ORIGINAL ARTICLE

Profile evaluation of women that attended reference health centers in Porto Alegre/RS and the relationship between cytological alterations detected in cytopathological examination and presence of HPV

Avaliação do perfil de mulheres atendidas em centros de referência em saúde de Porto Alegre/RS e relação de alterações citológicas detectadas no exame citopatológico e a presença do HPV

Evaluación del perfil de las mujeres atendidas en los centros de referencia de salud de Porto Alegre/RS y la relación de las alteraciones citológicas detectadas en el examen citopatológico con la presencia de VPH.

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ABSTRACT

Background and Objectives: Cervical cancer is considered a worldwide public health problem. Its diagnosis is made through cytopathological examination and its development related to human papillomavirus (HPV) infection. This study aims to evaluate the profile of women treated at reference health centers in Porto Alegre, Rio Grande do Sul, as well as the relation of changes observed to cytopathological examination with the presence of HPV. **Methods:** This is a cross-sectional study carried out in women treated at basic health units and a referral clinic of a public tertiary hospital, from July 2014 to January 2017. Representative samples of the endo/ectocervix were collected to perform the cytopathological examination and investigated for the molecular presence of HPV. **Results:** 169 women with mean age between 31 and 40 years were analyzed, of whom 125 (74%) reported that the onset of sexual activity occurred in the 15-20 years age group, and 37.9% reported having had three to five sexual partners. In relation to cytopathological examination, 71 (42%) had a negative result for intraepithelial lesion or malignancy and 98 (58%) some squamous cell abnormality: 20 (11.8%) atypical; 22 (13%) low-grade squamous intraepithelial lesion and 56 (32.6%) high-grade intraepithelial lesion (HSIL). Fifty (29.6%) were positive for HPV, of which 56.4% were

diagnosed with HSIL ($p < 0.01$). **Conclusion:** The results reveal a high frequency of HPV in samples with cytopathological changes, in young women and with a degree of exposure to HPV, reinforcing the importance of the role of its early identification in the investigation of cervical carcinogenesis.
Keywords: *Uterine Cervical Neoplasms. Pathology. Papillomaviridae. Papanicolaou Test. Polymerase Chain Reaction*

RESUMEN

Justificación y objetivos: El cáncer de cuello uterino se considera un problema de salud pública en todo el mundo. Su diagnóstico se realiza mediante el examen citopatológico (EC), y su desarrollo está relacionado con la infección por el virus del papiloma humano (VPH). Este estudio objetivó evaluar el perfil de mujeres atendidas en los centros de referencia en salud de Porto Alegre, Rio Grande do Sul (Brasil), así como la relación de las alteraciones observadas en el EC con la presencia del VPH. **Métodos**: Estudio transversal realizado en las mujeres atendidas por unidades de atención primaria y por una clínica ambulatoria de referencia del hospital público terciario en la ciudad de Porto Alegre, en el período de julio de 2014 a enero de 2017. Se recolectaron muestras representativas de endo/ectocérvice para realizar la CE, las cuales se clasificaron según el sistema Bethesda y se investigaron la presencia molecular del VPH. **Resultados**: Analizamos 169 mujeres con promedio de edad entre 31 y 40 años, de las cuales 125 (74%) informaron que el sexarche ocurrió en el rango de 15-20 años. La mayoría (37,9%) informó haber tenido de 3 a 5 parejas sexuales; y el 37,3% estaban usando anticonceptivos orales. Con respecto a la EC, 71 (42%) se clasificaron como negativos para lesión intraepitelial o malignidad; y el 98 (58%) tenían alguna anormalidad de células escamosas: 20 (11,8%) de atipias; 22 (13,0%) lesión intraepitelial escamosa de bajo grado y 56 (32,6%) lesión intraepitelial de alto grado (HSIL). La frecuencia de positividad del VPH encontrada fue de 50 (29,6%), de estas un 56,4% fueron diagnosticadas con HSIL (p<0,01). **Conclusiones:** Estos resultados revelan una alta frecuencia de VPH en muestras con alteraciones citopatológicas presentes en mujeres jóvenes con cierto grado de exposición al VPH, lo que refuerza la importancia de identificarse tempranamente en el análisis de la carcinogénesis cervical.
Palabras Clave: *Neoplasias del Cuello Uterino. Patología. Papillomaviridae. Prueba de Papanicolaou.*

