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Orapuh Journal

ORIGINAL ARTICLE

Periodontal health and human oral protozoa in parts of Enugu State, Nigeria

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ARTICLE INFO

Received: 18 January, 2020
Accepted: 28 May, 2020
Published: 31 May, 2020

Keywords:
Entamoeba gingivalis, Trichomonas tenax, human oral protozoa, periodontal health, mixed infection, oral microbiota

Peer-Review: Externally peer-reviewed

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To cite:
Adamu, V. E., Eneojo, N. I. F., Amaechi, A. A., Nwoke, B. E. B., Ajaero, C.M.U., & Ukaga, C. N. (2020). Periodontal health and human oral protozoa in parts of Enugu State, Nigeria. *Orapuh Journal*, *1*(1), e701.

ISSN: 2644-3740

ABSTRACT

Introduction
Periodontal disease, like dental caries and some forms of halitosis, is initiated by microbial activity in the oral cavity. It is well known that the presence of protozoa may be established both in persons with pathological alterations in the oral cavity and in those with no such symptoms.

Purpose
This study was carried out to assess the relationship between periodontal health status and infection with human oral protozoan parasites in Ogbeke-Nike Community of Enugu State, Nigeria.

Materials and methods
The study design adopted for this research work was cross-sectional survey. A total of 233 participants were selected, using the convenience non-probability sampling technique, from 6 rural villages in Ogebeke-Nike community in Enugu-East LGA of Enugu State and were studied, using questionnaires, clinical assessments and parasitological techniques.

Results
Analyses of data from the study revealed that participants who had gingivitis yielded a higher prevalence of human oral protozoan parasites (57.58%), *E. gingivalis* (24.24%), *T. tenax* (9.09%) and the mixed infections (24.24%) than participants with healthy periodontium (31.09%, 14.51%, 7.25%, 9.33%, respectively) or periodontitis (14.29%, 14.29%, 0.00%, 0.00%, respectively). These results suggested that the prevalence of human oral protozoan parasites was related to periodontal health status of participants. This relationship was statistically significant ($p<0.05$).

Conclusions
Human oral protozoan parasites infection is a reality in the study population. Strict attendance to daily oral hygiene and seeking professional help with incipient periodontal problems may help control the prevalence of the study organisms in the oral cavity of humans.

INTRODUCTION

Periodontal disease, like dental caries and some form of halitosis is initiated by microbial activity in the oral cavity. The natural history of this disease portends an interaction between oral tissues and microbial ecosystem (Baliga, 2013). It is an established fact that a disruption of the balance of the oral cavity's microbial ecosystem and the oral environment results in the prevention, arrest or the initiation and progression of the disease. A work has referred to this interesting metamorphosis as the 'ecological plaque hypothesis' (Marsh, 1994).

It is well known that the presence of protozoa may be established both in persons with pathological alterations in the oral cavity, and in those with no such symptoms (Feki & Molet, 1990). Human oral protozoan parasites cause gum itch, palate sore, unpleasant smell of mouth, fatigue, fever, headaches, and periodontal tissue damage (Gharavi et al., 2006).

Two types of protozoan parasites infect the oral cavity of humans. These are *Entamoeba gingivalis* and *Trichomonas tenax*.

E. gingivalis belong to Entamoebaidae family and sub-order Tubulinae (Albert et al, 1988; Gharavi, 2004). This parasite is found only in trophozoite form, which varies from 5-35 µm (Sonne & Gradwohl, 1980; Borwn & Neva, 1983; Dao et al., 1983; Gharavi, 2004). *T. tenax*, on the other hand, is a small trichomonad that usually occurs in the oral cavity of 5-10% of humans. This protozoon is of Trichomonadidae family (Albert et al, 1988; Gharavi et al, 2006).The flagellated organism is only found in the form of trophozoite and its size varies from 5µm to12 µm (Beaver, Jung, and Cupp, 1984).

According to Bergquist (2009), the presence of *E. gingivalis* and *T. tenax* in the oral cavity is related to poor oral hygiene and a low standard of living. Most parasites do not adapt successfully to the oral cavity of humans. In fact, many scholars think that it is not very easy for these organisms to infect the oral cavity of individuals who are not sick or do not have their immune system suppressed or compromised. Bergquist further stated that even though many scholars think parasites rarely affect the oral cavity, there is a growing body of evidence that proofs the opposite. The only problem is that sufficient number of studies are not yet available to prove this point.

