Original article

FACTORS ASSOCIATED WITH THE REPRESENTATIVENESS OF THE TRANSFORMATION ZONE IN CYTOPATHOLOGICAL CERVICAL TESTS

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ABSTRACT: The objective was to analyze the factors associated with the representativeness of the Transformation Zone in cervical screening tests. A retrospective and quantitative study was undertaken through the analyses of the cytopathology tests registered in the Cancer Information System in 2014. The final sample resulted in 1,157 reports. For the data analysis, the software Statistical Package for the Social Sciences was used. Of all tests,96.88% were undertaken for disease control and screening and 24.3% did not present the transformation zone. The factors that influenced the representativeness of the transformation zone were age between 25 and 64 years, use of oral contraception, hormone replacement therapy and metaplastic epithelium. These factors can be considered facilitators to capture a satisfactory sample with the possible reduction of false-negative results, which would entail a delay for the early treatment of cervical cancer.
DESCRIPTORS: Uterine cervical neoplasms; Mass screening; Vaginal smears.

FATORES ASSOCIADOS À REPRESENTATIVIDADE DA ZONA DE TRANSFORMAÇÃO EM EXAMES CITOPATOLÓGICOS DO COLO UTERINO

RESUMO: Objetivou-se analisar os fatores associados à representatividade da Zona de Transformação em exames citopatológicos para controle do câncer do colo do útero.Trata-se de um estudo retrospectivo de caráter quantitativo realizado por meio das análises dos exames citopatológicos registrados no Sistema de Informação do Câncer em 2014. A amostra final resultou em 1.157 laudos. Para a análise dos dados foi utilizado o software *Statistical Package for the Social Sciences*. Do total de exames, 96,88% foram realizados para o controle e rastreamento da doença e 24,3% não apresentaram a zona de transformação. Os fatores que influenciaram a representatividade da zona de transformação foram idade entre 25 a 64 anos, uso de contraceptivo oral, terapia de reposição hormonal e epitélio metaplásico. Tais fatores podem ser considerados facilitadores para a captação de uma amostra satisfatória com a possível diminuição de resultados falso-negativos, o que acarretaria o retardo do tratamento precoce do câncer do colo uterino.
DESCRITORES: Câncer do colo do útero; Programas de rastreamento; Esfregaço vaginal.

FACTORES ASOCIADOS A LA REPRESENTATIVIDAD DE LA ZONA DE TRANSFORMACIÓN EN EXÁMENES DE CITOPATOLOGÍA DEL CUELLO UTERINO

RESUMEN: Estudio cuya finalidad fue analizar los factores asociados a la representatividad de la Zona de Transformación en exámenes de citopatología para control del cáncer de cuello del útero. Es un estudio retrospectivo de carácter cuantitativo realizado por medio de los análisis de exámenes de citopatología registrados en el Sistema de Información del Cáncer en 2014. La muestra final resultó en 1.157 informes. Para el análisis de los datos, se utilizó el *software Statistical Package for the Social Sciences*. Del total de exámenes, 96,88% fueron realizados para el control y rastreo de la enfermedad y 24,3% no presentaron la zona de transformación. Los factores que influenciaron la representatividad de la zona de transformación fueron edad entre 25 y 64 años, uso de anticonceptivo oral, terapia de reposición hormonal y epitelio metaplásico. Se puede considerar esos factores como facilitadores para la captación de una muestra satisfactoria con la posible disminuición de resultados falso-negativos, lo que resultaría el retraso del tratamiento precoz del cáncer de cuello uterino.
DESCRIPTORES: Cáncer de cuello de útero; Programas de rastreo; Examen vaginal.

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INTRODUCTION

The cervical screening test is a strategy for the early detection of this type of cancer. Known also as the Papanicolau, Pap Test or Prevention test, its goal for the past 50 years has been to identify precursor lesions suggesting cancer based on epithelial cells, being the primary option around the world today for secondary cancer prevention[1,2]. Temporal comparative studies have demonstrated a significant reduction in morbidity and mortality rates after the introduction of this type of program[3]. In Brazil, the application of this test is recommended primarily in women between 25 and 64 years of age, being repeated every three years after two consecutive normal results in a one-year interval[4].

