CLINICAL RESEARCH

Detection of Oral Entamoeba *Gingivalis* and Trichomonas *Tenax* in Adult Quilombola Population with Periodontal Disease

Detección de Entamoeba *Gingivalis* oral y Trichomonas *Tenax* en población de quilombola adulta con enfermedad periodontal

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ABSTRACT: The objective was to analyze the periodontal condition severity and the occurrence of pathogenic microorganisms in the oral cavity of an adult population of an Afrodescent Community of northeastern Brazil. This is an observational and cross-sectional study performed through an oral clinical examination, using a standardized clinical record. For the subjects with periodontal disease, the bacterial biofilm was collected in a Petri dish containing 0.9% physiological solution to detect the presence of microorganisms Entamoeba *gingivalis* and Trichomonas *tenax*, and later observed under an optical microscope. Statistical analysis was performed by calculating the prevalence of periodontal disease and the frequency of the protozoa in the bacterial biofilm. Statistical significance of the relationships researched was verified by Fisher's exact test. It was evaluated 29 subjects pertaining to the Quilombola Patioba community, aged 35 to 44 years. The results showed that among the adults of the community, there was a high prevalence of periodontal disease (75.86%), being higher in the 1st and 6th sextants of the Community Periodontal Index (CPI). E. *gingivalis* positivity occurred in most sextants affected by gingivitis, while in the condition of periodontitis, this microorganism was not present in the 3rd, 4th and 6th sextants. In all sextants affected by periodontal disease, T. *Tenax* was observed when associated with gingivitis. It is worth mentioning the begging of the elaboration of health policies, social and professional commitment that foster a greater promotion of oral health and quality of life for the quilombolas of northeastern Brazil.

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RESUMEN: El objetivo fue analizar la severidad de la condición periodontal y la aparición de microorganismos patógenos en la cavidad oral de una población adulta de una comunidad afrodescente del noreste de Brasil. Este es un estudio observacional y transversal realizado a través de un examen clínico oral, utilizando un registro clínico estandarizado. Para los sujetos con enfermedad periodontal, la biopelícula bacteriana se recogió en una placa de Petri que contenía una solución fisiológica al 0,9% para detectar la presencia de microorganismos Entamoeba *gingivalis* y Trichomonas *tenax*, y luego se observó bajo un microscopio óptico. El análisis estadístico se realizó calculando la prevalencia de la enfermedad periodontal y la frecuencia de los protozoos en la biopelícula bacteriana. La significación estadística de las relaciones investigadas se verificó mediante la prueba exacta de Fisher. Se evaluaron 29 sujetos pertenecientes a la comunidad Quilombola Patioba, de 35 a 44 años. Los resultados mostraron que entre los adultos de la comunidad, hubo una alta prevalencia de enfermedad periodontal (75.86%), siendo mayor en el sexto sexto y sexto del Índice periodontal comunitario (IPC). La positividad de E. *gingivalis* se produjo en la mayoría de los sextantes afectados por gingivitis, mientras que en la condición de periodontitis, este microorganismo no estaba presente en los sextantes tercero, cuarto y sexto. En todos los sextantes afectados por enfermedad periodontal, se observó T. *Tenax* cuando se asoció con gingivitis. Vale la pena mencionar el inicio de la elaboración de políticas de salud, compromiso social y profesional que promuevan una mayor promoción de la salud oral y la calidad de vida de las quilombolas del noreste de Brasil.

INTRODUCTION

Presenting in various clinical forms, periodontal disease is considered one of the most common oral diseases, constituting one of the main public health problems, where about 5 to 20% of the world population suffers from severe generalized periodontitis (1). With a complex infectious and/or inflammatory etiology, periodontal disease has a large number of inflammatory mediators that contribute to tissue destruction and consequently bone loss (2).

The high prevalence of this disease in adults has been described in studies conducted in various community groups and age groups (3,4). Periodontal disease has a multifactorial etiology, with several potential risk factors for the development of the disease, affecting mainly black individuals exposed to low socioeconomic or educational conditions, diabetes, obesity and smoking (5-7). In this sense, authors pointed to the inclusion of blacks in the lower socioeconomic ranges of Brazilian and world society, including with important repercussions on their health and quality of life (8).

Microbiologically, periodontal lesions contain numerous neutrophils, mobile bacteria, spinning rods and protozoa (9). In this context, the high incidence

of E. gingivalis and T. *tenax* in periodontal disease has led some researchers to consider them as etiological agents of this condition, and have pointed out that the increased incidence of these protozoans may act as an indicator of disease progression (10,11). However, the etiology of periodontal disease is multifactorial and not yet well established. Thus, it is important to verify associations of the involvement of these microorganisms in this disease (12).

The scientific literature of population studies about the periodontal condition of the African descent population in Brazil is scarce, and does not focus on the theme associated with other possible biotic risk factors, such as the presence of protozoa. In this context, the objective of this study was to determine the prevalence of periodontal disease severity and the occurrence of E. *gingivalis* and T. *tenax* in bacterial biofilm samples from adult individuals with periodontal disease in a Quilombola population (descendants of Afro-Brazilian slaves), located in northeastern Brazil.

