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Herpes simplex virus type 1 is the main cause of genital herpes in women of Natal, Brazil

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ABSTRACT

Objectives: The purpose of this study was to assess the prevalence of HSV-1 and HSV-2 in sexually active women who participated in the cervical cancer screening program in Natal, Brazil.
Study design: The study included 261 sexually active women resident in the metropolitan area of Natal, Brazil and attending a public clinic for cervical screening. From each participant, a sample of exfoliated uterine cervical cells was collected, using a cytobrush which was conditioned in a tube containing a preserving solution (PBS + vancomycin + nystatin) and sent to a laboratory where it was processed for DNA extraction. The samples were analyzed for the presence of HSV-1 and HSV-2 DNA in separate reactions by PCRs using specific primers.
Results: HSV-1 in genital infection is four times more prevalent than HSV-2 in the population analyzed. The highest prevalence rates for both viruses were found in women aged 31–39 years. We did not observe any association between the presence of both virus serotypes and socio-demographic characteristics in the population studied, nor with some classical risk factors for sexually transmitted diseases.
Conclusions: HSV-1 was the major cause of genital infection by Herpes simplex virus in the women included in this study. No association was found between HSV infection and the socio-demographic characteristics or some classical risk factors for sexually transmitted diseases.

1. Introduction

The herpes simplex virus (HSV) belongs to the family Herpesviridae, subfamily Alphaherpesvirinae, and genus Simplexvirus, composed of two serotypes: Herpes simplex type 1 (HSV-1) and type 2 (HSV-2) [1]. Like other members of this family, these viruses can cause persistent infection and produce symptomatic and asymptomatic infections. They can cause infections of the skin and mucous membranes of the mouth, eyes, and genitals [2], being the main cause of genital ulcers worldwide [3,4].

HSV is a highly complex virus showing up as a particle of 186 nm diameter, composed of a genome of linear double-stranded DNA containing 152 kb [5]. HSV-1 has been primarily associated with orofacial infections, while HSV-2 is commonly associated with anogenital infection transmitted through sexual contact, and also an important source of vertical transmission of virus from mother to child. In recent studies, however, the proportion of cases of genital herpes caused by HSV-1 has increased considerably. Reasons for the seemingly increasing prevalence of anogenital HSV-1 are unclear. It is suggested that this reflects lower rates of childhood HSV-1 infections or possibly a change in sexual practices in regard to orogenital contact [2,6–9].

Genital infection with HSV is among the most common sexually transmitted diseases worldwide, with approximately 640,000 new cases diagnosed annually [10]. The epidemiological profile of genital infection by HSV suggests that the risk factors for the acquisition of the virus include age, sex, ethnic group, socioeconomic status, number of sexual partners, age at first intercourse, and a history of previous sexually transmitted infections (STIs) [11,12].

In Brazil, the reporting of diseases caused by herpes simplex virus is not mandatory. The few available data on the prevalence of infection were generally achieved in isolated studies on sexually transmitted diseases; those studies were conducted in public health clinics, and most were based on serology [13–15]. The present study evaluated the prevalence of genital infection with

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HSV-1, and HSV-2 in a segment of the female population of the metropolitan region of Natal, Brazil, and attempted to establish correlations with socio-demographics and with some conditions considered risk factors for sexually transmitted diseases.

2. Materials and methods

In this cross-sectional study, we included 261 sexually active women residents in the metropolitan area of Natal, Rio Grande do Norte state, Brazil, who self-referred for the program of cervical cancer screening at two public gynecological clinics in Natal city in the period between January 2000 and December 2003 and agreed to participate in the research. No patient presented clinical signs of the disease. All subjects participating in this study were informed about the methodology and objectives of the research before signing an informed consent. A standardised questionnaire, administered to the participants by a trained interviewer, included questions about socio-demographic characteristics, sexual and reproductive behavior and smoking habits. The patient's ethnicity was defined based on self-reports according to the criterion of the Instituto Brasileiro de Geografia e Estatística (IBGE), which classifies ethnicity into five categories: white, black, mulatto, Asian, and native. In this study, the black, mulatto, Asian, and native categories were combined into a non-white category.

From each participant, a sample of exfoliated uterine cervical cells was collected and conditioned in a tube containing a preserving solution (PBS + vancomycin + nystatin) and then sent to a laboratory where it was processed for DNA extraction.

The tubes containing the cervical specimens were submitted to vigorous agitation before removal of the brush and were centrifuged at 300 × *g* per 10 min. The supernatant was removed and the resulting pellet was processed for DNA extraction using rapid isolation of DNA from mammal protocol, with proteinase K [16]. Around 30 ng of the DNA samples were submitted to a polymerase chain reaction (PCR) to amplify a 110 bp fragment of the human β-globina gene, using the primers PCO3+/PCO4+ [17] to analyze the quality of target DNA and the absence of PCR inhibitors. The products of PCR were submitted to electrophoresis on 8% polyacrilamide gel, followed by silver staining [18].

