RISING PROFILE OF ORAL CAVITY PROTOZOA AMONGST DENTAL PATIENTS IN SOUTH EASTERN NIGERIA

Oggonna Christiana Ani
Department of Applied Biology, Faculty of Science, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria
Ogo_ani@yahoo.com

Emmanuel E. Agbo
Dental Clinic, Federal Teaching Hospital, Abakaliki, Ebonyi State, Nigeria
agboemanuelemenike@gmail.com

Emmanuel Ikechukwu Nnamonu*
Department of Biology, School of Science, Federal College of Education, Eha-Amufu, Enugu State, Nigeria
nnamonue@gmail.com

Samuel Osemedua Onyeidu
Department of Information and Communication Technology, ICT University Yaounde, Cameroon
samuel.onyeidu@ictuniversity.org

Blessing Uchechukwu Onyeidu
Department of Home Economics, Federal College of Education, Eha-Amufu, Enugu State, Nigeria
onyeidublessing@gmail.com

Nkiru Joy Okwerekwu
Department of Biology, School of Science, Federal College of Education, Eha-Amufu, Enugu State, Nigeria
Joynkiru2011@gmail.com

*Corresponding Author - Emmanuel Ikechukwu Nnamonu nnamonue@gmail.com
Abstract

The observed low knowledge of oral hygiene and the need to investigate the possible cause(s) of the existing oral diseases in our study area motivated this study. This study investigated the prevalence of human oral protozoan parasites amongst dental patients attending the clinic at Federal Teaching Hospital Abakaliki, South Eastern Nigeria. A total of 180 patients (72 males and 108 females) were sampled. Dental plaque, dental calculus and saliva samples were collected from the patients. Samples were analyzed following standard methods. An overall prevalence rate of 40% occurred. Entamoeba gingivalis was the most encountered 28 (15.56%), followed by Trichomonas tenax 24 (13.33%) while mixed infections were recorded in 20 (11.11%) patients. The prevalence of both protozoans was higher in females 44 (40.74%) than in males 28 (38.89%). The highest prevalence was recorded among patients within the age range of ≤ 50 years (22 (81.48 %)) and least within the age range of ≤ 20 years 4 (16.00%). There was an observed significant relationship between age and oral hygiene status. We, therefore report that poor oral hygiene is a predisposing factor that exposes the buccal cavity to frequent colonization by parasites. Constant oral health education should be encouraged especially among dental patients.

Keywords
Entamoeba Gingivalis, Trichomonas Tenax, Prevalence, Dental Patients, Abakaliki

1. Introduction

Oral diseases pose a major health burden for many countries and affect people throughout their lifetime, causing pain, discomfort, disfigurement and even death (WHO, 2020). Globally, it was estimated that 2.3 billion people suffer from caries of permanent teeth and more than 530 million children suffer from caries of primary teeth (Global Health Metrics, 2018). Severe periodontal (gum) disease, which may result in tooth loss, is also very common, with almost 10% of the global population affected (Ferlay et al., 2018). The Global Burden of Disease Study 2017 estimated that oral diseases affect close to 3.5 billion people worldwide, with caries of permanent teeth being the most common condition. Severe periodontitis and untreated caries in deciduous teeth (milk teeth) were respectively the sixth and tenth most prevalent conditions, affecting approximately 1 out of every 10 people globally (Marcenes et al., 2010).
Treatment for oral health conditions is expensive and usually not part of universal health coverage. In most high-income countries, dental treatment averages 5% of total health expenditure and 20% of out-of-pocket health expenditure (WHO, 2020).

Oral infections have been predicted to be on the lingering increase in most low-income and middle-income countries, with increasing urbanization and changes in living conditions (WHO, 2020).

Because quality oral health has remained a necessary building block for good living, fundamental to the ability to breathe, eat, swallow, speak or even smile and that ability to interact with others is weakened by dental infections, in addition to the observed low knowledge of oral hygiene and need to investigate the possible cause(s) of the existing oral diseases in our study area, this study was therefore designed to investigate the prevalence of oral cavity protozoa in dental patients, southeastern Nigeria.

