

Research Article

Anal cytology in immunocompetent patients with high-grade intraepithelial neoplasia (CIN II and CIN III)

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Submitted: 10 December 2019

Approved: 12 February 2020

Published: 13 February 2020

How to cite this article: Ribeiro MEF, Barbosa LCR, Schnaider TB. Anal cytology in immunocompetent patients with high-grade intraepithelial neoplasia (CIN II and CIN III). Clin J Obstet Gynaecol. 2020; 3: 007-012.

DOI: dx.doi.org/10.29328/journal.cjog.1001041

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Keywords: Papillomaviridae; Anus neoplasms; Uterine cervical neoplasms; Cytodiagnosis



Abstract

Introduction: Anal cancer is directly related to the presence of high-grade HPV. Immunocompromised patients have defined conducts, something which is not observed in immunocompetent patients.

Objective: To study the anal cytology of patients with high-grade intraepithelial neoplasia (CINII and III) in order to propose a protocol to be followed by the Oncology Gynaecology Service at the Samuel Libânio Clinical Hospital (HCSL).

Methods: Clinical, prospective, transversal and single-centered study. Sampling was by convenience within one year. A total of 150 patients were studied. Out of these, 76 were patients with high-grade cervical intraepithelial lesions diagnosed through histopathological examination of the uterine cervix (Study Group) and 74 without high-grade intraepithelial lesions and with cervical cytology negative for neoplasia (Control Group). The following variables were analyzed: age, sexarc, tobacco use, alcohol consumption, STD history (not HPV), menopause, Hormone Replacement Therapy, anal sex practice, parity, number of sexual partners and contraceptive use.

Results: There was no significant difference between the number of cases in altered anal cancer oncology in the study group, in comparison with the control group.

Conclusion: There were changes in the anal cytology of the study group and these should be evaluated due to the risk of dealing with pre-neoplastic anal lesion. Clinical Trials: NCT03241680.

Introduction

Uterine cervix cancer, also known as cervical, is caused by the persistent infection of some types of the Human Papillomavirus – HPV. Genital infection caused by this virus is very frequent and most of the times does not result in any disease. However, in some cases, it can cause cellular changes that may progress to a cancer. These changes are easily identified through the oncotoc colpocitology exam, which can be cured in almost all the cases. That's why carrying out this exam is so important [1].

Cervical intraepithelial neoplasia can be of low grade (CIN I) or high grade (CIN II and III). Women with CIN I have low potential in developing cervical malignancy in contrast to those with CIN II and III. Based on the lesion condition, being of both low grade or high grade, treatments or medical care can be proposed, based on the risk of a progression to an invading cancer and also to spare from indications of aggressive treatments for lesions with a potential of a spontaneous regression [2].

Human Papillomavirus (HPV) is a small desoxyribonucleic acid (DNA) virus of approximately 7900 base pairs. DNA sequencing techniques have eased HPV classification and characterization, with every type being distinct for having less than 90% of base pairs homologue with any other type of HPV. There are more than 40 types of HPV that infect the anogenital area [3,4].

The incidence of anal cancer in the overall population has increased a lot in the last 30 years, both in the United States and other countries. This can be related, especially in the female gender, to the infection by HPV, the number of sexual partners, genital warts, tobacco use, receptive anal intercourse and the infection by the immunodeficiency virus (HIV). From an etiological standpoint, anal cancer is more similar to malign genital diseases than gastrointestinal diseases [5].

Anal and cervical canals share characteristic embryological, histological and pathological similarities. Both develop from the embryonic cloacal membrane and are places of ectodermal and endodermal tissues fusion to form the squamocolumnar junction. Both can show normal metaplasia and dysplasia area related to the infection by HPV [6].

Thus, the National Cancer Institute (INCA, 2017) defines as risk group women with a high-grade uterine cervix lesion. The low but increasing incidence of anal cancer, particularly within vulnerable populations, make it a concerning disease, as well as being difficult to manage with the lack of access to a better care. Studies about its diagnosis and management are limited and almost all anal cancer guidelines avoid any direct recommendation in relation to routine screening [7].