The positive samples for β-globin were analyzed for the presence of DNA from HSV-1 and HSV-2 in separate reactions by PCRs specific for each type. Each reaction was composed of a Master Mix, 10 mM of each primer, and 2.5 mM of DNA sample, for a final volume of 25 μl. The conditions for both reactions were as follows: incubation at 50 °C for 2 min, denaturation of DNA at 95 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, 45 s at 58 °C for annealing, an extension step at 72 °C for 30 s, and a final extension step at 72 °C for 10 min. The primers HSV-1a (5′-CCCTGTCTCGCGCGAGCCAC-3′) and HSV-1b (5′-TCAGCCACCCA-TACGCGTAA-3′), which amplify a fragment of 142 bp [19], were used to detect DNA from HSV-1, while the primers HSV-2 (A) (5′-GGACGAGGCGCCAAAGCACACG-3′) and HSV-2 (B) (5′-TCCGTCCAGTCGTTTATCTTCAC-3′), which generate a product of 270 bp [20], were used for HSV-2. The products of PCRs underwent vertical electrophoresis on polyacrylamide gel at 8% [16], with subsequent revelation by silver [18].

Statistical analysis of the results was performed using the chi-square test and associations between risk factors and HPV infection were analyzed by calculating the odds ratios (odds ratio – OR) and their respective confidence intervals (ICs). Results were considered statistically significant at p value <0.05.

This study was carried out in accordance with The Code of Ethics of the Declaration of Helsinki. It was approved by the Ethical Committee on Research of the Federal University of Rio Grande do Norte.

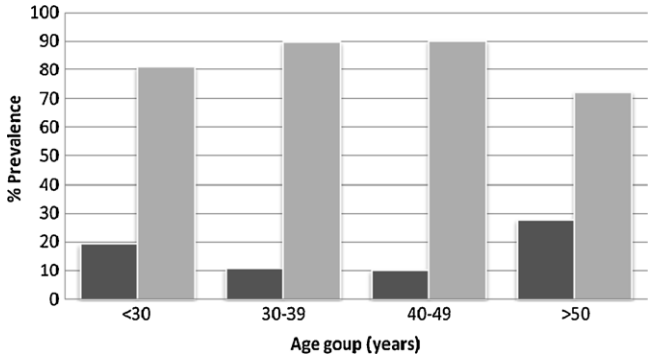

Fig. 1. Prevalence of HSV-1 and HSV-2 in women infected by herpes virus, according to age groups (■ HSV-2 and ■ HSV-1).

3. Results

The study group consisted of 261 sexually active women, ranging in age from 13 to 79 years, with an average of 38.7 years. The majority of the women were younger than 40 years old, non-white, married, with an education level of elementary or less, and with a family income of up to $330 U.S. dollars per month. Of the total samples analyzed, 60 were positive for HSV-1, of which six were also positive for HSV-2, revealing an overall prevalence of infection with HSV-1 of 23.0%, with 20.7% of the women having only HSV-1 and 2.3% having both HSV-1 and HSV-2. The presence of HSV-2 was detected in 14 samples, revealing an overall prevalence of 5.4%, with 3.1% of the cases of infection by HSV-2 alone and 2.3% in co-infection with HSV-1. The distribution of prevalence rates for different age groups showed that most cases of infection by both HSV-1 and HSV-2 occurred in women up to 39 years old, with no significant difference observed among prevalence rates for the different age groups, for HSV-1 or HSV-2 (Fig. 1). We also did not observe any association between ethnicity, marital status, education or family income and genital infection by both HSV-1 and HSV-2 (Table 1).

Regarding the variables related to behavior and sexual activity, we observed that most study participants initiated sexual activity before 17 years of age, had only one sexual partner throughout life, had not used or rarely used condoms during sexual intercourse, and had a maximum of two children. No association was observed between any of the variables analyzed with increasing rates of prevalence of infection by HSV-1 or HSV-2 (Table 2).

4. Comments

In this study, we analyzed specimens containing exfoliated epithelial cells of the uterine cervix of asymptomatic women and found a higher prevalence of HSV-1 compared with HSV-2. Considering only HSV for positive cases, a greater proportion of HSV-1 (88.2%) was observed when compared with HSV-2 (20.6%). These results contradict expectations, considering the previously established concept that in genital tract infections by HSV, the predominant type is HSV-2. This is due to the fact that HSV-2 is transmitted mainly through sexual contact as opposed to HSV-1, which is predominantly in the oral-facial region and is transmitted mainly by direct contact with saliva of asymptomatic carriers. However, a reversal in the distribution of those viruses in the human genital tract has been observed in recent decades by studies [2,7–9] of virus isolation showing a gradual increase in prevalence of HSV-1 in the genital tract. This could be due, at least in part, to using new methods of detecting the virus, since the previous studies that formed the basis for the establishment of these concepts were conducted primarily through serological methods.