Therefore, appropriate collection of the material is fundamental for a successful diagnosis. When correlated with a population coverage of 80% of sample fitness, it reduces the incidence rates of cervical cancer by up to 90%[2]. The factors that limit the effective screening potential of oncotic cytology are the insufficient cell sample and the inappropriate preparation of the swabs, being closely related with the way the professionals collect the material[5]. Therefore, the health professional should guarantee a satisfactory swab with a representative number of squamous and glandular cells, fixed and reaching the Transformation Zone (TZ), where more than 90% of the precursor lesions of cervical cancer are located[4].

The TZ is characterized as the intersection between the stratified epithelium of the ectocercix and the columnar epithelium of the endocervix and its presence in the collection of tests has been considered a quality indicator of satisfactory samples[6]. The unsatisfactory collection of test material represents expenses for the health system, as there is no restriction on the payment of these tests. In addition, it entails physical wear for the woman who, when she returns to the health service, will need to be submitted to a new collection[7].

The absence of the TZ in the sample is not used to classify it as unsatisfactory, but can demonstrate the need for constant monitoring and professional qualification in the collection of prevention tests[4]. In some studies in the literature, cytopathology reports were analyzed, outlining the profile of the microorganisms found and the prevailing degree of inflammation in the cervical cells[3,8]. Nevertheless, few studies have been published on the theme thus far that assess the quality of the sample by analyzing the representativeness of the transformation zone[9].

Despite the increased test coverage available at the health services and the easy access to those services, cervical cancer represents the third largest cause of cancer in women and the fourth cause of death in that group. Per year, 17,540 new cases are estimated, with an estimated risk of 17 cases for every 100 thousand women[4]. Therefore, this situation proposes two hypotheses: low quality in the test collection or faults in the monitoring of women in case of precursor lesions[5].

As it is mostly the nurse who collects the cytopathology material, the quality of the care offered needs to be assessed by analyzing the test results and the factors associated with the absence of epithelial cells collected, with a view to contributing for these professionals to insert new conducts and routines in woman's health care. This can enhance the problem solving ability of primary health care by identifying factors that can be improved. This study is justified in order to strengthen and qualify the actions in woman's health care for the prevention and early diagnosis of cervical cancer.

Thus, this study departs from the premise that the high rates of precursor lesions of cervical cancer can originate in the determinants of the false negative results in which the region of the transformation zone is not captured. In view of the above, the goal was to analyze the factors associated with the representativeness of the TZ in cervical screening tests for cervical cancer control in an important city in the West of the State of Santa Catarina.

METHOD

A descriptive, retrospective and quantitative study was undertaken based on the analysis of the cytopathology reports registered in the Cancer Information System (SISCAN) of the City of Chapecó-SC. Located in the West of the State, the city has 189,052 inhabitants, beings 95,700 women[10]. In addition,

there are 29 Family Health Centers for gynecological health care.

To determine the sample calculation, the total test production in the year before the collection of the data registered in the SISCAN of the city was considered, equivalent to 19,296 tests. To guarantee greater statistical reliability, a 3% statistical error and 97% reliability was added to define the sampling. Through proportional stratification representative of the tests produced at each health service, the number of tests to be analyzed at each service was defined. Thus, after calculating the sample, in total, 1,157 reports were accessed in the city.

The data were collection in August 2015 and the reports were selected by the ordinal sequence in which they appeared in the system, starting with the first, followed by the second and so forth, until reaching the total number appointed at each service by the sample calculation. Hence, the inclusion criteria were: all reports assessed registered in SISCAN and cleared for the patients in 2014. The anonymity of each report was guaranteed, identified by a code consisting of "R" for the word "Report", followed by an Arabic numeral indicating the order of the data collection (R1, R2, R3...).