Infection with human oral protozoan parasites occur more frequently in people with compromized integrity of the oral cavity's soft tissues. The burden of the infection on the oral cavity may justify with the assertion that the prevalence of *E. gingivalis* and *T. tenax* is enhanced amongst people who are sick, have lowered body immunity or poor oral hygiene, have lost the integrity of their oral cavity's mucous membranes or that have infection of the palatal tonsils and paranasal sinuses (Liu et al., 2001; Onyido et al., 2011). Cavalcanti et al. (2011) suggested that *Entamoeba gingivalis* were more common in the early stages of periodontitis. Wantland et al. (1958) inferred that both *E. gingivalis* and *T. tenax* were prevalent in individuals exhibiting pyorrhea and periodontitis in the population they studied. Bohmfalk (1996) posited that the prevalence of human oral protozoa, especially, *E. gingivalis* is positively correlated with advanced periodontal disease. While it is known that *E. gingivalis* and *T. tenax* are commensal protozoa commonly found in human oral cavity, it is most probable that they are opportunists especially in the lesions of gingivitis and periodontal pockets (Talaro & Talaro, 2002). Ghabanchi et al. (2010) reported that parasitic infections are relatively common among patients with periodontal disease.

Pomes et al. (2000) carried out a study of the risk for periodontal diseases in 17 different locations in Guatemala on 62 young adolescents. The study found *E. gingivalis* in 21% of the children in 11% of the sites investigated. Similarly, Favoreto and Machado (1995) studied 100 randomly selected patients presenting at a Brazilian hospital specialized in odontology, and found the prevalence of *E. gingivalis* to be as high as 62%. Thus, apart from an increasing number of reports of positive findings in malnourished people and in patients with immunodeficiency syndromes, *E. gingivalis* and *T. tenax* may be more common than originally thought (Bergquist, 2009). Corroborating this fact is the work of Roberts and Janovy, Jr. (2010), which inferred that oral protozoa are present in all populations. According to Jackson and Rawdin (1996), up to 50% of persons with healthy mouth may harbor oral protozoa. In fact, Roberts and Janovy, Jr. asserted that both *E. gingivalis* and *T. tenax* dwell only in the mouth and that they are present in all populations

Traditionally, periodontal disease diagnosis and magnitude estimation utilise clinical parameters like the Community Periodontal Index and radiographic loss of alveolar bone (Polson & Goodson, 1985).

MATERIALS AND METHODS

This study was carried out to assess the relationship between periodontal health status and infection with human oral protozoan parasites in Ogbeke-Nike Community of Enugu State, Nigeria.

Research Design:

The study design adopted for this research work was cross-sectional survey.

Administration of Questionnaires:

Data collection involved administration of a questionnaire with open and closed-ended questions based on literature, clinical assessments and dental samples collection. 233 questionnaires were distributed among the participants, who were selected from 6 rural villages in Ogbeke-Nike Community of Enugu-East LGA, Enugu State, Nigeria, using the convenience non-probability sampling method and all the questionnaires were returned (100% return rate).

The first section of the questionnaires was designed to elicit the demographic information of the participants. The other section was utilized as data collection schedule form (DCSF) to record observations from clinical assessments and laboratory investigations.

Inclusion & Exclusion Critteria:

Persons considered eligible for inclusion in this study were persons who had not had any form of antibiotic therapy within the 3 months preceding the dental sample collection days (this criterion was as described by Ibrahim and Abbas (2012), had not had any dental prophylactic treatment like scaling & polishing treatment within the previous 6 months preceding the sample collection days (this criterion was as described by Angelov et al. (2009)), had not had their daily oral hygiene like teeth brushing on the morning of the dental sample collection, as described by Ibrahim and Abbas, were permanent residents in the respective villages surveyed, who were not seriously sick with any form of systemic or debilitating illness that may have any influence on the oral environment (this criterion was as described by Angelov, et al. and Omale (2014) and who were not experiencing any cognitive impairment, as described by Omale.

Diagosis of Periodontal Disease:

Periodontal disease was diagnosed using the Community Periodontal Index (CPI) probe and criteria, as described by World Health Organization, WHO (1997).