Women with cervical and vulva cancer, as well as immunosuppressive drug users, showed an increase in risk of anal cancer, when compared to the overall population [8,9]. Anal cancer, when diagnosed in an initial stage, can be cured without having to go through a surgical treatment, but when diagnosed in an advanced stage, it's necessary to carry out an abdominoperineal amputation [10].

The objective of this paper was to study the anal cytology of patients with high-grade intraepithelial neoplasia (CINII and CINIII) in order to propose a protocol to be followed by the Oncology Gynaecology Service at the Samuel Libânio Clinical Hospital.

Material and methods

This is a clinical, prospective, transversal and single-centered study, carried out at the Oncology Gynaecology Service at the Samuel Libânio Clinical Hospital (HCSL) in Pouso Alegre, Minas Gerais, in Brazil. Sampling was by convenience within one year (from August 2016 to August 2017), which met the eligibility criteria.

The study was approved by the Research and Ethic Committee of the Vale do Sapucaí University, by the Approval

Report n. 1.922.447, with histopathological study revealing CIN II and CIN III at the Oncology Gynaecology Service and the Gynaecological Surgery ambulatory with negative cervical cytology.

The inclusion criteria referred to patients of female gender aged between 18 and 65 years old. The case-by-case basis corresponded to 150 patients. Out of these, 76 were diagnosed through a histopathological examination of the uterine cervix (Study Group) and 74 without high-grade intraepithelial lesion and with cervical cytology negative for neoplasia (Control Group).

There were 39 patients, 111 diagnosed with conventional anal cytology. Below is the flowchart:

Procedures

Collection method of anal oncotic cytology by conventional means

Patients were placed in lithotomy position, with the introduction of endocervical brush soaked in water distilled in the anal canal, with the brush rotating three times around the entire anal circumference and using glass slide smear and storing in glass vial with alcohol at 70%.

Samples of conventional anal cytology were received at HCSL and the Pathology Center of Pouso Alegre – CEPAPA. They were mounted in appropriate means and by using cover slips. They were also labeled and distributed to the professionals responsible for the glass slides analysis. The data were tabulated and submitted to statistical analysis. Using the programs SPSS 16.0 (Statistical Package for Social Sciences, Inc., Chicago, USA) and Bioestat 5.0 (Mamirauá Sustainable Development Institute, Belém, PA, Brazil), with rejection level of null hypothesis fixed at 5% ($p \leq 0.05$). For the numeric variables, descriptive statistics were used, with calculations of median, average and standard deviation.

Tests used:

1. Mann-Whitney Test (Siegel) to study the possible differences between the Study and Control groups for quantitative variables.
2. Chi-squared test or Fisher's exact Test (Siegel) to confront both Study and Control groups for the qualitative variables.
3. Cochran's G Test (Siegel) to study habits concomitances and characteristics of the Case and Control groups.
4. Kendall's Coefficient of Concordance (Siegel) in order to study the descending order of the characteristics' importance.

Results

Both Study and Control groups with respect to the variable

age are compatible and comparable, with the average age of the Control Group being 41.5 years old and of the Study Group being 40.3 years old, as well as the median age being 41 for the Control Group and 38 for the Study Group (Table 1).

When Mann-Whitney Test was carried out to compare the medians, no evidences were obtained, in a significance level of 5%, for the rejection of null hypothesis ($p > 0.05$) that the studied groups present statically different average ages. The result is satisfactory, because we can conclude that for both groups the patients' ages are compatible and comparable (Table 2).

Sexarc's median was of 18 years in the Control Group and 17 years in the Study Group. The average was of 18.8 years in the Control Group and 17.6 years in the Study Group, with no statistically considerable difference observed in both groups.

No statistically considerable difference was observed on the average number of observations through the application of the Mann-Whitney Test in the patients' sexarc comparison. ($p > 0.05$) (Table 3).

The Study Group presented an average of 2.9 children per patient and a median of 3 children per patient. The Control Group had an average of 2.1 children per patient and a median of 2 children per patient. Therefore, they present a statistically considerable difference between both groups, in benefit of the Study Group.