2. Materials and Methods

2.1 Study Area

This study was conducted in the Dental Clinic at Federal Teaching Hospital Abakaliki, Ebonyi State, Nigeria. Abakaliki is the capital city of Ebonyi state in southeastern Nigeria and has a population of 79,280 according to the 2006 census (NPC Ebonyi State, 2016). It is designated by the following coordinates 6°20’N 8°06’E / 6.333°N 8.100°E (Hoiberg, 2010). It has 1750-2500 mm rainfall per annum with an average atmospheric temperature of 27° C (Range 26° C - 29.6° C) (Nigeria Meteorological Agency).
Figure 1: Map of Ebonyi State Showing the Study Area “Abakaliki”

Source: Cartography Unit, Geology Department Ebonyi State University

2.2 Ethical Clearance

Ethical clearance was obtained from the Federal Teaching Hospital Abakaliki Ethical Committee. The consent of the patients was also sought, and those that gave their consent were part of the study. Ethical standards for the use of human subjects in line with the approval of the World Health Organization were strictly adhered to.

2.3 Sample Collection and Sampling Technique

One hundred and eighty (180) patients attending Dental Clinic at Federal Teaching Hospital Abakaliki were sampled using convenience non-probability sampling technique (Gharavi et al., 2006). The biodata such as age and sex were obtained from the patients’ folder. Dental plaque and dental Calculi were collected using scalers and preserved in 2 ml Ringer’s solution made by dissolving 9 g NaCl in 1 litre of water (0.9 g NaCl to 100 ml of water) as
described by (Hille, 1984). Saliva samples were also collected using sterile swabs and put in sterile containers. The samples were labelled and taken to Applied Biology Laboratory, Ebonyi State University for parasitological studies and identification.

2.4 Parasitological Studies

2.4.1 Wet Preparation

Wet preparation followed by Fotedar et al. (2007). From a homogenous mixture of each sample, a drop of the sample fluid was placed at the centre of a clean grease-free microscope slide. A coverslip was slowly lowered over the smear to minimize air bubbles while excess water was drained off using cotton wool. The slide was properly mounted on the microscope for the identification of the protozoan trophozoites (10x and 20x objectives).

2.4.2 Permanent (Giemsa) Staining Method

A smear of each of the samples was made, fixed in methanol and stained using Giemsa method. Thick and thin films were prepared and stained respectively. Microscopic observation was made using 10x and 20x objectives (Gharavi et al., 2006). The presence of *Trichomonas tenax* was recorded as a pear-shaped flagellated trophozoite of about 5-13 µm long with circular movement while the presence of trophozoite of size 10-20 µm with prominent pseudopodia and sluggish movement were recorded for *Entamoeba gingivalis*.

2.5 Statistical Analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) 20.0. Data on the relationship between age and sex of the participants and the presence of the parasites were analyzed using the Chi-square (χ²) test and simple percentages as appropriate. Probability values of $P<0.05$ was regarded as significant.

3. Results

3.1 Prevalence of the Oral Protozoan Parasites among Patients Concerning Sex

There was an overall 40% prevalence recorded. Out of the 180 patients sampled (72 males and 108 females), oral protozoa were isolated from 72(40.0%) of them. While 28(38.9%) occurred in males, 44(40.7%) occurred in females (Table 1). However, the sex difference was not statistically significant [$\chi^2 = 3.1725; P = 0.2047 (P > 0.05)$].
Table 1: Prevalence of the Oral Protozoan Parasites among Patients concerning Sex

| Sex     | Number examined | Number positive | % Positive | E. gingivalis | T. tenax | Mixed infection |
|---------|-----------------|-----------------|------------|---------------|----------|-----------------|
|         | Number          |                 |            | Number        | Number   | Number          |
|         |                 |                 |            | % | % | % | % |
| Male    | 72              | 28              | 38.9       | 12           | 6        | 10              |
|         |                 |                 |            | 16.7 | 8.3 | 13.9 |
| Female  | 108             | 44              | 40.7       | 16           | 18       | 10              |
|         |                 |                 |            | 14.8 | 16.7 | 9.3  |
| Total   | 180             | 72              | 40.0       | 28           | 24       | 20              |
|         |                 |                 |            | 15.6 | 13.3 | 11.1 |

$\chi^2 = 3.1725; P = 0.2047$ (Not significant at $P > 0.05$)

3.2 Prevalence of the Oral Protozoan Parasites among Age Groups

It was observed that individuals aged 20 years and less had the least prevalence of 4 (16.0 %). The highest prevalence of 22(81.5 %) occurred in the age group 50 years and above 22(81.5%), followed by age group 40–49 years 10(66.7 %) (Table 2). The differences were statistically significant ($\chi^2 = 27.7570; P = 0.0005$ Significant at $P < 0.05$).

