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Human salivary pH in the occurrence of human oral protozoan parasites in Ogbeke-Nike, Enugu State, Nigeria

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ABSTRACT

Introduction
The interplay between human salivary potential hydrogen (pH), which measures the acidity and alkalinity level of saliva, and the prevalence of human oral protozoan parasites should be of immense interest to clinicians who are wary of the role salivary pH plays in the regulation of the oral microbiota. Salivary pH affects the growth of microorganisms and helps maintain the oral environment.

Purpose
This study was undertaken to assess the interplay between human salivary pH and infection with human oral protozoan parasites in the Ogbeke-Nike community of Enugu State, Nigeria, to enhance clinical decisions.

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

Results
Analyses of data from the study revealed that the prevalence of human oral protozoan parasites, *Entamoeba gingivalis*, and the mixed infections (*E.gingivalis* & *Tichomonas tenax*) were higher in participants with salivary pH of 6.0 – 6.5 (36.51%, 17.99 & and 13.23%, respectively) and zero in participants with salivary pH of 5.0 – 5.5, 8 – 8.5 and 9.0 – 9.5 (0.00% all through). *T. tenax* infection was more common in participants with salivary pH of 7.0 – 7.5 (16.67%). 5.29% of participants with the 6.0 – 6.5 salivary pH also manifested *T. tenax*, whereas the infection was not found in all other pH values. Results also indicated that there was no significant relationship between salivary pH and infection with human oral protozoan parasites **(p>0.05)**.

Conclusions
Even though there was no significant relationship between salivary pH and the occurrence of infection with human oral protozoan parasites, the peak incidence of these commensals may be positively associated with the pH value of 6.0 – 6.5. Maintaining the ideal salivary pH may be key to regulating oral microbiota.

INTRODUCTION

The role salivary potential hydrogen (pH) plays in the regulation of oral microbiota, including the protozoan parasites colonizing the human oral cavity, has been a subject of oral scientific discussion over the years. Saliva is a dilute oral fluid secreted by oral salivary glands. About

99.9% of saliva is water (Baliga et al., 2013). A lot of literature is emerging that infer that oral protozoan parasites are very common and infest oral cavities (Bergquist, 2009). Salivary pH measures the acidity and alkalinity level of saliva. The pH of human saliva helps to maintain sanity in the oral cavity. The normal pH of saliva is 6.2 – 7.6 (Frothingham, 2018), and the average pH of saliva is 6.3. According to Baliga, et al, salivary pH is maintained near neutrality in the oral cavity. It is noteworthy that food, fluid, or other activity centred on the oral cavity, dehydration and daily oral hygiene may change salivary pH considerably and salivary pH has been considered an important easy-to-use inexpensive chair-side biomarker in the diagnosis of periodontal disease (Baliga, et al., 2013). Salivary pH affects the growth of microorganisms and helps maintain the oral environment (Takahashi & Schachtele, 1990; Takahashi et al. (1997).

It is not very easy for most parasites to adapt to the environment of the oral cavity. That is why oral health authorities, parasitologists, and researchers consider parasitic infestation of the oral cavity to be very low in people who are not sick or have their immune system suppressed. The indigenous works of researchers like Onyido et al. (2011) and Ozumba et al. (2004) and other empirical literature on the subject matter from around the world (Sonne & Gradwohl, 1980; Borwn & Neva, 1983; Dao et al., 1983; Gharavi, 2004) have long established the fact that human oral protozoan parasites exist. These parasites are the amoeba, *Entamoeba gingivalis*, and the flagellate, *Trichomonas tenax*. Roberts & Janovy, Jr. (2010) opined that oral protozoa are ubiquitous. According to Jackson and Rawdin (1996), up to half of all persons whose oral cavity may be considered healthy may be infected with oral protozoa. Roberts and Janovy, Jr. asserted that the niche of both *E. gingivalis* and *T. tenax* is the oral cavity and that they are present in the oral cavity of persons from all races, ages, and contexts.

E. gingivalis is a member of the Entamoebaidae family and sub-order Tubulinae (Albert et al., 1988; Gharavi, 2004). This parasite cannot exist outside the trophozoite form. *E. gingivalis'* trophozoite varies from 5-35 µm (Sonne & Gradwohl, 1980; Borwn & Neva, 1983; Dao et al., 1983; Gharavi, 2004). *Trichomonas tenax*, on the other hand, is a small trichomonad. This trichomonad usually infects the oral cavity of 5-10% of humans. This protozoon is a member of the Trichomonadidae family (Albert, et al, 1988; Gharavi et al, 2006). The organism is a flagellate, which, like the *E. gingivalis*, exists only in the trophozoite form, but unlike the *E. gingivalis*, the size of *T. tenax* varies from 5µm to12 µm (Beaver et al., 1984).

