DOI: https://doi.org/10.53555/8zw8wr51 Publication URL: https://nnpub.org/index.php/PBS/article/view/1864

AN EPIDEMIOLOGICAL STUDY TO DETERMINE THE PREVALENCE OF E. GINGIVALIS IN ORAL OF DIABETIC MELLITUS PATIENTS IN CITY OF KERBALA

Assistant Teacher Zahra Abdul Khader Hashem*

**University of Kerbala, College of Dentistry, Medical Sciences Branch, Iraq. Email: zahra.a@uokerbala.edu.iq*

Corresponding Author:
zahra.a@uokerbala.edu.iq

Abstract

Background: *Many people suffer from infection with Entamoeba gingivalis which mainly causes for periodontitis and other dental diseases specially in patient suffering from Diabetic mellitus.*

Aim of study: *To determine the prevalence of the Entamoeba gingivalis among diabetes mellitus patients in Kerbala city.*

Methodology: *oral swap samples were collected from 300 of respondents (patients were diagnosed clinically by physician suffering from DM) attended to Imam Hassan Center for Endocrinology and Diabetes and some of outpatients clinics in Kerbala Province of Iraq from May to August 2023. Oral swap specimens were collected and immediately introduced for microscopic examination for diagnosis of Entamoeba gingivalis parasites.*

Results: *the results of current study showed that DM patients with Periodontitis were increased in age groups 50-59 years and accounting (68.18%), and in 40-49 years accounting (60.31%) while in DM without Periodontitis were most common in age groups 30-39 Years and accounting (63.01%), and in 40-49 years accounting (39.69%). The distribution of patients according to gender, in which male and female of DM patients with Periodontitis accounted for (54.05%) and (46.05%) respectively, while DM Without Periodontitis in which male and female accounted for (45.95%) and (53.95%) respectively. The residence of DM patients with Periodontitis was more common in rural, 57.66% compared to DM Without Periodontitis was more common in urban, 56.45%. Out of (300) oral swap samples that which examined microscopically for E.gingivalis by using light microscope, E.gingivalis infection detected in 45 (30.0%) out of 150 DM Patients with Periodontitis, and in 15 (10.0%) out of 150 DM Patients without Periodontitis.*

Conclusions: *the rate incidence of Entamoeba gingivalis infevtion in the current study 40%. The residence of DM patients with Periodontitis was more common in rural, compared to DM Without Periodontitis was more common in urban.*

Keywords: *Entamoeba gingivalis*, Periodontitis, Diabetic mellitus.

INTRODUCTION:

Entamoeba gingivalis was the first amoeba of humans, discovered by Gros in 1849. It is global in distribution and only the trophozoite is found; the cystic stage being apparently absent. The trophozoite is about 10–20 μm, actively motile with multiple pseudopodia [1].

The cytoplasm contains food vacuoles with ingested bacteria, leuocytes, and epithelial cells. Nucleus is round with central karyosome lined by coarse chromatin granules. The amoeba lives in gingival tissues and is abundant in unhygienic mouths [2].

It is a commensal and is not considered to cause any disease. It is transmitted by direct oral contact [3]. *E. gingivalis* have been found in bronchial washings [4] and vaginal and cervical smears, where it can be mistaken for E. Histolytica [5].

Periodontitis is one of the most prevalent diseases worldwide. Periodontal disease, also known as gum disease, is a set of inflammatory conditions affecting the tissues surrounding the teeth. In its early stage, called gingivitis, the gums become swollen and red and may bleed [6].

It is considered the main cause of tooth loss for adults worldwide. In its more serious form, called periodontitis, the gums can pull away from the tooth, bone can be lost, the teeth may loosen or fall out, and bad breath may also occur [7].

The pathology is characterized by gum inflammation with bone loss, often associated with pain, halitosis and gingival bleeding. In frequent cases, alveolysis can lead to tooth loosening or even loss. Though these clinical manifestations are obvious signs of disease, patients habitually tolerate discomfort or suffering; the impact of oral health-related quality of life is indeed often underestimated [8].