Table 2: Prevalence of the Oral Protozoan Parasites among Age Groups

| Age grp (Yr.) | Num. Examined | Num. +ve | % +ve | E.gingivalis | T.tenax | Mixed infection |
|---------------|---------------|----------|-------|--------------|---------|-----------------|
|               | Num. | % | Num. | % | Num. | % | Num. | % |
| ≤ 20          | 25.0 | 16.0 | 2.0 | 8.0 | 2.0 | 8.0 | 0 | 0 |
| 20-29         | 55.0 | 29.1 | 10.0 | 18.2 | 6.0 | 10.9 | 0 | 0 |
| 30-39         | 58.0 | 34.5 | 6.0 | 10.3 | 8.0 | 13.8 | 6.0 | 10.3 |
| 40-49         | 15.0 | 66.7 | 8.0 | 53.3 | 0.0 | 0.0 | 2.0 | 13.3 |
| 50 & above    | 27.0 | 81.5 | 2.0 | 7.4 | 8.0 | 29.6 | 12.0 | 44.4 |
| Total         | 180.0 | 40.0 | 28.0 | 15.6 | 28.0 | 13.3 | 20.0 | 11.1 |

$\chi^2 = 27.7570; P = 0.0005$ (Significant at $P > 0.05$)

4. Discussion

The 40% overall prevalence reported in the present study consonants with Ullah et al. (2006) and Adamu et al. (2015) but at variance with Ozumba et al. (2004) and Onyido et al. (2011). We report that the high prevalence observed in this study could be attributed to the fact that many of the patients had oral deposits and disease conditions as observed during the
collection of samples. Previous studies with high oral protozoa prevalence had similarly associated with poor oral hygiene and dental diseases with high protozoa prevalence (Onyido et al. 2011).

The isolated protozoans (Entamoeba gingivalis and Trichomonas tenax) in the present study was also reported by previous indigenous researchers such as Ozumba et al. (2004), Onyido et al. (2011) and Adamu et al. (2015). Our study further revealed that Entamoeba gingivalis was more prevalent than Trichomonas tenax. This was highlighted by the 28.0 % prevalence of Entamoeba gingivalis against the 24.0 % of Trichomonas tenax in the study population. This consonant with Ibrahim and Abbas (2012) and varied with Onyido et al. (2011). The exact reason for this variation is not certain at this moment but the use of a polymerase chain reaction (PCR) protocol could produce a better yield of the organisms.

Sex-specific prevalence showed that the parasites occurred more in females than males (40.7 % and 38.9 % respectively). This finding corroborates with Adamu et al. (2015) but varied with Sumaiah & Rasha (2012). Though the exact cause of the variations may not be exactly adduced, in an attempt to say the possible cause Gharavi et al. (2006) opined that oral care in females is more respected hence the infection with E. gingivalis is less prevalent. However, the sex difference prevalence was not statistically significant in this study.

The findings of this study showed that the age group of 50 years and above recorded the highest prevalence of the protozoa while the age bracket of 20 years and less recorded the least prevalence. This consonant with Jawad, 2011. Age group 40-49 years specifically yielded the highest prevalence of E. gingivalis and this finding is at variance with some studies Onyido et al. (2011) and Adamu et al., 2015. A possible explanation for this may be because there is no significant difference between the oral hygiene statuses of the two-age groups (40-49 years and 50 years and above). The low prevalence recorded for the age group of 20 years and fewer years (16.0 %) collaborates the long assertion that human oral protozoa are rarely found in children. This is in line with the reports of Adamu et al. (2015), Onyido et al. (2011) and Gharavi et al. (2006). The significance in the relationship between age and infection with these protozoa is supported by Sumaiah & Rasha, 2012.

