations, systemic alterations may be associated with invasive uterine cervical cancer. There is a tumor inflammatory response, with the release of cytokines by the tumor, capable of controlling leukocyte migration and function.[8] Other studies have also demonstrated that systemic hematological factors, such as neutrophil-lymphocyte ratio and hemoglobin levels, may be associated with prognosis and recurrence of CIN.[9,10] But to our knowledge, there are no studies in the literature relating the presence of HPV with systemic changes and blood count parameters. Thus, the objective of this study was to verify whether there is an association of low and high-risk HPV with blood count parameters in patients diagnosed with cervical intraepithelial neoplasia.

MATERIAL AND METHOD

Patients

We prospectively evaluated 48 patients treated at the Colposcopy and Gynecological Oncology outpatient clinics of the Department of Gynecology and Obstetrics between 2018 and 2020. The inclusion criterion was a woman with Pap smear showing low and high - grade squamous intraepithelial lesion, submitted to collection of material to perform PCR for low and high-risk HPV. Exclusion criteria were pregnant patients and immunosuppressive diseases.

The following blood count parameters were evaluated: hemoglobin, red blood cells, hematocrit, absolute number of leukocytes, neutrophils, lymphocytes, eosinophils, basophils, monocytes and platelets, neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and Red Cell Distribution Width (RDW). NLR was calculated by dividing the absolute number of neutrophils by the absolute number of lymphocytes. PLR was calculated by dividing the absolute number of platelets by the absolute number of lymphocytes. PCR technique was performed for detection of high and low-risk HPV, as described below.

The study was approved by the Research Ethics Committee (CEP) under CAAE 84963118.2.0000.5154, number 2,599,027.

PCR technique

The biopsy fragments suffered mechanical rupture and followed a DNA isolation protocol according to instructions contained in the manufacturer's package insert (TRIzol®). The DNA samples obtained from the biopsies were submitted to a PCR protocol for β-actin (450 bp), low-grade HPV (302 bp) and high-grade HPV (175 bp), using Taq DNA Polymerase, Recombinant (Invitrogen®, catalog number kit) 11165-036), in an AB Applied Biosystems – Veriti TM 96 Well Thermal Cycler. The technique was performed according to the manufacturer's instructions. The samples were submitted to electrophoresis in 10% polyacrylamide gel to reveal / evaluate the products amplified in the PCR. A 50pb DNA ladder control (Invitrogen®, catalog number 10416-014) was used.

Statistical analysis

Data were analyzed using MedCalc 19.0.4 and IBM SPSS Statistics 20 programs. Receiver operating characteristic (ROC) curve was used to obtain the area under the curve (AUC) relating the positivity or not of low and high-risk HPV with the blood count parameters evaluated. Subsequently, the multivariate analysis was performed (multiple linear regression). The level of significance was considered less than 0.05.

RESULTS

The characteristics of the patients included in the study are shown in Table 1. All 48 patients with an initial diagnosis of intraepithelial lesion by the Pap smear underwent colposcopy, biopsy, blood collection for complete blood count, and collection of material for performing single-probe PCR for high-risk and low-risk HPV. Regarding the results of the biopsies, 21 (43.75%) were normal or had CIN 1 or HPV alterations, 6 (12.5%) had CIN 2 and 21 (43.75%) had CIN 3. Regarding PCR, 36 (75%) had positive high-risk HPV and 23 had HPV had positive low-risk HPV.

Receiver operating characteristic (ROC) curve was used to obtain the area under the curve (AUC) relating the positivity or not of HPV with the blood count parameters evaluated. There was

statistical significance only between high-risk HPV and platelet count (sensitivity=75%, specificity=66.7%, AUC=0.674, p=0.039). That is, absolute platelet value ≤ 177,000/mm^3 was associated with high-risk HPV (figure 1). This association was not found with low-risk HPV, nor with other blood count parameters.

Subsequently, the multivariate analysis was performed (multiple linear regression), and the absolute value of platelets was considered an independent variable in this association with high-risk oncogenic HPV. The covariates used were age, smoking, and the presence of high-grade CIN.

Table 1. Characteristics of patients included in the study (median, minimum and maximum values, or *n* and %).