INTRODUCTION

Cervical cancer (CC) is the fourth most frequent type of cancer in women worldwide. In Southern Brazil, it is also the fourth most frequent type of cancer (15.17 per 100,000). Estimates point to 22,211 new cases in Brazil in 2020, which will lead to death of 10,383 women; by 2035, about 261,206 new cases of cervical cancer will be diagnosed.[1,2]

The main etiological agent for the development of cervical cancer is the human papillomavirus (HPV), an infectious agent transmitted by fomites and sexual contact. Associated with HPV, several other factors are involved in cervical carcinogenesis, such as early initiation of sexual activities, multiple sexual partners, number of pregnancies, and smoking, showing that the persistence of HPV infection is a necessary but not sufficient condition for the development of neoplasia.[1-4]

Currently, there are more than a hundred HPV genotypes, which differ in their tissue tropism and carcinogenicity, thus being classified into one of four groups: group 1 (carcinogenic to humans); group 2A (probably carcinogenic); group 2B (possibly carcinogenic); group 3 (non-classifiable); group 4 (probably non-carcinogenic to humans).[3,5,6]

In 1940, based on professional initiatives, cervical cancer screening was introduced in Brazil through cytopathological examination, whose objective is to track intraepithelial lesions early to reduce mortality. The quality of cytopathological results depends on the technique and experience in the interpretation of morphological findings. Consequently, achieving greater sensitivity is a challenge due to the intra- and interobserver discrepancies reported.[1,7,8]

Cervical cancer evolves slowly, presenting benign and detectable precursor lesions that are curable even in asymptomatic women. About half of HPV infections are undetectable in cytopathological exams up to one year after contact with the virus, and the virus is commonly eliminated in a period of approximately two years without causing damage or symptoms. To reinforce this, approximately 34% of all squamous intraepithelial lesions regress, 41% persist, and 25% progress to high-grade squamous lesions. Of these, 10% progress to *in situ* carcinoma and 1% to invasive cancer. Thus, 75% of squamous intraepithelial lesions of all grades do not progress.[6,8,9]

Some techniques were developed to detect HPV and contribute to the early identification of this type of neoplasm, including hybrid capture and polymerase chain reaction. Nevertheless, these methodologies also present diagnostic limitations, especially lower specificity due to the impossibility of inferring whether the infection is transient or persistent, since it is known that the persistence of HPV, characterized by detection in two or more tests, is a prerequisite for the development of precursor lesions.[10-12]

In developed countries, such as the United States, there are three approaches for cervical cancer screening: the abovementioned cytopathological exam; HPV test, which identifies viral DNA in epithelial cells; and simultaneous evaluation between both methods, which verifies the same cell sample for both high-risk HPV types and cervical cell changes. Cytopathological examination is used as the only screening method for intraepithelial lesions in women aged 21 years or older due to the transience of HPV infection and the low specificity of the HPV-DNA test. When any cellular alteration is detected in this age group, HPV test is recommended to discover its origin. From the age of 30 onwards, cytopathological examination with molecular biology examination – a procedure known as co-test –, aims to increase screening intervals of up to five years for women who do not have HPV and have normal cytopathological results, even if they engage with new sexual partners safely.[13.14]

Although well-established in international programs, HPV-DNA tests are still being studied as a screening method in Brazil; despite being more sensitive than cytopathological examination, they are less specific, leading more women to colposcopy. This limitation can be circumvented if performed in women over the age of 30. The so-called co-test seems to be the most interesting approach, as only positive results for HPV-DNA accompanied by altered cytopathological results will be referred for colposcopy. The same recommendation occurs in the post-treatment of high-grade squamous intraepithelial lesion (HSIL), emphasizing that residual disease can be excluded in patients with negative results, but the positive test is not specific for indication of disease, rather recommending periodic follow-up for a longer time.[2,14,15]

In view of this scenario, the objective of this study was to evaluate the profile of women treated at reference health centers in Porto Alegre, Rio Grande do Sul, Brazil, as well as the relationship of alterations observed in the cytopathological exams with the presence of HPV.

METHODS

This is a cross-sectional study, in which cervical samples of 169 women treated in basic health units and reference outpatient clinic of a tertiary public hospital in the city of Porto Alegre were evaluated from July 2014 to January 2017.