The research variables were the items described in the system, i.e. age, confirmed pregnancy, use of Intrauterine Device (IUD), use of oral contraception, bleeding after sexual intercourse or after menopause, hormone replacement therapy, signs of Sexually Transmitted Disease (STD), reason for test, previous test, cervical inspection, assessment of sample, representative epithelial cells, presence of TZ, descriptive diagnosis, microbiology and cytological cell alterations. It should be highlighted that the results of reports demonstrating cytolysis, hypotrophy, dyskeratosis, macronucleosis, escamas córneas, macrocytosis, binucleationand perinuclear halos were considered as cytological findings, being specific to clinical pathology.

For the data analysis, the softwareStatisticalPackage for the Social Sciences, version 20.0 was used. To study the association between the independent variables and the occurrence of not of the outcome, the variables at each determination level were subject to bivariate analysis, using Pearson's chi-squared test in case of normal distribution and Fisher's Exact Test for non-parametric data.

The intensity of the association was assessed by estimating the odds ratio. To control for the effect of potentially confounding variables, multivariate analysis (multiple logistic regression) was applied. For all inferential statistical tests, a significance level of $p<0.05$ was set. The quality of the adjustment was assessed by means of the Hosmer-Lemeshow test. The development of this research followed all standards. Approval was obtained from the Ethics Committee for Research involving Human Beings at Universidade Federal da Fronteira Sul (UFFS) on 08/05/2015, under Opinion 059401/2015and Ethical Evaluation Certificate (CAAE) 46421815.0.0000.5564.

RESULTS

In total, 1,157 reports of cervical screening tests were analyzed. In this group, 70.1% (n= 810) referred to women between 25 and 64 years of age. This age range is the target of health actions in Brazil. In addition, 22% (n= 254) involved women between 15 and 24 years of age and 65% over 65 years of age (Figure 1). The mean age was 37.5 (+/- 15.4 years).

The women's main characteristics according to the variables studied based on the test reports are displayed in Table 1. It could be identified that 96.7% (n= 1,121) of the tests were carried out for screening purposes and that 11.6% (n=134) of the women had never been subject to the test earlier.

As regards the test results, 99% of the selected sample was satisfactory and 75.6% presented squamous and glandular epithelium, showing the absence of the TZ in 24.3% of the tests. Concerning the diagnosis, 41.4% presented results within the limits of normality, followed by atrophy with inflammation (27.1%) and cytological findings (11.7%). The prevalent microbiota were *Lactobacillussp* (60.3%) and the infectious agent *Gardnerellavaginalis* (16.9%) (Table 2).

In Table 3, the association between the research variables and the representativeness of the transformation zone is displayed. In this initial analysis, the associated variables were: age between 25 and 64 years old, use of oral contraception, confirmed diagnosis of pregnancy, Hormone Replacement

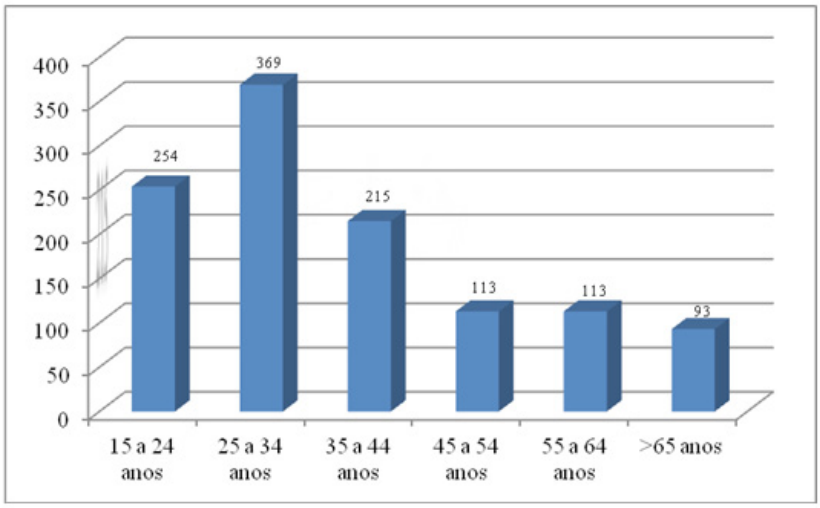

Figure 1 – Distribution of cervical screening tests according to age group. Chapecó, 2015

Therapy (HRT) and metaplastic epithelium.