MATERIALS AND METHODS

This observational and cross-sectional study was conducted in the Quilombola Patioba Community, located in the municipality of Japaratuba in the state of Sergipe, located in northeastern Brazil. The community physical space (Patioba 1 and 2) was distributed on different streets, named A,B,C and D to facilitate the survey. All of these streets were equally selected for research. This distribution was used as a strategy to identify community residences during the development of the research, since there was no official record of the public places in the community.

In 2009 the population of this afrodescent quilombola community was made up of 593 individuals, corresponding to 186 families registered in the SUS Primary Care Information System (SIAB). All individuals aged 35 to 44 years were included in the study, following the guidance of the current Ministry of Health guideline for epidemiological survey of oral health of the adult population (13). Hospitalized individuals were excluded. After selection, the sample totaled 29 adults.

A clinical oral examination was performed to investigate the occurrence of periodontal disease using the Community Periodontal Index (CPI), performed according to the diagnostic criteria of the National Oral Health Survey (13). The CPI allows the periodontal condition to be evaluated for health, bleeding on probing and presence of stone or pouch (14). In the subjects with periodontal disease, the bacterial biofilm was collected and placed in a Petri dish containing 0.9% physiological solution to determine the presence of the protozoa E. *gingivalis* and T. *tenax*. The biological sample was extended on slide, stained with lugol and observed under light microscopy (A=400X).

The survey was conducted by previously calibrated examiners using the following tools recommended by the World Health Organization (1999): odontoscope, North Carolina probe, clinical forceps, cotton, wooden spatula and procedure gloves (15). We considered the diagnostic criteria of the SB-2010 Project, which is part of a historical process of diagnosing the oral health of Brazilians, marking the field of action of the health surveillance component of the National Oral Health Policy.

Statistical analysis was performed using the software SPSS 22.0, with calculation of the prevalence of oral diseases, and absolute frequency of protozoa E. *gingivalis* and T. *tenax*. Fisher's exact test with significance level of 95% ($p<0.05$) was applied. The study was approved by the Research Ethics Committee of the Universidade Tiradentes, under protocol number 270610.

RESULTS

Of the 29 adult subjects, 24.1% (n=7) were male and 75.9% (n=22) female. The prevalence of periodontal disease was 75.86% (CPI≠0), being higher in the 1st (p=0.040) and 6th sextant (p=0.060), with null percentage of people with CPI=0. In the 2nd and 6th sextants, there were no significant differences in the severity stages of periodontal disease (p>0.005).

In the first sextant, the significant majority of individuals were in the gingivitis stage 58.6% (p=0.040), others (13.8%) in the periodontitis stage, and 27.6% had the excluded sextant. There was edentulism (CPI=X) in all sextants, especially in the 1st (27.6%), 2nd (31%) and 3rd (27.6%) sextants (Table 1).

Most individuals are in the gingivitis stage, especially when considering 1st, 3rd, 4th and 5th sextants. The occurrence of E. *gingivalis* and / or T. *Tenax* microorganisms was observed in 77.06% of patients with periodontal disease, in addition to the association of these two protozoans in the condition of gingivitis and periodontitis in all sextants (p <0.001) (Table 2).

Most individuals are in the gingivitis stage, especially when considering 1st, 3rd, 4th and 5th sextants. The occurrence of E. *gingivalis* and/or T. *Tenax* microorganisms was observed in 77.06% of patients with periodontal disease. The highest prevalence of these two protozoa, individually or both combined, was observed in gingivitis when compared with the periodontitis setting (p<0.001) (Table 2).

Table 1. Absolute and relative frequency distribution of CPI index scores in the upper and lower arch sextants of adult quilombola individuals.

Sextants	CPI=0		CPI=1		CPI=2		CPI=3		CPI=4		CPI=X		p-value*
	n	%	n	%	n	%	n	%	n	%	n	%	
1º	0	0.0	4	13.8	13	44.8	2	6.9	2	6.9	8	27.6	0.040
2º	3	10.3	6	20.7	8	27.6	3	10.3	0	0.0	9	31.0	0.250
3º	2	6.9	2	6.9	13	44.8	4	13.8	0	0.0	8	27.6	0.040
4º	1	3.4	3	10.3	17	58.6	2	6.9	1	3.4	5	17.2	<0.001
5º	1	3.4	1	3.4	21	72.4	4	13.8	1	3.4	1	3.4	<0.001
6º	0	0.0	4	13.8	13	44.8	4	13.8	1	3.4	7	24.1	0.060

* Fisher's Exact Test.
Note: (0) Gingival health; (1) Bleeding on probing; (2) Detection of calculus with or without bleeding, excess or lack of restorative material; (3) Probing depth between 3.5 mm and 5.5 mm; (4) Probing depth greater than 5.5 mm; (X) Excluded sextant.

Table 2. Absolute frequency distribution of periodontal disease severity according to the CPI with respect to the occurrence of the microorganism.