After carrying out the Mann-Whitney Test to identify a statistically considerable difference between both groups' patients' parity, the rejection of the test's null hypothesis is observed ($p \leq 0.05$) and we can conclude there is a statistically considerable difference of this characteristic in both groups. Different parities for each group are considered (Table 4).

The Study Group had 52 patients who used contraceptive methods and 11 who did not. In the Control Group 25 patients used and 23 did not, which shows a tendency of patients who use contraceptive to belong to the Study Group.

Chi-squared Test for Independence was used to test the independence between the use of contraceptive methods by the analyzed groups' patients. The p value obtained, lower than 0.05, presents indication of a hypothesis' rejection that the observations are independent from each other. Thus, we can conclude that the use of contraceptive methods is related to the fact that patients belong either to the Study or Control groups, in which the ratio of patients who use them is higher in the Study Group (Table 5).

Nineteen patients of the Study Group had 4 or more sexual partners and 44 had less than 4 partners, and in the Control Group 10 patients had 4 or more partners and 38 patients had less than 4 partners. Therefore, no statistically considerable difference is presented between the groups.

The number of partners between patients does not present any indications that relates it to its presence in each of the groups. The p value obtained, of 0.268, using the Chi-squared Test, does not reject null hypothesis of independence, thus concluding that the number of partners does not increase the risk for patients to belong to the Study Group (Table 6).

Nine patients from the Study Group claimed to have had anal sex intercourse, whilst 54 denied. In the Control Group, all patients denied having ever had anal sex intercourse. We can affirm that there is a greater number of positive replies within the Study Group.

By carrying out the Chi-squared Test for Independence, with Fisher's correction, the p value obtained in the correlation between anal sex practice according to the considered groups provides evidence of rejection of null hypothesis of

Table 1: Median and Average age (in years) of Control and Study Groups' Patients.

Age	Control	Study
Median	41	38
Average	41.52	40.3

Z = 1407; p = 0.532 Mann-Whitney Test.

Table 2: Sexarc's Median and Average years of Control and Study Groups' patients.

Sexarc	Control	Study
Median	18	17
Average	18.83	17.65

Z = 1224.0; p = 0.206; Mann-Whitney Test.

Table 3: Parity's Median and Average of Control and Study Groups' patients.

Parity	Control	Study
Median	2	3
Average	2.10	2.98

Z = 1109.5; p = 0.015*; Mann-Whitney Test.

Table 4: Number of patients who use any sort of contraceptive method between Control and Study Groups' patients.

	Yes	No	Total	% of Yes
Study	52	11	63	83%
Control	25	23	48	52%
Total	77	34	111	69%

X² = 11.893; p = 0.001*; Chi-squared Test.

Table 5: Number of patients with a number of partners equal or higher than 4 (>= 4) between Control and Study Groups' patients.

	Yes	No	Total	% of Yes
Study	19	44	63	30%
Control	10	38	48	21%
Total	29	82	111	26%

X² = 1.228; p = 0.268; Chi-squared Test.

Table 6: Number of patients in relation to anal sex practice between Control and Study Groups' patients.

	Yes	No	Total	% of Yes
Study	9	54	63	14%
Control	0	48	48	0%
Total	9	102	111	8%

X² = 7.462; p = 0.006*; Chi-squared Test.

independence. We can affirm that there is a greater number of positive replies within the Study Group's patients (Table 7).

Only one patient of the Study Group presented a prior history of sexually transmitted disease, that is to say, 62 patients didn't present any STD history. In the Control Group all patients denied having a STD history.

The *p* value's result of 0.381 from the Chi-squared Test with Fisher's correction in the association between the presence of sexually transmitted disease in patients of both groups does not present evidence of rejection of null hypothesis, leading to the conclusion that the presence or not of sexually transmitted disease does not depend on the fact that a patient is part of the Study Group or Control Group (Table 8).

Only 2 patients of the Study Group and one patient of the Control Group took hormone replacement therapy. These data show that there is no relation between taking hormone replacement therapy and belonging to one of the groups.