In Table 4, the result of the multiple regression analysis is displayed to control for the effect of potentially confounding variables. The variables that were statistically associated with the representativeness of the TZ in this final model were: age between 25 and 64 years, use of oral contraception, HRT and presence of metaplastic epithelium.

Table 1 – Characteristics of women according to variables studies based on cervical screening tests for cervical cancer control. Chapecó, SC, Brazil,2015

Variables	n= 1157 reports	
	Freq.	%
Reason test		
Screening	1121	96.7
Follow-up	22	1.9
Repetition	14	1.2
Previous test		
Yes	1023	88.4
No	134	11.6
Use IUD*		
Yes	12	1
No	1145	99
Use contraception		
Yes	549	47.5
No	608	52.5
Diagnosis pregnancy		
Yes	11	1
No	1146	99
Treatment Radiotherapy		
Yes	1	0.1
No	1156	99.9
Hormone Replacement Therapy		
Yes	22	1.9
No	1135	98.1
Bleeding after sexual intercourse		
Yes	35	3
No	1122	97
Bleeding after menopause		
Yes	8	0.7
No	1149	99.3

*Intrauterine Device

Table 2 – Aspects of cytopathology reports of cervical cancer control. Chapecó, SC, Brazil, 2015

Variables	n= 1157 reports	
	Freq.	%
Cervix aspect		
Absent	30	2.6
Normal	1042	90.1
Altered	80	6.9
Not visible	5	0.4
Suggesting STD*		
Yes	30	2.6
No	1127	97.4
Assessment of sample		
Rejected	3	0.3
Satisfactory	1146	99
Unsatisfactory	8	0.7
Epithelium of sample		
Squamous	1146	99
Glandular	866	75.6
Metaplastic	203	17.7
Transformation Zone		
Present	865	74.8
Absent	281	24.3
Diagnosis		
Within normal limits	479	41.4
Immature squamous metaplasia	67	5.8
Cytological findings**	135	11.7
Atrophy with inflammation	313	27.1
Low-grade intra-epithelial lesion	12	1
Atypical cells of undetermined meaning	20	1.7
High-grade intra-epithelial lesion	3	0.3
>2 associated	117	10.2
Microbiota		
Lactobacillussp	698	60.3
Cocos	2	0.2
Chlamydiasp	1	0.1
Gardnerellavaginalis	195	16.9
Candidaalbicans	44	3.8
Bacilli	145	12.5
Trichomonasvaginalis	2	0.2
> 2 associated	105	9.1

* Sexually Transmitted Disease
**cytolysis, hypotrophy, dyskeratosis, macronucleosis, escamas córneas, macrocytosis, binucleationand perinuclear halos.

Table 3 – Bivariate analysis of association between variables studied based on cervical screening test and representativeness of transformation zone found in the sample epithelium. Chapecó, SC, Brazil, 2015