Periodontal disease is generally due to bacteria in the mouth infecting the tissue around the teeth also the presence of *E. gingivalis* killed live epithelial cells by trogocytosis, demonstrating strong pathogenic potential for Periodontitis. Factors that increase the risk of disease include smoking, immunocompromised family history, certain medications and diabetes. Diagnosis is by inspecting the gum tissue around the teeth both visually and with a probe and X-rays looking for bone loss around the teeth [1].

E. gingivalis is considered an oral commensal but demonstrates a pathogenic potential associated with periodontal disease in diabetic mellitus individuals, therefore *E.gingivalis* can be an important agent in the pathophysiology of periodontitis [9].

E. gingivalis exists as a trophozoite and is transmitted through oral contact. Its occurrence can vary according to age, presence of periodontal disease, and immunosuppression conditions [10].

E. gingivalis is considered a harmless commensal organism in humans and is commonly found in the calculus and bacterial plaques, crevicular fluid, and saliva. There are controversies concerning its pathogenicity because it has been detected in healthy individuals but has also been associated with periodontal disease.

In the early 1980s, new interest in *E. gingivalis* after T. Lyons detected amoeboid organisms in periodontal pockets, while they were absent from healthy sites assuming these parasites were responsible for periodontal disease [11], also In a recent study conducted in 2021 it was mentioned about 15% of the healthy oral cavities of adults are infected with *E. Gingivalis* and the prevalence of this protozoan strongly increases in periodontal inflammation, and it colonizes up to 80% of inflamed pockets of patients with periodontitis [12].

The parasite *E. gingivalis* is more prevalent and more abundant in periodontal pockets, suggesting that this ecological niche is either propitious for its survival, or that the parasite induces changes leading to this environment [13]. Further studies will have to take into consideration the physicochemical and biological characteristics of the periodontal pockets to allow relevant studies of the biology of the parasites, either in vitro or in animal models [14] , [15].

Methodology:

Patients Group

Samples collection (300 Oral swap speciemens) were conducted for diabetes patients with periodentitis (150 participitant) and diabetes patients without periodentitis (150 participitant) during the period from the beginning of May 2023 to the end of August 2023 at Imam Hassan Center for Endocrinology and Diabetes and some of outpatients clinics in Kerbala and their ages ranged between (20 - ≥ 60) years.

Samples Collection (Oral swap)

Oral swap for mouth is directly done using a sterile cotton swab and and transferred to the parasitology department for direct examination method under light microscope.

Excluded Criteria

Comprised consuming systemic antibiotics in the past two months, using immunosuppressive drugs, smoking, being pregnant and having systemic, heart or respiratory diseases.

Ethical Standards

The current study obtained ethical approval by Kerbala Health Department / Training and Development Center, and written consent was taken from all participants in the research.

Microscopic Examination

Oral swap for mouth is directly done using a sterile cotton swab and the sample is placed on a glass slide, a drop of normal saline solution is placed on the sample, and the sample is examined under a light microscope under the forces of

(10x) , (40x). The positive result can be observed by seeing the parasite movement characteristic of it, where the mouth parasites move in a spiral manner.

Results and Discussion

Basic Demographic of cases and Control Subjects

This study was conducted on a total of (300) individual in different gender and age group cases (148 males and 152 females) enrolled in this study composed the two studied groups, namely, first group included the diabetic mellitus patients with peridontitis (150), the second group included diabetic mellitus patients without peridontitis (150) were selected from Imam Hassan Center for Endocrinology and diabetes and some of outpatients clinics in Kerbala, during the period from the beginning of May 2023 to the end of August 2023.

Table (1) shows their demographic characteristics, where that DM patients with Periodontitis were increased in age group 50-59 years were accounting (68.18%), and in 40-49 years accounting (60.31%) while in DM without Periodontitis were most common in age groups 30-39 Years and accounting (63.01%), and in 40-49 years accounting (39.69%).

Periodontitis is one of the important dental diseases in many parts of the world. The prevalence of the disease increases by age and it is estimated that 50% of people above 30 years of age are involved with this condition [3].