Cavalcanti et al. (2011) studied a group of individuals in order to understand the interplay between salivary pH and human oral protozoan parasites. The results of their study indicated that the salivary pH of his study participants ranged from 6.0 to 8.0, but the peak incidence of commensals in salivary samples occurred between pH 6.0 and 6.5. Souza (1982) and Zdero et al. (1999) also studied groups of people to observe this interesting interplay. Results of their studies showed findings similar to those of Cavalcanti, et al. The study of Ponce de León et al., (2001) found no relationship between salivary pH and the presence of human oral protozoan parasites.

This study was aimed at unraveling any significant relationship between human salivary pH and the prevalence of human oral protozoan parasites. Information garnered from this study may help enhance clinical decisions.

MATERIALS AND METHODS

Study Area

The study area is Ogbeke-Nike, a rural community in the Enugu-East Local Government Area of Enugu State, Nigeria. The geographical coordinates of Enugu are 6^0 26′0″ North, $7^0$29′0″ East (Maplandia, 2005). The villages of Ogbeke-Nike surveyed are Akparata, 4 corners, Njesike, Ugbo-Mike, Ugbo-James, and Aguofu. These villages have no health facilities of any kind (except that they are periodically served by an unskilled mobile drug retailer) and have very poor housing structures/patterns ("field observation", n.d.).

Residents of the villages are of very low socioeconomic status, and are, consequently, prone to many health and related challenges ("field observation", n.d.). According to Nweze (2002), about 85% of the rural population of Enugu State is engaged in small-scale farming and animal husbandry.

Research Design

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

Study Population

The study population consisted of all persons living in 6 villages (Akparata, 4 corners, Njesike, Ugbo-Mike, Ugbo-James, and Aguofu) of Ogbeke-Nike, a rural community in

Enugu-East Local Government Areas of Enugu State, Nigeria.

Sample size & Sampling techniques

A total of 233 persons were selected from the 6 villages, using the convenience non-probability sampling technique.

Inclusion & Exclusion Criteria

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 (2012), 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. (2009)) and Omale (2014) and who were not experiencing any cognitive impairment, as described by Omale (2014).

Administration of Questionnaires

Questionnaires were distributed among the study participants. The first section of the questionnaire was designed to elicit the demographic information of the participants. All the participants filled and returned the questionnaires (100% return rate). The second section was utilized as a data collection schedule form (DCSF) to record observations from clinical assessments and laboratory investigations.

Samples

Samples collected consisted of participants' saliva and 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). Participants' saliva was collected from the sublingual fossa, which bears the minor sublingual ducts of the sublingual salivary glands. This saliva sample was utilized for salivary pH analysis. This method was as described by Ibrahim and Abbas (2012), with modification. This modification was the choice of the site of Saliva collection (sublingual fossa). The dental plaque/material alba samples were collected by swabbing teeth and gingival surfaces using an oral swab. This method was as described by Onyido et al. (2011).

Clinical Assessments

Universal indicator strips (pH 0-14, Lab Star®) were used to determine the pH of the participants' saliva by smearing the indicator strip with the participants' saliva and observing the strip for colour change, which was matched to a standard colour marker and the corresponding pH level was read and recorded. This method was as described by Ponce de León et al. (2001) and Ibrahim and Abbas (2012).

Parasitological Analysis

Parasitological analyses were 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 coverslip. Thereafter, the wet smears were examined immediately under a 10x objective lens 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 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 generating 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 was analyzed using descriptive statistics of prevalence rates and inferential statistics of the 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

Results revealed that most of the participants were within the age range of '<20 years' and '50+' years (46.35% & 27.47%, respectively). More females than males participated in the study (57.5% & 42.5%, respectively). Most of the participants had only the nursery/primary school education (65.2%). The majority of the participants were not married (48.9%) and were farmers (51.9%) (**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+	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)

Table 1 shows that most of the participants surveyed were within the age range of '<20 years' and '50+ years', females, not married, farmers and had only the nursery/primary school education.