Variables	Transformation Zone (TZ)				
	Yes (n=865)		No (n= 281)		p-Value
	n	%	n	%	
Target public					**0.010**
Yes	620	71.7	183	65.1	
No	245	28.3	98	34.9	
Use IUD*					0.769
Yes	2	0.7	10	1.2	
No	855	98.8	279	99.3	
Use oral contraception					**0.000****
Yes	476	55.0	71	25.3	
No	389	75.0	210	74.7	
Radiotherapy treatment					0.210
Yes	0	0.0	1	0.4	
No	865	100.0	280	99.6	
Diagnosis pregnancy					**0.004****
Yes	5	0.6	5	1.8	
No	860	99.4	276	98.2	
Bleeding after sexual intercourse					0.481
Yes	9	3.2	25	2.9	
No	840	97.1	272	96.8	
Bleeding after menopause					0.320
Yes	0	0.0	7	2.5	
No	865	100.0	274	97.5	
HRT**					**0.000****
Yes	12	1.4	8	2.8	
No	853	98.6	273	97.2	
Cervix aspect					0.870
Normal	799	92.8	235	93.3	
Altered	62	7.2	17	6.7	
Signs suggesting STD***					0.727
Yes	24	2.8	6	2.1	
No	841	97.2	275	97.9	
Metaplastic epithelium					**0.000****
Yes	202	23.4	1	0.4	
No	661	76.6	280	99.6	
Diagnosis					0.503
Within normal parameters	362	41.8	117	41.6	
Positive cases	503	58.2	164	58.4	

*Intrauterine device
**Hormone Replacement Therapy
***Sexually Transmitted Disease
**** Significant difference between age group of the sample for p<0.05 (Fisher's exact test with Yates' correction).

Table 4 – Logistic regression of factors associated with representativeness of Transformation Zone in epithelial cells of cytopathology reports. Chapecó, SC, Brazil, 2015

Variable	Adjusted OR	95% CI	p-value
Use of oral contraception	1.2	(1.37 – 2.98)	0.0000
Hormone replacement therapy	0.4	(0.15 – 1.02)	0.0270
Metaplastic epithelium	1.3	(0.19 – 2.96)	0.0000
Age between 25 and 64 years	0.6	(0.51 – 1.93)	0.0250

DISCUSSION

Despite the large coverage of women attended in primary care and the easy access to the test, the percentage of women who took the preventive test in this study corresponded to 70.1%, below the proposed guideline of 80% to influence the epidemiological profile of cervical cancer in the country[11]. Other studies also evidenced the low coverage rate of tests in women between 25 and 64 years of age[11,12].

Applying the test to women younger than 25 years would imply a significant increase in diagnoses of low-grade lesions that are not precursors of cancer and will spontaneously reverse in most cases, resulting in unnecessary colposcopies and diagnostic and therapeutic procedures[13]. Various facts indicate that, direct or indirectly, the screening in women under 25 years of age does not influence the reduction in the incidence and/or mortality due to cervical cancer, as 1.1% of the cases of invasive injuries are found in these women[14]. On the other hand, there is no evidence that screening is useful in women over 64 years of age[15].

Some women in this study had never been subject to the test, similar to other studies[16,17]. The reasons why women do not adhere to this practice include: fear, shame, physical discomfort, absence of symptoms or complaints and lack of information(18).

Also concerning the characteristics of the study population, only 1% of the women who took the tests in this study were in the pregnancy period, similar to other studies[19,20]. Pregnancy does not impede that women develop cancer, nor that they take the preventive test, nor does it interfere in a favorable outcome, to the extent that routine screening for precursor lesions of cervical cancer in pregnant women is the same as in non-pregnant women(4).In the city studied, either the pregnant women took their cervical screening tests within the interval recommended by the Ministry of Health or the professionals responsible for collecting the tests were not doing so during this period.

In this study, 41.4% of the samples presented diagnoses within normal parameters, similar to the findings in studies undertaken in Mato Grosso do Sul and São Paulo[21,22]. Benign cell alterations were the most frequent, particularly inflammations and cytopathological findings, like in other studies[8,23]. It can be inferred that the reproductive health of the women in questions exceeds the expectations, as less than 1% of the population who took the test presented complications like bleeding after the menopause, radiotherapy treatment, low or high-grade atypical squamous cells, also illustrated by the small proportion of signs suggesting STD and visibly altered cervices.