Abbass et al. reported depend on the age the higher percentage of E.gingivalis parasite was (23.52%) in the age group (9-10 y) by microscopic method and (35.29%) in the same age group by PCR technique with statistically significant correlation between the infection and age at $p \leq 0.05$ [16].

Gender distribution of patients as shown in Table (1), in which male and female of DM patients with Periodontitis accounted for (54.05%) and (46.05%) respectively, while DM Without Periodontitis in which male and female accounted for (45.95%) and (53.95%) respectively.

Mohammed et al. found the prevalence of oral parasite *E.gingivalis* in male was higher than females, its due to females dental and mouth care is more respected therefore the infection to *E. gingivalis* were less prevalent than males [17].

Sharifi et al. reported that the rate of *E. gingivalis* infection in males was 2.8 times higher than that in females ($P<0.001$) [18].

High prevalence of mouth amoebiasis in the study reflects poor food hygiene and wrong social habits concerning food consumption in our Society [17].

The residence of DM patients with Periodontitis was more common in rural, 57.66% compared to DM Without Periodontitis was more common in urban, 56.45% .

In comparison with other studies, [19] found the average number of *E. gingivalis* in urban children was 12.84%, whereas a smaller amount of amoebae was found in rural children (10.74%).

Higher levels of amoeba in urban children than in rural children can be explained by the frequency of staying in larger groups, and contacts with a lot of children. In cities there is a greater microbiological contamination [20].

According to Doni et al. the place of residence has significant impact on the frequency and type of hygiene procedures performed in the oral cavity. Urban children more often clean their teeth and use additional means for dental hygiene [21].

Some authors claim that the use of additional mechanical products for plaque removal, such as dental floss, toothpicks or brushes for cleaning interdental spaces, increases the occurrence of amoebae [22]. This could explain the presented finding that more amoeba were found in urban.

Table (1): Demographics of Respondents according to Age groups and Sex

Age groups		GROUPS		
		With Periodontitis	Without Periodontitis	Total
20-29 Y	Count	24	56	80
	% within	30%	70%	100.0%
30-39 Y	Count	27	46	73
	% within	36.99%	63.01%	100.0%
40-49 Y	Count	38	25	63
	% within	60.31%	39.69%	100.0%
50-59 Y	Count	45	21	66
	% within	68.18%	31.82%	100.0%
60-69 Y	Count	16	2	18
	% within	88.89%	11.11%	100.0%
Total	Count	150	150	300
	% within	50.0%	50.0%	100.0%
Gender				
Male	Count	80	68	148
	% within	54.05%	45.95%	100.0%
Female	Count	70	82	152

	% within	46.05%	53.95%	100.0%
Total	Count	150	150	300
	% within	50.0%	50.0%	100.0%
Residence				
Urban	Count	71	92	163
	% within	43.55%	56.45%	100.0%
Rural	Count	79	58	137
	% within	57.66%	42.34%	100.0%
Total	Count	150	150	300
	% within	50.0%	50.0%	100.0%

Isolation and Identification of *Ent*amoeba *gingivalis* from Diabetes Patients with and Without Periodontitis

Out of (300) oral swap samples that which examined microscopically for *E.gingivalis* by using light microscope as shown in Figure (1) under (40x), *E.gingivalis* infection detected in 45 (30.0%) out of 150 DM Patients with Periodontitis, and in 15 (10.0%) out of 150 DM Patients without Periodontitis. As shown in Table (2) and Figure (1).

Many pathogenic agents as well as systemic and immunosuppressive diseases can cause major periodontal problems, among the current protozoa of the oral cavity, *E. gingivalis* is the most frequent parasite. The role of this parasite in inducing periodontal diseases is not exactly clear and remains controversial. To define the pathogenic role of the parasite, appropriate treatment schedules should be set up by dentists [3].

The total percentage of *E.gingivalis* in the presented study was 40% this nearly similar to [23] where found *Entamoeba gingivalis* in Babylon Province was (42.9 %).

The results of the current study was much higher than what [3] found in his study that conducted in Iran, reported that only 16% of the patients were infected with *E. gingivalis.*

The study presented by [24] found the prevalence of oral *E.gingivalis* in southern Iran was (66.7%), while [18] found the prevalence of *E. gingivalis* was 11.7% also in Southeastern Iran, and this results that reported by [18] was much lower than what we found in our study.

