

IMPACT OF VAGINAL ESTROGENIZATION IN FOLLOW-UP OF ONCOTIC CYTOLOGY OF UNDETERMINED SIGNIFICANCE IN POSTMENOPAUSAL WOMEN

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ABSTRACT: Current paper evaluates topic vaginal estrogenization following oncotoc cytology of undetermined significance, type ASC-H, in postmenopausal women. A case-control study evaluated menopausal women diagnosed with ASC-H, divided into two groups, with and without the use of topic vaginal estrogen (GE and GNE). Odds ratios were calculated from contingency tables built with robust variation. 128 medical records were analyzed, 27% of which were from the GNE and 72% from the GE. There was an increase in ASC-H diagnoses in GNE. In GE, approximately 68 times less inappropriate colposcopies and a decrease in the number of colposcopies and High Frequency Surgery (HFS), with a reduction of 21 and 12.5 times, respectively. Decrease in the number of colposcopies with better adaptation to the exam, unchanged number of biopsies and decrease in the number of high-frequency surgeries, in the group using topic vaginal estrogen during the follow-up.

KEY WORDS: Atypical squamous cells of the cervix; Estriol; Menopause; Squamous intraepithelial lesions of the cervix.

IMPACTO DA ESTROGENIZAÇÃO VAGINAL NO SEGUIMENTO DE CITOLOGIA ONCÓTICA DE SIGNIFICADO INDETERMINADO NA PÓS-MENOPAUSA

RESUMO: Avaliar a estrogenização tópica vaginal no seguimento de citologia oncológica de significado indeterminado, tipo ASC-H, na pós-menopausa. Estudo, tipo caso-controle, que avaliou mulheres menopausadas com diagnóstico de ASC-H, divididas em dois grupos, com e sem uso de estrogênio tópico vaginal (GE e GNE). Foi calculada razão de chance a partir de tabelas de contingência construídas com variação robusta. Foram analisados 128 prontuários, sendo 27% de pacientes do GNE e 72% do GE. Houve aumento de diagnósticos ASC-H no GNE. No GE, aproximadamente 68 vezes menos colposcopias inadequadas e diminuição do número de colposcopias e Cirurgia de Alta Frequência (CAF), com evidência na redução em 21 e 12,5 vezes, respectivamente. Diminuição do número de colposcopias com melhor adequação ao exame, número de biópsias inalterado e diminuição do número de cirurgias de alta frequência, no grupo com uso de estrogênio tópico vaginal durante o seguimento.

PALAVRAS-CHAVE: Células escamosas atípicas do colo uterino; Estriol; Lesões intraepiteliais escamosas cervicais; Menopausa.

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INTRODUCTION

It has been estimated that approximately one million females worldwide currently suffer from uterine cervix cancer. In 2012, some 528,000 new cases of uterine cervix cancer were diagnosed and 266,000 females died of the disease, most of whom in low and medium income countries¹.

Papanicolau smear test is a simple and low-cost technique commonly employed for screening cervical cancer and pre-invasive lesions of the uterine cervix by the cytology technique for diagnosis. Due to the association of cytology and other techniques for the tracing of uterine cervical lesions, there has been a decrease in death rates by cervical cancer owing to the detection of lesions within the pre-invasive phase^{2,3}.

Besides high and low grade lesions by cytology, the Bethesda Classification System currently takes into account ASC-H (Atypical Squamous Cells of Undetermined Significance, without the exclusion of High Grade Lesions) which occurs when there is an atypical cell division of squamous-like cells, with undetermined significance for the lesion⁴.

Since patients forwarded for cervical colposcopy and biopsy may overload the health system if caution is omitted, the Ministry of Health and the Brazilian Cancer Institute (INCA) recommend colposcopy for all patients with ASC-H cytology, regardless of their age⁵. Consequently, the identification of lesions that require care prevents the use of unnecessary aggressive treatments and enhances the treatment of early lesions⁶.

Further, estrogen, as a steroid hormone, is directly linked to several organic functions, such as sexual differentiation, growth, metabolism and sexual activity. Ovarian production is the main source of estrogen in the premenopausal females, in which androgens, derived from theca cells, are transformed into estrogens in the granulosa cells by aromatase enzyme. In the postmenopausal female, the main estrogen source is extragonadal, produced by the adipose tissue. However, production has a lower quality and quantity than those in the premenopausal phase⁷.