After agreeing to participate in the study by signing the informed consent form, the women answered a questionnaire on sociodemographic, behavioral and clinical aspects and were then submitted to the collection of two representative samples of the squamocolumnar junction. One of the samples was placed on a glass slide for cellular exams and the second was stored in a liquid medium kit (Digene DNA with PAP® – DNA Collection Device – HC2 HPV; Qiagen, USA) for molecular analysis of HPV-DNA. Exclusion criteria were pregnancy or other alterations that compromised sample quality such as hemorrhage and or intense leukorrhea.

Cytopathology was carried out as described by Papanicolaou. Microscopy was performed by two independent cytologists to evaluate cellular and nuclear alterations, which were interpreted and classified according to the Bethesda 2014 system, in: negative, for intraepithelial injury or malignancy (NILM); atypical squamous cells of undetermined significance (Ascus) and atypia of indeterminate significance that cannot exclude high-grade intraepithelial injury (ASCH); cytopathological changes, which include low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL).[15,16]

Molecular detection occurred from the extraction of nucleic acids using TRIzol (Invitrogen), as described by the manufacturer. Subsequently, a qualitative test was performed

using the technique of reverse transcription polymerase chain reaction (RT-PCR), for simultaneous detection of HPV of different carcinogenic groups in the samples by amplification of the L1 region of the viral genome using the primers MY09/11 and GP5+/6+.[10,17] As quality control of sample collection and nucleic acid extraction, the β-actin gene was also amplified.[18]Only positive samples for the control gene were sent for HPV screening. Finally, fragments of 450 base pairs for MY09/11 and 150 base pairs for GP5+/6+ were visualized in 2% agarose gel. Positivity in at least one of the reactions was already considered as positive for HPV as a whole.[10,17,18]

This study met all the recommendations of Resolution 466/2012 of the National Health Council and was approved by the Human Ethics Committee of the Federal University of Rio Grande do Sul, the Municipal Health Department of Porto Alegre and the Hospital de Clínicas de Porto Alegre under opinions no. 708,354; 781,338 and 1,472,443 (CAAE 18868514.1.0000.5347).

The Statistical Package for the Social Sciences (SPSS v.20.0) was used for statistical analysis. The qualitative variables were described as means and standard deviation and compared by Fisher's exact test. Variable association was evaluated by Pearson's coefficient, with confidence interval of 95% ($p < 0.05$)

RESULTS

The study included 169 women, with a mean age of 40 (± 13) years, who had their first sexual intercourse at 16.7 (± 2.83) years. Of these, 129 (76.3%) had previous pregnancies, 118 (69.8%) had a history of contraceptive use, 20 (11.8%) had a history of sexually transmitted infections (STI), 103 (60.9%) were married or in a stable relationship, 45 (26.6%) were smokers, 140 (82.8%) had attended elementary/high school and 41 (24.2%) reported more than ten sexual partners up to the time of the interview. The sociodemographic and clinical variables are presented in Table 1.

Table 1: Sociodemographic and clinical characteristics of patients (n = 44).

Variables	N (%)	N cytopathological changes
Schooling level		
Elementary school	66 (39.1)	40
High school	74 (43.8)	37
Higher education	29 (17.1)	21
Smoker		
No	124 (73.4)	67
Yes	45 (26.6)	29**
Age of first intercourse		

10-14	30 (17.8)	17
15-20	125 (74)	70
21-30	13 (7.7)	8
31-40	1 (0.6)	1
Number of sexual partners		
1-2	37 (21.9)	19
3-5	64 (37.9)	34
6-10	42 (24.9)	26*
11-15	7 (4.1)	6 *
>16	19 (11.2)	11
Parity		
0	40 (24.3)	21
1-2	88 (52.1)	49
3-5	32 (19.5)	22
6-10	5 (3)	3
Not informed	2 (1.2)	1
Contraceptive method		
None	51 (30.2)	30
Oral contraceptive	63 (37.3)	40
IUD	4 (2.4)	3
Condom	19 (11.2)	7
Sterilization	10 (5.9)	7
Injection	22 (13)	10
Previous HPV infection		
Yes	54 (32)	44**
No	115 (68)	54
Previous CC occurrence		
Yes	7 (4.1)	6
No	163 (95.3)	93
Not informed	1 (0.6)	1
Family history of CC		
Yes	36 (21.3)	23
No	130 (76.9)	73
Not informed	3 (1.8)	2

IUD: intrauterine device; CC: cervical cancer; *($p < 0.05$); ** ($p < 0.01$).