The study presented by Badri revealed a high prevalence rate of the infection among periodontal disease patients with 37% (95% CI 20-57%). To conclude, it must be considered that E. gingivalis can be a risk factor associated with oral diseases and a wide range of research is needed to specify its role in the pathogenesis of these disorders [25].

Table (2): Distribution of respondents according to microscopically diagnosis for *Entamoeba gingivalis*

GROUPS	No. of patients examined microscopically	No. of patients infected with *E.gingivalis*	Incidence rate
DM With Periodontitis	150	45	30.0%
DM Without Periodontitis	150	15	10.0%
Total	300	60	40.0%

Figure (1): *Entamoeba gingivalis* under Light Microscope (The arrows→ refer to trophozoite of parasite 40X).

Conclusions

the rate incidence of *Entamoeba gingivalis* infevtion in the current study 40%. The residence of DM patients with Periodontitis was more common in rural, compared to DM Without Periodontitis was more common in urban.

Acknowledgements
I would like to thank all physicians and staff members of Imam Hassan Center for Endocrinology and Diabetes and some of outpatients clinics in Kerbala Province of Iraq for their help in samples collection.
Also my deepest appreciation is directed to the patients who expressed their assistance and made this work possible.

Funding
The source of funding for this work was personal finance.

References

[1]. Bonner, M., Fresno, M., Gironès, N., Guillén, N., & Santi-Rocca, J. (2018). Reassessing the Role of Entamoeba gingivalis in Periodontitis. Frontiers in cellular and infection microbiology, 379.
[2]. Patel, N. (2019). The Prevalence of Protozoa During Canine Periodontal Disease (Doctoral dissertation, University of Leicester).
[3]. Rahdar, M., Abolfazli-Karizi, S., & Pedram, H. (2019). The comparison of Entamoeba gingivalis presence in healthy and periodontitis patients by using direct examination and PCR methods. Jundishapur Journal of Health Sciences, 11(1).
[4]. Stensvold, C. R., Nielsen, M., Baraka, V., Lood, R., Fuursted, K., & Nielsen, H. V. (2021). Entamoeba gingivalis: epidemiology, genetic diversity and association with oral microbiota signatures in North Eastern Tanzania. Journal of oral microbiology, 13(1), 1924598.
[5]. Bradbury, R. S., Roy, S., Ali, I. K., Morrison, J. R., Waldner, D., Hebbeln, K., ... & Bishop, H. S. (2019). Case Report: Cervicovaginal co-colonization with Entamoeba gingivalis and Entamoeba polecki in association with an intrauterine device. The American journal of tropical medicine and hygiene, 100(2), 311.
[6]. Mann, J., Bernstein, Y., & Findler, M. (2020). Periodontal disease and its prevention, by traditional and new avenues. Experimental and therapeutic medicine, 19(2), 1504-1506.
[7]. Frencken, J. E., Sharma, P., Stenhouse, L., Green, D., Laverty, D., & Dietrich, T. (2017). Global epidemiology of dental caries and severe periodontitis–a comprehensive review. Journal of clinical periodontology, 44, S94-S105.
[8]. Mehrotra, N., & Singh, S. (2021). Periodontitis. StatPearls [Internet].
[9]. Cembranelli, S. B., Souto, F. O., Ferreira-Paim, K., Richinho, T. T., Nunes, P. L., Nascentes, G. A., ... & Lages-Silva, E. (2013). First evidence of genetic intraspecific variability and occurrence of Entamoeba gingivalis in HIV (+)/AIDS. PLoS One, 8(12), e82864.
[10]. EL-Dardiry, M. A., Abdel-Aal, A. A., Nahnoush, R. K., Elmallawany, M. A., Akmal, M., Afife, A. A., & Badr, M. S. (2021). Structural and Genetic Diversity of Entamoeba Gingivalis Trophozoites Isolated From Diseased and Healthy Periodontal Sites.