The climacteric and menopausal phases integrate the female reproduction cycle. The climacteric phase

antecedes menopause and is a transition phase between the reproductive and non-reproductive phases in which hormonal changes, vaginal alteration and the ceasing of menstruation occur^{8,9}. Menopause is characterized by the interruption of the menstrual cycles for more than a year^{10,11} and may occur between 40 and 65 years of age¹¹. Due to a decrease in hormone levels of the endogenous estradiol during this period, such symptoms as vaginal atrophy, loss of urine due to efforts, dysuria, nocturia, urine urgency, feeling of incomplete vesicle emptying and replicated urinary infections¹² may occur with great discomfort. Further, vaginal atrophy may mask diagnosis of cervix lesions in the cytological exam due to difficulties in collection and thus contributes towards an increase in false-positive results^{2,3,12}. Consequently, therapy with vaginal topical estradiol in postmenopausal females proves to be efficient to mitigate symptoms linked to vaginal atrophy^{3,13}.

Current research evaluates the impact of vaginal estrogenization after type ASC-H oncotic cytology in postmenopausal patients attended to by specialized service in the pathology of the genital tract.

METHODOLOGY

Current analysis is a controlled case involving the evaluation of clinical cards on the Pathology of the Lower Genital Tract (PLGT) of the Regional Hospital Hans Dieter Schmidt (HRHDS) in Joinville, state of Santa Catarina, Brazil. HRHDS is a national health institution, featuring specialized PLGT service with outpatient and surgical attendance. Current study is a section of a master's dissertation by the main author, Natacha Machado de Araújo, and was undertaken with patients between February 2013 and July 2017. They were forwarded by the basic health network to the PLGT service of the hospital due to cytological alterations. The period for data retrieval comprises the start of the PLGT outpatient sector in the HRHDS till July 2017 at the end of the study. All patients were forwarded by the public health service to the PLGT of HRHDS.

After the analysis of the clinical charts of users enrolled in the system and which were available in the outpatient system (767 charts), only those charts featuring

patients with cervical pathologies were selected for the study groups. Further, only patients with results of the colposcycological exam, type ASC-H, and, concomitantly, in the menopause phase and with urogenital atrophy during the first colposcopy evaluation when enrolled were selected. Consequently, due to the above criteria, 128 charts were included in the study, whilst a database was established on Microsoft Excel.

Patients with a previous history of high grade cytological changes and patients without follow-up (last visit occurred a year ago) were not included. Patients with incomplete data in the charts were also excluded. It should be underscored that the interval between cytological alteration and evaluation by reference service never exceeded six months in any patient.

Due to their specificity for pathologies of the lower genital tract, the charts have full data, including the use or recommendation of estriol. Consequently, the sample was divided into two groups: patients who used topic vaginal estriol (GE) and patients who did not (GNE).

GE comprised patients who used estriol cream 1mg/g at least once a week, during 30 days, even irregularly. GNE comprised patients who did not use topic vaginal estriol prior to follow-up. The non-use was due to the patient's personal reasons which did not interfere in current study. Estriol cream mentioned in current study is available free of charge to all patients attended by the Brazilian health service.

Variables analyzed comprised age, time of menopause, number of pregnancies, births, menarche, sexarche, schooling, smoking, contraceptive method, systemic hormone therapy, results of cytological,

colposcopy and histopathological tests in semester follow-ups. Data were retrieved manually, transcribed and stored in a database within Microsoft Excel (2016).

Data analysis was performed with IBM-SPSS 21.0. Quantitative variables were given in means and standard deviation and qualitative variables were given in absolute and relative frequencies. When normal distribution of clinical characteristics by Kolmogorov-Smirnov's test in continuous variables was confirmed, test t for quantitative variables and chi-square test for qualitative variables were performed, except when frequency was lower than 5. In this case, Fisher's test was applied. The Mann-Whitney test was applied when normality was missing. Multivariate logistic regressions were constructed to evaluate the treatment's different outcomes, whilst chance ratios were calculated from tables to adjust biases. All tests were performed at $p < 0.05$ significance level. Adjustment factors for age, menopause period, systemic TRH and smoking were taken into account. Current study was approved by the Committee for Ethics in Research, n. 2.290.005.