Cytopathological results, obtained from the analysis of 169 ecto- and endocervical samples, is represented, according to the Bethesda 2014 system, in Figure 1. Of the samples included in the study, 71 (42%) women had negative cytological results (NILM), 98 (58%) some cytological alteration: 20 (11.8%) atypias, 22 (13%) LSIL and 56 (33.2%) HSIL (Table 2).

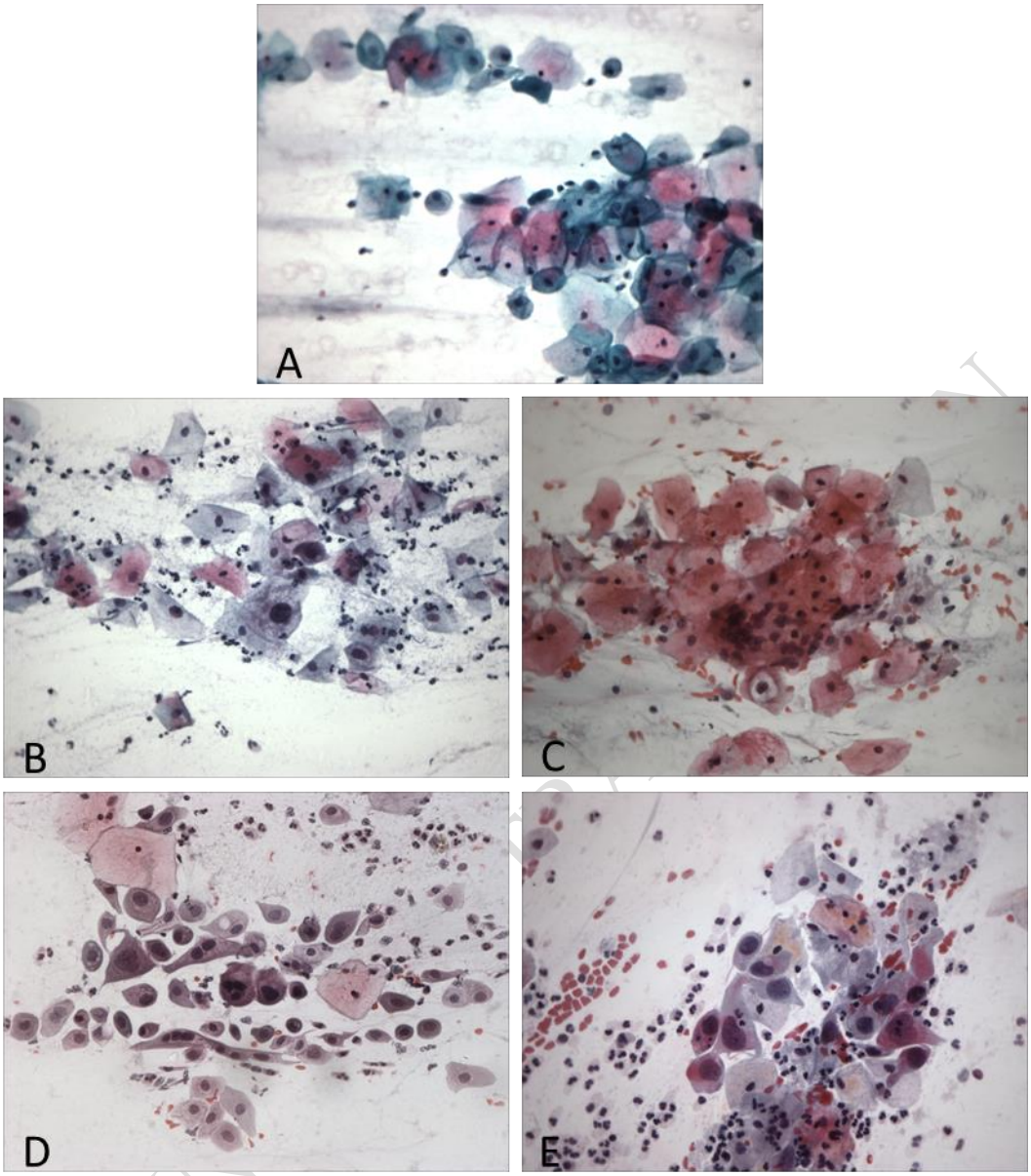

Figure 1. Cytological samples stained by Pap smearing and classified according to the Bethesda 2014 system (400X). A: negative for intraepithelial injury or malignancy (NILM); B: Atypical squamous cells of undetermined significance (ASCUS); C: low-grade squamous intraepithelial lesion (LSIL); D: atypical cells of indeterminate significance that cannot exclude high-grade intraepithelial lesion (ASCH); E: high-grade squamous intraepithelial lesion (HSIL).[15]

Of the 169 samples, 50 (29.6%) were positive for HPV. This result, when correlated with the cytopathological diagnosis (Table 2), demonstrates that 3 (4.2%) had no atypical changes or precursor lesions, 6 (30%) had the diagnosis of squamous atypia, 9 (38.1%) had low-grade squamous intraepithelial lesion and 32 (56.4%) had high-grade squamous intraepithelial lesion. Association between the variables was statistically significant, indicating that, when HPV is positive, around 94% of cytopathological analyses have some cellular alteration and 6% are classified as NILM.

Table 2: Age group correlated to cytopathological changes and HPV detection.

Age group	N (%)	N Cytopathological changes (%)	NILM	Atypia	LSIL	HSIL	Frequency (HPV identification)
15-20	17 (10.1)	2 (11.8)	15	1	1	0	3 (17.6)
21-30	37 (21.9)	24 (64.9)	12	4	6	14	13 (35.1)
31-40	41 (24.3)	25 (61)	16	4	5	16	14 (34.1)
41-50	36 (21.3)	25 (69.4)	11	7	6	12	9 (25)
>50	38 (22.5)	22 (57.9)*	17	4	4	14	11 (28.9)
Total (%)	169 (100)	98 (58)	71 (42)	20 (11.8)	22 (13)		56 (33.2)
Positive for HPV (%)			3 (4.2)	6 (30)	9 (38.1)	32 (56.4)*	50 (29.6)*
Negative for HPV (%)			68 (95.8)	14 (70)	13 (61.9)	24 (43.6)	119 (70.4)

NILM: negative for intraepithelial lesion or malignancy; ASCUS: atypical squamous cells of indeterminate significance; ASCH: atypical cells of indeterminate significance that cannot exclude high-grade intraepithelial injury; LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; *($p < 0.05$).

DISCUSSION

This study aimed to evaluate the profile of women assisted in primary healthcare and cervical cancer screening services, as well as a possible relationship of cytopathological alterations observed in examinations with the presence of HPV, thus seeking to broaden the understanding of cervical carcinogenesis.