[11]. Lyons, T. (1983). Oral amoebiasis: the role of Entamoeba gingivalis in periodontal disease. Quintessence International, 12, 1245-1248.
[12]. Bao, X., Weiner 3rd, J., Meckes, O., Dommisch, H., & Schaefer, A. S. (2021). Entamoeba gingivalis Exerts Severe Pathogenic Effects on the Oral Mucosa. Journal of Dental Research, 100(7), 771-776.
[13]. Hassan, S. S., Madkour, G. G., Henin, R. W., Gad, S. W. F., & Abd El-Aal, A. (2019). Is Entamoeba gingivalis a risk factor for periodontal diseases? A case-control study. Perio J, 3(1), 18-28.
[14]. Zięba, M., Chaber, P., Duale, K., Martinka Maksymiak, M., Basczok, M., Kowalczuk, M., & Adamus, G. (2020). Polymeric carriers for delivery systems in the treatment of chronic periodontal disease. Polymers, 12(7), 1574.
[15]. Zhuang, Y., Lin, K., & Yu, H. (2019). Advance of nano-composite electrospun fibers in periodontal regeneration. Frontiers in Chemistry, 7, 495.
[16]. Abbass, Z. A. A., Hanoon, S. A., & Kadhim, T. A. (2020). The Prevalence of Entamoeba Gingivalis and Trichomonas Tenax in Children Treated with Orthodontic Appliances in AL Muthanna Province, Iraq. Indian Journal of Public Health, 11(02), 1983.
[17]. Mohammed, S. T., Sulaiman, N. M., & Kamal, S. B. (2015). PREVALENCE OF ENTAMOEBA GINGIVALIS AMONG STUDENTS OF COLLAGE OF SCIENCE IN AI-MUSTASYRIA UNIVERSITY, BAGHDAD, IRAQ.
[18]. Sharifi, M., Jahanimoghadam, F., Babaei, Z., Mohammadi, M. A., Sharifi, F., Hatami, N., ... & Poureslami, H. (2020). Prevalence and Associated-Factors for Entamoeba gingivalis in Adolescents in Southeastern Iran by Culture and PCR, 2017. Iranian Journal of Public Health, 49(2), 351.
[19]. Mielnik-Blaszczak, M., Rzymowska, J., Michalowski, A., Skawinska-Bednarczyk, A., & Blaszczak, J. (2018). Entamoeba gingivalis-prevalence and correlation with dental caries in children from rural and urban regions of Lublin province, Eastern Poland. Annals of Agricultural and Environmental Medicine, 25(4).
[20]. Nooraldeen, K. (2015). Contamination of public squares and parks with parasites in Erbil city, Iraq. Annals of Agricultural and Environmental Medicine, 22(3).
[21]. Doni, N. Y., Gurses, G., Simsek, Z., & Zeyrek, F. Y. (2015). Prevalence and associated risk factors of intestial parasites among children of farm workers in the southeastern Anatolian region of Turkey. Annals of Agricultural and Environmental Medicine, 22(3).
[22]. Gaszynska, E., Wierzbicka, M., Marczak, M., & Szatko, F. (2014). Thirty years of evolution of oral health behaviours and dental caries in urban and rural areas in Poland. Annals of Agricultural and Environmental Medicine, 21(3).

[23]. Al-Dulaimi, F. H. A., Alajeely, A. A. A., & Ail, Y. M. (2020). Incidence of Entamoeba Gingivalis and Trichomonas Tenax in Periodontitis and Gingivitis Patients Who Attended to Private Clincs in Babylon Province. Medico-Legal Update, 20(1), 906-910.
[24]. Ghabanchi, J., Zibaei, M., Afkar, M. D., & Sarbazie, A. H. (2010). Prevalence of oral Entamoeba gingivalis and Trichomonas tenax in patients with periodontal disease and healthy population in Shiraz, southern Iran. Indian Journal of Dental Research, 21(1), 89.
[25]. Badri, M., Olfatifar, M., Abdoli, A., Houshmand, E., Zarabadipour, M., Abadi, P. A., ... & Eslahi, A. V. (2021). Current global Status and the Epidemiology of Entamoeba gingivalis in humans: a systematic review and meta-analysis. Acta Parasitologica, 1-12.