RESULTS

Ninety-two (72%) out of 128 patients, used topic vaginal estriol, whilst 36 (28%) did not use topic vaginal estriol. When the two groups are compared, there was no significant difference with regard to age, number of pregnancies, time of menopause, age at menarche, age at sexarche, smoking and schooling level. GE used more systemic hormonal reposition prior to females who did not use topic vaginal estriol ($p=0.005$), as Table 1 shows.

Table 1. Epidemiologic profile of patients analyzed

	With Estriol n=92	Without Estriol n=36	P Rate
Age (years) (DP)	61.4 ± 8.2	58.6 ± 6.7	0.081*
Number of pregnancies (DP)	3.5 ± 2.1	3.25 ± 1.4	0.852**
Time of menopause (months) (DP)	128.2 ± 93.6	96.5 ± 75.1	0.075**
Menarche (mean age) (DP)	12.9 ± 1.6	12.9 ± 1.7	0.878**
Sexarche (mean age) (DP)	16.4 ± 2.4	16.6 ± 2.2	0.282**
Systemic TRH (N; %)	45 (49.5)	8 (22.2)	0.005***
Smoking (N; %)	24 (26.1)	12 (33.3)	0.412***

(Continuation)

	With Estriol n=92	Without Estriol n=36	(Conclusion) P Rate
Schooling (N; %)			0.562***
Literate	6 (6.5)	1 (2.7)	
Illiterate	9 (9.8)	3 (8.3)	
Incomplete primary schooling	45 (48.9)	13 (36.1)	
Complete primary schooling	21 (22.8)	12 (33.3)	
Incomplete high schooling	5 (5.4)	1 (2.7)	
Complete high schooling	0	3 (8.3)	
Incomplete higher education	5 (5.4)	3 (8.3)	
Complete higher education	1 (1.1)	0 (0)	

*Test t ** Mann-Whitney *** Chi-square test

Source: the author

The group that used topic vaginal estrogen (GE) had a smaller number of ASC-H cases, with no statistical difference with regard to cytology of Low and High Grade Squamous Intraepithelial Lesion (LGSIL and HGSEL), biopsy (normal, non-specific/reactionary alterations, (LGSIL and HGSEL), as Table 2 shows.

Table 2. ASC-H post-diagnosis semester outpatient follow-up with and without topic vaginal estriol

	Vaginal estriol (Yes) N=92 (%)	Vaginal estriol (No) N=36 (%)	(Continuation) P rate
Cytology			
Normal	37 (40.2)	3 (8.3)	0.000*
ASCUS ¹	29 (31.5)	1 (2.8)	0.000*
ASC-H ²	11 (12.0)	28 (77.8)	0.001**
LGSIL ³	0 (0)	1 (2.8)	0.283*
HGSEL ⁴	15 (16.3)	3 (8.3)	0.396*
Colposcopy			
No need	12 (13.0)	2 (5.6)	0.347*
Adequate	66 (71.1)	3 (8.3)	0.000*
Not adequate	14 (15.2)	31 (86.1)	0.000**
Biopsy			
Not done	68 (73.9)	30 (83.3)	0.258**
Normal	0 (0)	2 (5.6)	0.078*
Non-specific/reactionary alterations	7 (7.6)	1 (2.8)	0.440*
LGSIL ³	1 (1.1)	1 (2.8)	0.485*
HGSEL ⁴	16 (17.4)	2 (5.6)	0.097*
HFS			
Not done	73 (79.3)	8 (22.9)	0.000**

(Conclusion)

Normal	0 (0)	17 (48.6)	0.000*
Low grade	0 (0)	4 (11.4)	0.006*
High grade	16 (17.4)	5 (14.3)	0.793*
Cancer	3 (3.3)	2 (2.9)	1.000*

* Fisher's exact test ** Chi-square test.

1. ASCUS: Atypical Squamous Cells of Undetermined Significance; 2. ASC-H: Atypical Squamous Cells of Undetermined Significance without the exclusion of High Grade Lesion. 3. LGSIL: Low Grade Squamous Intraepithelial Lesion. 4. HGSEL: High Grade Squamous Intraepithelial Lesion.

Source: the author.