From the interpretation of sociodemographic, behavioral and clinical characteristics of patients, we observed that most women were aged between 31 and 40 years, although a homogeneous distribution between the different age groups was also noted. The largest number of patients aged 30 years and over (> 68%) is probably due to the fact that this study also includes women already investigating cervical lesions, which displaces the mean age, corroborating the significant association ($p < 0.05$) with the diagnosis of atypia and precursor lesions found in women older than 50 years.

Regarding level of education, our data are in agreement with studies on the Southern Brazilian population, in which low and medium schooling were associated with precursor lesions of cervical cancer. This aspect emphasizes that an important portion of the population does not have access to basic information related to healthcare or participate in women's health programs.[9,19]

Similarly, we observed a considerable frequency of smokers, since 29% of them had some atypia or precursor lesion ($p < 0.01$). This result corroborates several studies that reinforce the important relation between smoking and HPV persistence, leading to the development of precursor lesions.[9,12]

Another important data refer the first sexual intercourse, since 125 (74%) women

included were in the range that had their first sexual intercourse at 15-20 years, of these, 70 (56%) had some atypia in the cytopathological exam, and this variable is considered an important factor for the development of cervical neoplasms. Several studies indicate that the early onset of sexual activity contributes to the increase in STIs, such as HPV, due to the greater opportunity of multiple partners throughout life. Reinforcing this, the literature has described that this period of puberty allows greater biological vulnerability to HPV infection due to exposure of the squamocolumnar junction, also known as cervical transformation zone.[6,12,19,20]

In addition to this data, it was observed that, when correlated with HPV positivity, the number of sexual partners presented a statistically significant association in the categories of 6-10 and 11-15 partners. This reinforces published results that observed a trend of early onset of sexual activity and the relevance of partner multiplicity, already attributed to the high prevalence of cervical cancer in developing countries.[9,14] Still, 54 (32.0%) participants already had previous HPV infection.