		E. *gingivalis*	T. *tenax*	E. *gingivalis* and T. *tenax*	Absence of protozoa	Total	p-value*
1st sextant	Gingivitis	3	4	4	2	13	<0.001
	Periodontitis	1	1	1	1	4	
2nd sextant	Gingivitis	2	1	3	2	8	<0.001
	Periodontitis	1	0	1	1	3	
3rd sextant	Gingivitis	4	4	4	1	13	<0.001
	Periodontitis	0	1	0	3	4	
4th sextant	Gingivitis	5	6	4	2	17	<0.001
	Periodontitis	0	1	0	2	3	
5th sextant	Gingivitis	5	6	7	3	21	<0.001
	Periodontitis	1	1	0	3	5	
6th sextant	Gingivitis	2	6	3	2	13	<0.001
	Periodontitis	0	1	1	3	5	

*Fisher's Exact Test

DISCUSSION

In the present study, the prevalence of periodontal disease in the quilombola adult population was high (75.8%), being more prevalent in the 1st and 6th sextants. Most individuals are in the gingivitis stage, especially when considering 1st, 3rd, 4th and 5th sextants. The division into sextants allows an adequate view of disease severity in the various sites, which is a differential of this research, since most previous studies that do not show occurrence by sextants.

A study conducted in a rural area of the state of Bahia estimated the prevalence and factors associated with periodontitis in 172 adult individuals, and showed that gingivitis was diagnosed in 97.7% of the population, corresponding to the percentage of those with bleeding. Periodontitis, however, was observed in 24.4% of this population (16).

A systematic review of the international literature verified that oral diseases with the presence of calculus and shallow pockets are most prevalent among adult population, especially in the 35 to 44 age group (17). However, it is known that in Brazil, especially in rural populations, this situation is even more severe and has shown high levels of edentulism, such as the study conducted by Macêdo (16), who found 50.0% of the rural population of Bahia with more than 4 missing teeth.

Studies demonstrate that the presence of protozoa may be associated with periodontal disease (11,17-19). In this study, the occurrence of parasitic infection is similar to the results of the study by Albuquerque Júnior (10), who pointed to the protozoan E. *gingivalis* being more common than the protozoan T. *Tenax*. These authors also found positivity for E. *gingivalis* in 31.37% of biofilm samples (50.0% with gingivitis and 50.0% with periodontitis). Regarding T. *tenax*, there was positivity in 22.53% of biofilm samples (16.66% gingivitis, 41.67% periodontitis and 41.67% healthy).

In the researched community, the subjects presented periodontal disease in most sextants and single or multiple infection with E. *gingivalis* and T. *tenax*. However, the presence of these potentially pathogenic microorganisms in the

oral cavity was more prevalent in the gingivitis condition when compared to periodontitis. It has been demonstrated that T. *tenax* presents activity mediated by cysteine endopeptidases which hydrolyze collagen molecules types I, III, IV, V and also lyse human eritrocytes (18-20).

In the present study, the concomitant presence of E. *gingivalis* and T. *tenax* was most prevalent in second and fifth sextant gingivitis. In a periodontitis, we could not report a concomitant occurrence of those microorganisms in the third, fourth or fifth sextants. This clinical setting could indicate the involvement of E. *gingivalis* in the formation of the dental biofilm, probably in an opportunistic manner, with the ability to reproduce itself in the oral microenvironment. It was also found that this microorganism enabled disease progression in individuals undergoing immunosuppressant drug therapies (21).

Ghabanchi (22) performed a study on gingival health with patients presenting with periodontal disease and a control group in southeastern Iran, and also concluded that E. *gingivalis* and T. *tenax* infection is more common in patients with periodontal disease. One other study performed on 120 patients in eastern Nigeria reported that these two microorganisms are significantly associated with the presence of dental calculus, plaque, and cavities (11).

The transmission mechanisms of T. *tenax* and E. *gingivalis* are direct contact-through saliva particles, kissing, or sputtering- and indirect contact, such as sharing utensils or toothbrushes from infected individuals (23). Another important factor lies in the detection of T. *tenax* in the human oral cavity, which serves as a marker for poor oral hygiene (24,25).

Ghabanchi (22) had already stated that parasite infections were relatively common in patients with periodontal disease, and the more widespread the disease became, the larger the number of infestations detected, especially of E. *gingivalis* e T. *tenax*, thereby indicating the necessity for a careful instruction on oral and general hygiene for the population as a control mechanism.

CONCLUSION

Thus, we observed that the residents of the Patioba Community, included in the age group of 35 to 44 years, have high prevalence of periodontal disease, particularly in the 5th sextant. The excluded sextants, especially in the upper arch, show partial edentulism present in these individuals. The prevalence of E. *gingivalis* and/or T. *tenax* microorganisms, including their association with periodontal disease was high, showing a higher prevalence in gingivitis compared to periodontitis, hypothesizing an association with poor oral hygiene conditions. Thus, this study highlights the need for public health policies that can promote health by stimulating the development of healthy oral hygiene habits.

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