Samples:

Samples collected consisted of dental plaque/materia alba from the region of un-stimulated saliva in the participants' oral cavities. The utility of the unstimulated saliva stemmed from the description of Navazesh (1993). The dental plaque/material alba samples were collected by swabbing teeth and gingival surfaces using oral swab. This method was as described by Onyido, et al. (2011).

Parasitological Analysis:

Parasitological analysis aspect of the study was done using the method of Ozumba et al. (2004) and Cavalcanti et al. (2011) with a modification. This modification was the addition of material alba to the sample.

The dental plaque/material alba samples were placed on individual glass microscope slides immediately after collection. Individual samples were diluted with normal saline at room temperature (25 to 28°C) to about 0.1ml volume. Immediately after dilution, a drop of standard eosin: C.I. 548-265 (BDH® England) was added to the slide preparation and the preparation was covered with a cover slip. Thereafter, the wet smears were examined immediately under a 10x objective of a compound microscope for the presence or absence of the motile amoeba trophozoites, *E. gingivalis*, identified by their morphologic characteristics (pseudopodia, a small central endosome and sphenoid nucleus) or flagellates, *T. tenax*, identified by their characteristic 4 anterior flagella, an undulating membrane and a posterior flagella. Observations were recorded accordingly.

All laboratory investigations were carried out under natural daylight at a standardized time of the day, as recommended by WHO (1997), plus illumination from an artificial illumination source, the electric generator set, which improved the compound microscope use. All analyses were carried out in the respective villages, right inside the side (make-shift) laboratory/clinic.

Data Analysis:

Data obtained were analyzed using descriptive statistics of prevalence rates and inferential statistics of non-parametric chi square test. The significance level was set at 5% ($p<0.05$). The inferential analyses were done using the Social Science Statistics® software authored by Stangroom (2015).

RESULTS

Analysis of the results indicated that most of the participants were within the age range of '<20 years' and '50 years and above' (36.49% & 32.03%, respectively). More females than males participated in the study (59.9%, 40.1%). Most of the participants had only the nursery/primary school education (54.6%). Majority of the participants were single (42.1%) and were farmers (49.3%). (**Table 1**)

Table1
Demographic characteristics of the participants

Variables	Variable category	Participants (*N* = 233)
		n (%)
Age (in years)	< 20	108 (46.35)
	20 – 29	16 (6.87)
	30 – 39	18 (7.72)
	40 – 49	27 (11.59)
	50 & above	64 (27.47)
Gender	Male	99 (42.5)
	Female	134 (57.5)
Education	Nursery/primary	152 (65.2)
	Secondary	26 (11.2)
	Tertiary	1 (0.4)
	Vocational	11 (4.7)
	Non	43 (18.5)
Marital status	Single	114 (48.9)
	Married	80 (34.3)
	Widowed	36 (15.5)
	Separated	2 (0.9)
	Divorced	1 (0.4)
Occupation	Farming	121 (51.9)
	Trading	3 (1.3)
	Civil service	3 (1.3)
	Self-employment	5 (2.2)
	Student/pupil	95 (40.8)
	Dependent	5 (2.1)
	Pensioner	1 (0.4)

Results further revealed that participants who had gingivitis yielded a higher prevalence of human oral protozoan parasites (57.58%), *E. gingivalis* (24.24%), *T. tenax* (9.09%) and the mixed infections (24.24%) than participants with healthy periodontium (31.09%, 14.51%, 7.25%, 9.33%, respectively) or periodontitis (14.29%, 14.29%, 0.00%, 0.00%, respectively). The only participant who manifested human oral protozoan parasites among those who were diagnosed with periodontitis manifested *E. gingivalis* (14.29%). Further inferential analysis of data revealed a relationship between periodontal health status of participants and infection with human oral protozoan parasites, and this relationship was statistically significant (**χ^2(2, N=233) = 10.06, p=0.007** (significant at P<0.05)) (**Table 2**).