There is no evidence of rejection of hypothesis of independence within the taking of hormone therapy between patients according to their groups. The *p* value provided by the test shows that there is no relation between taking hormone therapy and belonging to the Study Group or Control Group (Table 9).

Twenty-three patients out of a total of 63 of the Study Group consumed alcoholic beverages, whilst only 2 patients out of a total of 46 had this habit in the Control Group. Therefore, the fact they consume alcohol, increase the patients' chances of belonging to the Study Group.

Chi-squared Test's null hypothesis of independence is rejected for the relation between alcohol consumption and the group that a patient belongs to. There is a higher probability that patients who consume alcohol are part of the Study group (Table 10).

Thirty-five patients out of 63 were smokers in the Study Group. In the Control Group 11 out of 48 were smokers. This comparison provides a hypothesis that patients who smoke belong to the Study Group.

Hypothesis of independence between tobacco use and its presence in a certain group in this study is rejected. The *p* value considerably lower than the critical value of 0.05 confirms that patients who smoke present a higher tendency to be in the Study group (Table 11).

Four patients out of 63 of the Study Group had results in cytology as ASC-US. In the Control Group, 3 patients out of 48 had ASC-US.

As the *p* value obtained for the independence test between the result of the patients' oncotic cytology was

Table 7: Number of patients in relation to the presence of Sexually Transmitted Disease (not HPV) between Control and Study Groups' patients.

	Yes	No	Total	% of Yes
Study	1	62	63	2%
Control	0	48	48	0%
Total	1	110	111	1%

$\chi^2 = 0.769$; *p* = 0.381; Chi-squared Test (Fisher).

Table 8: Number of patients in relation to the use of hormone therapy between Control and Study Groups' patients.

	Yes	No	Total	% of Yes
Study	2	61	63	3%
Control	1	47	48	2%
Total	3	108	111	3%

$\chi^2 = 0.123$; *p* = 0.725; Chi-squared Test (Fisher).

Table 9: Number of patients in relation to alcohol consumption between Control and Study Groups' patients.

	Yes	No	Total	% of Yes
Study	23	40	63	37%
Control	2	46	48	4%
Total	25	86	111	23%

$\chi^2 = 16.330$; *p* - value = 0.0001* Chi-squared Test.

Table 10: Number of patients in relation to tobacco use between Control and Study Groups' patients.

	Yes	No	Total	% of Yes
Study	35	28	63	56%
Control	11	37	48	23%
Total	46	65	111	41%

$\chi^2 = 11.959$; *p* - value = 0.001* Chi-squared Test.

Table 11: Anal oncotic cytology results of Control and Study Groups' patients.

	BCC	ASC	Total	Percentage
Study	59	4	63	94%
Control	45	3	48	94%
Total	104	7	111	94%

$\chi^2 = 5.699$; *p* - value = 0.058 Chi-squared Test.

BCC: Benign Cellular Changes; ASC: Atypias of Squamous Cells of indeterminate meaning – possibly non-neoplastic.

Table 12: Number of patients in relation to menopause between Control and Study Groups' patients.

	Yes	No	Total	% of Yes
Study	12	51	63	19%
Control	12	36	48	25%
Total	24	87	111	22%

$\chi^2 = 0.570$; *p* = 0.450; Chi-squared Test.

higher than 0.05, even under a small margin, the hypothesis of independence won't be rejected and we consider that the result is independent of whether the patients are part of the Study group or Control group (Table 12).

Twelve patients out of a group of 63 were menopausal in the Study Group. Twelve patients out of 48 were menopausal in the Control Group.

There is no evidence of an association between menopause and the group to which a patient belongs. The *p* value obtained of 0.450 is not enough to reject the hypothesis of independence.

Discussion

Most studies about anal intraepithelial neoplasia are performed in HIV-positive patients and in men who have sex with men. Studies in patients with immunocompetent CIN II and CIN III, as those in this study, are scarce [11].