5. Conclusion

The Amoeba Entamoeba gingivalis and flagellates Trichomonas tenax were the two protozoan parasites isolated from the oral cavity of dental patients in this study. Although the
exact contribution of these organisms to dental diseases is not known, their prevalence among dental patients especially those with poor oral hygiene probably underscores their correlation with dental diseases. In light of the findings of this study, attention should be paid to the prevention of oral amoebiasis and trichomoniasis. Intensive oral hygiene awareness campaigns should be carried out to enlighten the populace especially dental patients to curb the prevalence of oral parasitic infections.

REFERENCES

Adamu, V. E., Nwoke, B. E. B., Amaechi, A. A. & Ukaga, C. N. (2015). Prevalence of Human Oral Protozoan Parasites in selected LGAs in Enugu State Nigeria. Nigerian Journal of Oral Health, 2 (1), 31–36.

Ferlay, J. E. M., Lam, F., Colombet, M., Mery, L., Piñeros, M., Znaor, A., Soerjomataram, I. & Bray, F. (2018). Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Published in 2018. Accessed May 10, 2020.

Foteder, R., Stark, D., Beebe, N., Marriott, D., Ellis, J. & Harkness J. (2007). Laboratory Diagnostic Techniques for Entamoeba Species. Clinical Microbiology Reviews, 20 (3), 514-515. https://doi.org/10.1128/CMR.00004-07

Gharavi, M. J., Hekmat, S., Ebrahimi, A. & Jahani, M. R. (2006). Buccal cavity protozoa in patients referred to the Faculty of Dentistry in Tehran, Iran. Iranian Journal of Parasitology, 1, 43-46.

Global Health Metrics (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet, 392: 1789–8583.

Hille, B. (1984). Ionic Channels of Excitable Membranes. Sunderland, Sinauer. Associates, Inc. 1375.

Hoiberg, D. H. (2010). “Abakaliki”. Encyclopaedia Britannica. 15th edition. Chicago, II. Encyclopaedia Britannica, inc.

Ibrahim, S. & Abass, R. (2012). Evaluation of Entamoeba gingivalis and Trichomonas tenax in patients with periodontitis and gingivitis and its correlation with some risk factor. Journal of Basic Sciences, 24 (3), 158–162.
Jawad, S. Q. (2011). Frequency of Entamoeba gingivalis and Trichomonas tenax among patients with dental prosthesis fixed or removable. Journal of College of Basic Education, 68, 97–100.

Marcenes, W., Kassebaum, N. J., Bernabé, E., Flaxman, A., Naghavi, M., Lopez, A., & Murray, C. J. L. (2013). Global burden of oral conditions in 1990–2010. A systematic analysis. Journal Dental Research, 92 (7), 592–7. https://doi.org/10.1177/0022034513490168

Onyido, A. E., Amadi, E. S., Olofin, I., Onwuma, A. A., Okoh, I. C. & Chikwendu, C. I. (2011). Prevalence of Entamoeba gingivalis and Trichomonas tenax among dental patients attending Federal School of Dental Technology and Therapy clinic, Enugu, Nigeria. Nature and Science, 9 (9), 59–62.

Ozumba, U. C., Ozumba, N. & Ndiokwelu, E. M. (2004). Oral protozoa in a Nigeria population. African Journal of Clinical and Experimental Microbiology 5 (1), 15–19. https://doi.org/10.4314/ajcem.v5i1.7355

Sumaiah, I. & Rasha, A. (2012). Evaluation of Entamoeba gingivalis and Trichomonas tenax in patients with periodontitis and gingivitis and its correlation with some risk factors. Journal Baghdad College of Dentistry, 24 (3), 158–162.

Ullah, Z., Khan, M., Jan, A. H. & Ali, I. (2006). Mouth protozoa in North-West Frontier Province of Pakistan. Pakistan Oral & Dental Journal, 27 (2), 245–248.

World Health Organization (2020). Oral Health. Accessed May 10, 2020. https://www.who.int/news-room/fact-sheets/detail/oral-health