	Median, minimum and maximum values, or *n* and %
Age (years)	33 (16-71)
Parity (births)	2 (0-8)
First sexual intercourse (years)	16 (10-23)
Number of partners	4 (1-20)
Smoking	21 (34,42%)
Contraceptive method	
No method	18 (29,5%)
Hormonal contraceptives	22 (36,06%)
Tubal ligation	15 (24,59%)
Condom	5 (8,19%)
DIU	1 (1,63%)

Table 2. Multivariate analysis of the variables age, smoking, CIN 2/3 and absolute platelet value considering high-risk oncogenic HPV.

Variable	*Multivariate analysis*	
	OR (95% CI)	***p-value***
Age (> 50y *vs* ≤ 50y)	2.261 (0.260-19.654)	0.460
Smoking (yes *vs* no)	0.968 (0.186-5.041)	0.970
CIN 2/3 (yes *vs* no)	1.679 (0.325-8.674)	0.536
Platelets (≤ 277,000/mm^3 *vs* > 277,000/mm^3)	0.152 (0.034-0.677)	0.013

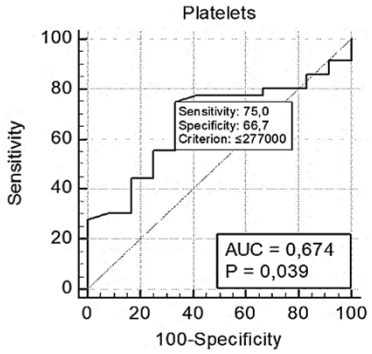

Figure 1: Receiver operating characteristic (ROC) curve was used to obtain the area under the curve (AUC) relating the positivity or not of high-risk HPV with the blood count parameters.

DISCUSSION

The present study evaluated the relationship between blood count parameters in a group of patients diagnosed with CIN. The following relationships between blood count parameters and the diagnosis of cervical cancer are already known, suggesting a systemic alteration related to the release of cytokines in the tumor inflammatory response.[8,11] In addition, several studies have already demonstrated the association of blood count parameters with prognosis and recurrence in CIN.[10]

NLR and PLR may also be associated with prognosis in cervical cancer, demonstrating a systemic inflammatory change related to the behavior of these tumors.[11,12] These ratios are also higher in patients with invasive cancer compared to CIN.[11,13] Furthermore, NLR, PLR, and platelet count may be predictive factors of response to chemo radiation in women with cervical cancer.[14] In our study, there was no statistically significant assessment of NLR and PLR. However, our study compared this data with HPV positivity by PCR in patients with CIN, and not the association with CIN.

It is already known that thrombocytosis can be a prognostic factor in gynecological tumors.[15-17] One study found that pretreatment thrombocytosis may be an independent prognosis predictor in women with cervical cancer and may be a prognostic biomarker.[18] Another study demonstrated that the association of platelet count and FIGO stage increased the prediction performance of FIGO staging and may provide additional risk stratification for operable cervical cancer patients.[19]

Our study demonstrated that platelet count ≤ 177,000/mm^3 is associated with high-risk HPV.This association was not found with low-risk HPV. The cut-off value found by the ROC curve was 177,000/mm3 (sensitivity=75%, specificity=66.7%, AUC=0.674, p=0.039). When the multivariate analysis was performed, platelet count was considered an independent variable in this association with high-risk HPV. This suggests that the presence of high-risk (but not low-risk) HPV, regardless of the presence of high-grade CIN, may already be associated with systemic changes seen in the blood count.

The main limitation of the study was that genotyping was not performed to determine whether any type of high-risk HPV has a greater impact on the change in the absolute platelet count, and even on the other parameters of the blood count. On the other hand, to our knowledge, this is the first study in the literature that studied the association of high-risk HPV with blood count parameters. And it also demonstrates that this finding is independent of the presence of high-grade CIN. New studies on this topic could help to better understand why the mere presence of high-risk HPV in the cervix could already generate systemic changes, and what impact this would have on the evolution of these lesions to CIN. We can hypothesize that initially, high-risk HPV can lead to a reduction in platelets, and with the inflammatory response, after progression from a CIN to invasive cancer, the response reverses to an increase in platelet count that will influence the prognosis of the disease. The blood count is an easy-to-collect, accessible and low-cost test, and its use in the evaluation of the systemic response in diseases of

the lower genital tract is promising.

Therefore, systemic response is found with the presence of high-risk HPV in patients with CIN. Thus, platelet count ≤ 177,000/mm^3 is associated with high-risk HPV, regardless of the presence of high-grade CIN.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interests.

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