In the cytopathological examination, 44% had some atypia or precursor lesion in the sample ($p < 0.01$). This result corroborates the abovementioned data, indicating that early onset of sexual activity and multiplicity of sexual partners are very significant risk factors for both squamous cell carcinoma and adenocarcinomas.

From the exams, among the 169 samples, only 71 (42%) were diagnosed as NILM. This result includes, in addition to completely normal samples, benign cellular alterations (active or reparative), usually resulting from the action of physical agents, which can be radioactive, mechanical or thermal, and chemical, such as abrasive or caustic and chemotherapy drugs, as well as vaginal acidity on the epithelium.[8]

The frequency of cytopathological alterations found was 98 (58.0%), classified as: 20 (11.8%) atypias; 22 (13%) LSIL and 56 (33.2%) HSIL; these findings are interesting due to the high rate of squamous alterations. However, we note that this study also included women already investigating cervical lesions, which probably contributed to the increased frequency of atypia and precursor lesions.[19,21]

The prevalence of HPV-DNA was 50 (29.6%). According to a systematic review on prevalence in Brazil, the overall prevalence of cervical HPV infection ranged from 13.7% to 54.3%. Some authors detected a 15.5% prevalence in Rio Grande do Sul in 2007 at the Hospital das Clínicas de Porto Alegre; 27.5% in 2009, in gynecology and obstetrics outpatient clinics of the University Hospital of Rio Grande; 18.2% in 2013, in Gynecology and Obstetrics Outpatient Clinics and in a basic health unit in Rio Grande; 15.7% in 2013, at another healthcare unit in Cruz Alta; and 20.7% in 2016, in a private medical unit in Carazinho. These data considered

patients with characteristics similar to those observed in our sample, since they included women under investigation in reference services.[9,19,22-25]

When correlating positive HPV samples and cytopathological diagnosis (Table 2), demonstrates that 3 (4.2%) had no atypical changes or precursor lesions, 6 (30%) had the diagnosis of squamous atypia, 9 (38.1%) had low-grade squamous intraepithelial lesion, and 32 (56.4%) had high-grade squamous intraepithelial lesion. These data demonstrate that HPV detection is predominantly concomitant with the detection of precursor lesions in cytopathological examination, considering the statistical correlation ($p < 0.05$). The evolution of the viral infection corroborates this; the HPV integrates with host cells, accumulating several mutations and predisposing them to the development of precursor lesions of cervical cancer.[2,5,11,13] According to a 2010 study, research projects that only included women that attended health units have an increased number of abnormal cytology due to previous alterations or clinical complaints, compared to the prevalence of infection in the general population.[22]

We note that this study has limitations such as sample size and the non-association with histopathological diagnosis, considered standard. Another important aspect was the non-genotyping of HPV, which would provide the identification of the main viral types present in these samples, allowing progress in the understanding of local epidemiology.[5]

Advancement of technologies has improved screening programs, as well as the detection of HPV-DNA, which already has its proven importance and, according to instructions and use in developed countries, can be considered in the future for inclusion in screening programs in developing countries, as these factors are linked to the early diagnosis of patients with precursor lesions of cervical cancer.

This study, conducted in reference services, recorded high frequency and persistence of HPV due to the association with cytopathological alterations, which reinforces the importance of identifying this agent in the investigation of cervical carcinogenesis.

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Authors' contribution:

Andréia Buffon, Diogo André Pilger, Aline Daniele Schuster, Lúcia Maria Kliemann, Márcia Luiza Montalvão Appel Binda, Luciane Noal Calil and Débora Renz Barreto Vianna contributed to the design, analysis and writing of the article; Aline Daniele Schuster, Andréia Buffon, Diogo André Pilger contributed to the planning and design, review and final approval of the article. All authors approved the final version of the manuscript and declared themselves responsible for all aspects of the work, guaranteeing their accuracy and integrity.

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