The use of topic vaginal estriol decreased 13 times the number of colposcopy and 21 times when adjustment factors were used. The table reveals that there was no need of colposcopy in 12 patients since their cytological exams were normal. There was also a greater adequacy to colposcopy in the GE group when compared to the GNE, as Table 3 demonstrates.

Table 3. Need for colposcopy in the group of patients that used vaginal estriol and in the group that did not use vaginal estriol

	N		Crude			Adjusted		
	without estriol	with estriol	P	CR	IC95%	P rate	CR	IC95%
No need	2	12	0.014	13.286	2.617-67.440	0.001	20.644	3.305-128.932
Adequate	3	66	0.000	48.714	13.040-181.985	0.000	68.024	15.607-296.482
Not adequate	31	14	0.000	0.029	0.010-0.087	0.000	0.020	0.006-0.072

Notes: Adjustment factors: age, time of menopause, systemic HRT (Hormone Replacement Therapy) and smoking.

CR = Chance ratio.

Source: the author

There was no significant difference in biopsies for crude and adjusted rates when compared to the two groups studied.

Nineteen GE patients underwent High Frequency Surgery (HFS), whereas 28 GNE patients required surgery. Table 4 shows that there were less chances of invasive procedure in GE.

Table 4. Need of biopsy and HFS in GE and GNE patients

	N		Crude			Adjusted		
	Without estriol	With estriol	P rate	CR	IC95%	P rate	CR	IC95%
No biopsy	30	68	0.006	0.002	0.567-1.529	0.097	0.401	0.136-1.181
No HFS	8	73	0.000	11.526	4.650-28.569	0.000	12.540	4.661-33.740

Notes: Adjustment factors: age, time of menopause, systemic TRH (Hormone Replacement Therapy) and smoking.

CR = Chance ratio.

Source: the author

All ASC-H patients underwent colposcopy at enrolment. Patients with unsatisfactory colposcopy or with negative biopsy used or did not use (depending on the study group) of topic vaginal estriol and underwent another intervention after six months. Patients who used topic estriol had a lower index of cytolposcopy discrepancy, without any need for HFS. On the other hand, patients who did not use the therapy had a greater number of HFS, albeit without the intervention in a greater number of cervical cancer diagnoses.

DISCUSSION

Gynecologists have reported that, in clinical practice, atrophy may impair the collection of cytological material due to the difficulties in inserting the speculum and, consequently, adequate visualization of the Squamous Columnar Junction (SCJ) and the uterine cervix. However, no studies have proved the relationship between these findings. In fact, there is a basic gap in the literature on the theme.

Current analysis evaluated the correction of vaginal trophism by topic vaginal estriol in menopausal females. Data revealed that improvement occurred not only for ASC-H diagnosis, but also for the decrease in the number of invasive procedures such as High Frequency Surgery (HFS) and colposcopies. Further, a greater adequacy of colposcopy was highly relevant and showed a smaller chance in non-adequacy when patients use topic vaginal estriol at least once prior to exam.

Mello & Aguiar¹⁴ and Wilbur & Nayar¹⁵ underscored changes in classification related to cytology of the uterine cervix throughout the history of gynecological pathology. This statement is basic so that one may conclude that actually the insertion of the terms ASC-H and ASCUS for Atypical Squamous Cells of Undetermined Significance are comprehensive of a recent gynecological pathological science.

Analysis by Yang and collaborators¹⁶ demonstrated a 10.9% prevalence of LGSIL in females with positive ASCUS cytology. The same authors quoted a study by Sherman, Solomon & Schiffman identifying "equivocal HSIL" (ASCUS [atypical squamous cells of undetermined

significance]-H¹⁷, who demonstrated that 12% of females with ASCUS also had HGSEL, corroborated by current study with similar data and revealed the importance of HGSEL in the development of uterine cervical cancer.

In a study with a sample of 100 patients diagnosed with ASC-H, Siddiqui, Cohen & Nassar¹⁸ concluded that the updating of diagnostic tests is required due to the great number of false positive tests which fail to show the true situation of the disease. Current study showed similar results with regard to false positive tests. The correction of vaginal trophism decreases the number of ASC-H diagnoses considered positive results without the use of topic vaginal therapy.