It should be highlighted that the cytological findings found in the tests in this study, including cytolysis, dyskeratosis, macrocytosis, macronucleation, binucleation, among others, are morphological cell alterations that facilitate infection by the Papillomavirus (HPV)[24]. The microbiological and infectious findings in this study, such as the presence of *Lactobacillussp* and *Gardnerellavaginalis*, respectively, were also found in studies undertaken in Paraíba and Sergipe, classified as normal findings as they are part of the female vaginal flora and represent alterations in the vaginal pH[23,25].

The assessment of the epithelia in the sample of this study revealed that 24.3% did not present the glandular epithelium, reaffirming that the inexistence of this epithelium also reveals the inexistence of the TZ. The absence of this zone limits the visibility and interpretation of the collected sample, which

contributes to a high rate of false negative results, associated with a bad quality of the collection of these cells[26]. It is highlighted that 62% of the causes related to the false negative results refer to errors in the collection of the test material[27]. In this study, less than 1% of the samples were rejected or unsatisfactory, characterizing the nurses' potential to collect the tests.

Among the variables that revealed statistical associations with the study outcome, the use of oral contraception presented 1.2 times more chance of presenting cells that represent the TZ in the test samples. It is believed that the hormonal action of estrogen influences the distribution of the basal and subcolumnar cells of the cervical epithelium, externalizing the location of this zone to a region easy to capture during the collection[7]. Like the hormonal action of oral contraception use, the hormones resulting from HRT were also associated with the presence of TZ cells, supporting that the hormonal action facilitates the externalization of these cells, facilitating their capture during the collection[4]. These findings could not be compared with other studies due to the lack of studies that used this approach.

Another aspect evidenced was the age range of the women between 25 and 64 years of age who presented a greater chance that the TZ would easily be captured during the test collection in comparison with the other age groups. A similar result was found in a study that, after the analysis of 24,316 reports, identified a drop in the number of TZ cells collected as the age range of the target population increased[7].

The presence of metaplastic epithelium also resulted in a 1.3 times higher probability of an association with the representativeness of the TZ. Squamous metaplasia is a transformation process of the glandular epithelium to squamous epithelium, predominantly occurring in younger women, making them vulnerable to the development of cervical intra-epithelial lesions and to the risk of infection by HPV[28]. In addition, the representativeness of the metaplastic epithelium can be equivalent to the presence of TZ and can also be used as a quality parameter of the test collection[26]. Some studies appoint the small proportion of reports in which this metaplastic epithelium was captured in the collected samples, which may result in false negative reports that retard the treatment of women with cervical cancer[25,29].

As 24.3% of the tests did not present cells representing the TZ and as the Brazilian guidelines establish that these cells should be present in all samples, the effectiveness of the screening method for precursor lesions of cervical cancer needs to be monitored. The scientific knowledge gained can permeate the introduction of different practical measures.

CONCLUSION

In this study, 70% of the cervical screening tests were applied to women between 25 and 64 years of age, the target population of health promotion actions in primary health care. If, on the one hand, age, oral contraception, HRT and metaplastic epithelium acted as facilitators for the capturing of TZ cells, on the other, weaknesses in the collection technique of the screening can demonstrate failures in the effectiveness of actions and services in clinical practice. That can be verified in this study, where TZ cells were not captured in the test for almost a quarter of the study population, demonstrating that the professionals can and have to improve and constantly assess the collection technique. The absence of two epithelia in the test samples can contribute to the incidence of false negative results and, thus, a delay in the early diagnosis of precursor lesions of cervical cancer, in accordance with the initial research hypothesis.

In this context, nursing should play a central role in the planning of actions to strengthen the work process, in view of the need to diagnose this type of cancer early. These findings underline the need to constantly monitor the quality of the cervical screening test collections, with a view to providing qualified and problem-solving care to the female population attended.

Nevertheless, it is highlighted that the reports analyzed only came from the public health system, which means a partial view of the reality and represents a study limitation. Further studies should use other quality and monitoring indicators of the preventive test samples.

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