Table 2
Prevalence of human oral protozoan parasites according to the periodontal health status of the participants

Periodontal health status	No examined	Positive samples	*E. gingivalis*	*T. tenax*	Mixed infection
	n (%)	n (%)	n (%)	n (%)	n (%)
Healthy	193(83.83)	60(31.09)	28(14.51)	14(7.25)	18(9.33)
Periodontitis	7(3.00)	1(14.29)	1(14.29)	0(0.00)	0(0.00)
Gingivitis	33(14.16)	19(57.58)	8(24.24)	3(9.09)	8(24.24)
Total	**233 (100)**	**80 (34.33)**	**37 (15.88)**	**17 (7.30)**	**26 (11.16)**

χ^2(2, N=233) = 10.06, p=0.007 (significant at P<0.05).

Many parasites cannot adapt to the oral cavity. This is the reason why many researchers think that people who are health are not likely to attract oral parasite infections.

DISCUSSION

This study indicated that the prevalence of human oral protozoan parasites was higher in participants who had gingivitis (57.58%) than those with healthy periodontium or periodontitis (31.09% and 14.29%, respectively), with *Entamoeba gingivalis* accounting for 24.24% of that lot. This result is similar to the result of the Pomes et al. (2000) study of the risk for periodontal diseases in Guatemala. The study found *E. gingivalis* in 21% of the participants in 11% of the sites investigated. Similarly, Favoreto and Machado (1995)'s study in a Brazilian hospital specialized in odontology found the prevalence of *E. gingivalis* to be as high as 62%. If gingivitis is not treated, it can precipitate periodontitis. These findings are also similar to the findings of Cavalcanti et al. (2011) that suggested that *Entamoeba gingivalis* is more common in the early stages of periodontitis. According to Bergquist (2009), the occurrence of human oral protozoan parasites in the oral cavity is associated with poor oral hygiene, which precedes some form of gingivitis, and these parasites may be quite common in people who have a low standard of living. It is most probable that these oral protozoa are opportunists especially in the lesions of gingivitis and periodontal pockets (Talaro & Talaro, 2002). Having studied participants from rural villages, who are

predominantly farmers (and, probably, have a low standard of living), it no surprise, then, that the results of the study were the way they were. The results of the present study were validated by the assertions of Talaro & Talaro that human oral protozoan parasites may be quite common in people who have a low standard of living.

This study further indicated that 1 out of the 12 participants that manifested periodontitis tested positive to oral protozoa, specifically, *Entamoeba gingivalis*. Ghabanchi et al. (2010) reported that parasitic infections are relatively common among patients with periodontal diseases. Moreover, Wantland et al. (1958) inferred that both *E. gingivalis* and *T. tenax* were prevalent in individuals exhibiting pyorrhea and periodontitis. These findings agree with the inference of Bohmfalk (1996), who inferred that the prevalence of human oral protozoa, especially, *E. gingivalis* is positively correlated with advanced periodontal disease.

Up to 31.09% of participants without any form of periodontal disease manifested infection with human oral protozoan parasites. This result corroborates the findings of Jackson and Rawdin (1996) who posited that up to 50% of persons with healthy mouth may harbor oral protozoa. In fact, Roberts and Janovy, Jr. (2010) asserted that both *E. gingivalis* and *T. tenax* dwell only in the mouth and that they are present in all populations.

This present study established a statistically significant relationship between the periodontal health status of participants and infection with human oral protozoan parasites. The impact of the infection with human oral protozoan parasites on the course of inflammatory processes in the oral cavity may be supported by the fact that *E. gingivalis* and *T. tenax* occur more frequently amongst people with alterations of the mucous membrane of the oral cavity, inflammation of the palatal tonsils and paranasal sinuses, as well as amongst those with bad oral cavity hygiene, which catalyses periodontal diseases (Liu et al., 2001; Onyido et al., 2011).

CONCLUSIONS

Human oral protozoan parasites infection is a reality in the study population. The prevalence of these parasites is related to periodontal health status. And this relationship was statistically significant. Strict attendance to daily oral hygiene and seeking professional help with incipient periodontal problems may help control the prevalence of the study organisms in the oral cavity of humans.

Acknowledgment: The authors wish to acknowledge the authorities of the Enugu State Ministry of Health, the Ogbeke-Nike Community Head and the village heads for ethical approvals, permissions and co-operations to ensure the success of this work.

Ethics Approval: Ethical approval for this work was obtained from the Ministry of Health, Enugu State, Nigeria.

Conflict of Interest: None declared.

OrCID iDs: Nil identified.

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