Studies on chance ratio identified as a protecting factor the employment of topic vaginal estriol to avoid unnecessary invasive exams and even cytological and colposcopy exams with a false positive diagnosis. In the case of colposcopy, due to factors of chance ratio adjustments, one may perceive that there were approximately 21 times less chances for the performance of colposcopy in patients using topic vaginal estriol when compared to patients who did not use it.

The influence of hypoestrogenism on vaginal atrophy may be confirmed. The Brazilian Institute of Cancer Research (INCA)³ and Backes et al.¹⁹ state that the decrease in estriol levels alters the epithelium's cytological analysis which reveals a smaller number of superficial cells and an increase in basal and parabasal layers. In their systematic review, Lethaby et al.²⁰ insist that the therapy mode concerned was highly efficacious in the treatment of vaginal atrophy symptoms and showed slight differences in cream preparations, vaginal ring and vaginal pills going beyond oral hormone replacement therapy methods.

Results showed that patients who used topic vaginal estriol had an approximately 68 less chance of not presenting adequacy in the exams when compared to patients who did not use it. Since vaginal atrophy causes unsatisfactory or inadequate colposcopy according to the Handbook of Diagnoses and Behaviors of the Brazilian Association of Pathology of the Lower Genital Tract and Colposcopy (ABPTGIC)²¹ and other authors^{2,3,19}, the reconstitution of cell architecture and cervical epithelium

is crucial and makes possible a trustworthy exam and adequate screening of possible cervical lesions.

Data have shown that there was no greater chance in crude or adjusted rates with regard to biopsy among patients that used topic vaginal estriol when compared to patients who did not use it. Although it does not affect lesions, estriol may elucidate false positive cases of ASC-H. When there is a clear case of uterine cervical lesion, the atrophy of vaginal epithelium does not interfere in undertaking biopsy since the cause of the lesion should be made clear by the method to continue the treatment. According to Muller et al.²² and Nam²³, standard exam for the diagnosis and differentiation of cervical lesions is the direct biopsy of the affected area which may be also visualized by colposcopy.

In the case of the number of High Frequency Surgery (HFS), there was a decrease in chances for the undertaking of the invasive procedure for patients who used topic vaginal estriol when compared to those who did not use it. Crude rates were adjusted for the factors age, time of menopause, systemic TRH and smoking, with approximately 12.5 times (adjusted rate) less chance for HFS in patients who used topic estriol when compared to patients who did not use it.

An increase in HFS indication has been currently detected due to cytolposcopy discrepancy, or rather, results for the cytological exam and colposcopy are different and, therefore, the invasive procedure has been adopted for elucidation.

Current research shows that decrease in HFS was caused by a decrease in cases of cytolposcopy discrepancy since topic estrogenization improved the correct identification in the lesions' cytology in its stages. According to Ciavattini et al.,²⁴ HFS may be indicated for patients in whom SCJ is not visible (inadequate colpocytology) and if there is any suspicion of endocervical lesion, such as in cases of cytolposcopy discrepancy, positive curettage of the endocervical canal and unsatisfactory colposcopy. When there is an improvement in the adequacy of colpocytology post-topic vaginal therapy and decrease of cytolposcopy discrepancy, decrease in HFS is corroborated by indicating the above-mentioned invasive procedure^{3,24}.

Oliveira et al.⁵ have insisted that the excessive referral of patients to colposcopy and biopsy may overload the health system, especially the National Health System (SUS) focused in current study. Since resources in Brazil, a developing country, are scarce, the possibility of a new protocol should be undertaken in the follow-up of postmenstrual patients with ASC-H cytology. The protocol would decrease unnecessary costs, surgical comorbidities and an increase in the rapidity of secondary service. A prospective study is required in the form of a randomized clinical assay to support the statement. Here lies the scientific relevance of current study and the manner it may be a precursor for further analyses and possible modifications of future clinical behavior.

The limiting factor in current study is the lack of a clinical assay model, or rather, tests with control and treatment groups have not been performed. An assay with such a design would be highly interesting.

CONCLUSION

Current study has revealed that the number of colposcopies decreased and their adequacy improved. No changes in the number of biopsies occurred but there was a decrease in the number of High Frequency Surgeries in the group that used topic vaginal estriol during ASC-H follow-up in the population studied.

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