Results also revealed that the prevalence of human oral protozoan parasites, *E. gingivalis*, and the mixed infections *were* higher in participants with salivary pH of 6.0 – 6.5 (36.51%, 17.99 & 13.23%, respectively) and zero in participants with saliva pH of 5.0 – 5.5, 8.0 – 8.5 and 9.0 – 9.5 (0.00% all through) (**Table 2**).

T.tenax infection was more common in participants with salivary pH of 7.0 – 7.5 (16.67%). 5.29% of participants with the 6.0 – 6.5 salivary pH also manifested *T. tenax*, whereas the infection was not found in all other pH values (**Table 2**).

Inferential analyses of data revealed no significant relationship between salivary pH and the prevalence of human oral protozoan parasites **(p>0.05)** (**Table 2**).

Table 2
Prevalence of human oral protozoan parasites according to salivary pH of the participants

Salivary pH *(6.2 – 7.4)*	**No examined**	**No of positive samples**	***E. gingivalis***	***T. tenax***	**Mixed infection**
	n (%)	*n (%)*	*n (%)*	*n (%)*	*n (%)*
5.0 – 5.5	1(0.43)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
6.0 – 6.5	189(81.12)	69(36.51)	34(17.99)	10(5.29)	25(13.23)
7.0 – 7.5	42(18.03)	11(26.19)	3(7.14)	7(16.67)	1(2.38)
8.0 – 8.5	1(0.43)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
9.0 – 9.5	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Total	**233 (100)**	**80 (34.33)**	**37 (15.88)**	**17 (7.30)**	**26 (11.16)**

$\chi^2(3, N=233) = 1.757, p=0.6242$ (not significant at p>0.05)

Table 2 shows that the prevalence of human oral protozoan parasites, *Entamoeba gingivalis*, and the mixed infections (*E.gingivalis* & *Tichomonas tenax*) were higher in participants with salivary pH of 6.0 – 6.5 and zero in participants with salivary pH of 5.0 – 5.5, 8 – 8.5 and 9.0 – 9.5. *T. tenax* infection was more common in participants with salivary pH of 7.0 – 7.5. There was no statistically significant relationship between salivary pH and infection with human oral protozoan parasites (p>0.05).

DISCUSSIONS

This study indicated that the prevalence of human oral protozoan parasites was higher in participants with salivary pH of 6 – 6.5 (36.51%, 17.99 & and 13.23%, respectively) and zero in participants with saliva pH of 5 – 5.5, 8 – 8.5, and 9 – 9.5 (0.00% all through). As a rural community, these statistics were not too surprising. These results are in agreement with the findings of Cavalcanti et al. (2011) whose study findings also indicated that the salivary pH of his study participants ranged from 6.0 to 8.0, but the peak incidence of commensals in salivary samples occurred between salivary pH 6.0 and 6.5. The works of other researchers like Souza (1982) and Zdero, et al. (1999) also corroborate these findings.

This study inferred that there was no significant relationship between salivary pH and infection with human oral protozoan parasites. This inferential result agrees with the study of Ponce de León et al. (2001) that found no statistically significant relationship between salivary pH and the presence of these protozoa. What is unclear here is whether their context, oral hygiene statuses, or nutrition had anything to do with the results of this study.

The presence of the study organisms in salivary pH considered normal (6.2 – 7.6) and their absence in salivary

pH considered too acidic (5.0 – 5.5) and too alkaline (7.0 – 9.5) suggests that human oral protozoan parasites cannot thrive in media with extreme potential hydrogen (pH) levels. On the other hand, *T. tenax* seems able to thrive in neutral or near-neutral salivary pH levels (7.0 – 7.5). Knowledge of this fact may help clinicians (temporarily) alter salivary pH to achieve certain therapeutic outcomes.

Frothingham (2018) opined that the pH of the oral cavity must be balanced (fall within the normal range) to support intact anatomy and optimum physiology. Oral health challenges like dental caries, persistent halitosis, and tooth hypersensitivity, just to mention a few are associated with extreme salivary pH values. Consequently, oral intervention strategies may not only assuage pain, restore function, or improve quality of life; they also help to keep the oral environment intact and preserve life.

CONCLUSION

Even though there was no significant relationship between salivary pH and the occurrence of infection with human oral protozoan parasites, the peak incidence of these commensals may be positively associated with the pH value of 6.0 – 6.5. Maintaining the ideal salivary pH may be key to regulating oral microbiota. Clinicians may leverage the results of this study to develop or promote oral intervention strategies that support this (regulatory) ideal for consumers of oral health care services.

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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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