In the cases analyzed in this study, no statistically considerable difference was observed between the number of cases in altered anal oncotic cytology in the group of patients with CIN II and CIN III in comparison to the group of patients not carrying this lesion. In literature, three studies were found which seek to estimate the prevalence of AIN in women with lower genital tract neoplasia; two were linked to the execution of high resolution anoscopy and the study from Park, et al., provides data related to the prevalence of anal changes in anal cytology [12,13].

Park, et al. [12], found changes in anal cytology of 7.35% (four out of 68 women) with cervical pre-neoplastic lesions. By analyzing the results of this study, four cases of altered cytology were found in a group of 63 patients, revealing a prevalence of 6.34% of altered cytology.

This research sample profile, composed by 63 patients in the study group and 48 in the control group, is based on patients who make use of the Brazilian Public Healthcare System, starting their sexual lives before turning 20 and having a clear association with alcohol beverages consumption, tobacco use and oral contraceptive in patients that are part of the Study group. This profile characterizes women with CIN II and CIN III who make use of the Oncology Gynaecology Service at HCSL.

In contrast, two other studies which seek to determine the prevalence of precursor lesions of anal cancer in women with genital neoplasia, carried out in Brazil, didn't show any considerable association between anal intercourse and anal lesion precursor to neoplasia [14,15]. A considerable association between alcohol beverages consumption and tobacco use was found between patients of the study group.

The cultural context in which the patients live nowadays end up stimulating the regular consumption of tobacco and other drugs. Alcohol when abusively consumed leads to a risky sexual behavior, e.g., unprotected sex [16]. Moreover, studies suggest considerable associations between cervix cancer and HPV infection in consumers of alcohol, tobacco and other drugs [17].

Data similar to the ones in the literature were found in this research, as a considerable higher number of patients who consume alcohol beverages was observed in the study group. Heráclio, et al. [11] cited more than 50% of patients with CIN II and CIN III being alcohol consumers [18].

The high proportion in the study group of patients who smoke, in this research, can be linked to the fact that tobacco

reduces considerably the amount of functions of Langerhans cells, which are responsible for the defense of the epithelial tissue [19].

However, there is a mechanism of interaction of sexual steroids, although not yet clarified, in the sense of stimulating the viral transcription of HPV. For this reason, it is hypothesized that the use of exogenous sexual hormones is a risk factor to the genesis of cervical carcinoma. It is also known that the uterine cervix transformation area is highly sensible to estrogens, in comparison to other areas of the female reproductive system. However, studies reveal that there doesn't seem to be any increased risk in the most common formulations [20]. This observation was pertinent when assessing the data, as there is a similarity between the cervix and anal epitheliums [21].

Menopause does not represent a risk factor in this study for patients of both groups. According to Jacyntho [22], HPV occurs with less frequency in hypoestrogenic environments, probably due to the difficulty of the virus proliferation in people with low oestrogenic rate, as with men, that suppress virus diseases more easily.

Anal cytology in patients with CIN II and CIN III can be carried out routinely in all gynaecological services, at the same moment when cervix cytology is collected (Pap test), in a painless, quick and cheap way, and can be conducted in Community Health Centers.

In the case of patients with anal intraepithelial neoplasia, screened by cytology and confirmed biopsy, a simpler treatment with local chemical agents could be put in practice with a possible complete remission of the disease and its cure. This procedure could avoid its progression to invasive lesions and their possible consequences, thus avoiding high expenses with complex treatments, serving a considerable higher number of people, with a great reduction in morbidity and improvement of these people's quality of life.

From this study and its protocol, anal cytology collection can be added to the gynecological routine of doctors and other professionals involved in the care of patients with high-grade intraepithelial neoplasia by seizing the chance of carrying it out during a gynecological exam.

By expanding the knowledge in order to inform the community about the pathophysiology and the consequences of anal cancer and the propose of screening being drawn up, desmystification in relation to this exam's routine screening may occur, making women more aware in relation to the need for its collection.

Conclusion

Changes in anal cytology of the Study Group were identified and these must be considered due to the risk of dealing with pre-neoplastic anal lesion.

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