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RESEARCH ARTICLE

PREVALENCE OF *ENTAMOEBA GINGIVALIS* IN PATIENTS WITH GINGIVITIS AND PERIODONTITIS AND HEALTHY INDIVIDUALS AND ITS ASSOCIATED FACTORS

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Abstract

The present study was carried out to determine the prevalence of *Entamoeba gingivalis* among 300 (156 males and 144 females) participants divided into three groups as follows: 100 patients with periodontitis (periodontitis group), 100 patients with gingivitis (gingivitis group), and 100 individuals with healthy gingiva (healthy control group), using wet mount technique with physiological saline and Giemsa stain. The overall prevalence of *E. gingivalis* was 18.3% and the highest prevalence of *E. gingivalis* was among periodontitis group (29%) followed by gingivitis group (18%), and healthy group (8%). The highest prevalence (23.7%) of *E. gingivalis* was found among the age group 40-49 years. Infection was highly significant in males than females (23.7% vs.12.5%). With regard to risk factors, the prevalence of *E. gingivalis* was common in non-tooth brush users, smokers, qat chewers, and diabetic patients and there were significant differences between tooth-brush users and non-tooth brush users, smokers and non-smokers, qat chewers and non-gat chewers, and between diabetic and non-diabetic patients and occurrence of *E. gingivalis*. We conclude that the higher prevalence of *E. gingivalis* in periodontal disease compared to healthy individuals may be an indicator of the potential role *E. gingivalis* in the pathogenesis of periodontal diseases.

Keywords: Prevalence, *Entamoeba gingivalis*, Periodontal disease, Aden.

Introduction

Entamoeba gingivalis was the first commensal parasite detected in the human oral cavity. It is a cosmopolitan anaerobic amoebic protozoan [1], which exists as trophozoite form that has variable size ranging from 10 to 35 µm in diameter. *E. gingivalis* has no cyst form; therefore, it spreads either directly via direct contacts, by kissing or indirectly through contaminated food, or by sharing eating utensils [2] *E. gingivalis* is found most frequently in tartar between and around the teeth, tonsillar crypts and gingival tissues, particularly in suppurative, inflammatory processes, due to its preference for anaerobic environments [3].

E. gingivalis is more commonly found in persons with bad oral hygiene. Also, *E. gingivalis* is generally considered as an oral commensal but reports show that it displays a pathogenic role in periodontal disease [4]. In addition, *E. gingivalis* has been detected in conditions of immune suppression. At present, there is evidence to propose an association of *E. gingivalis* in pathogenesis of periodontal disease [4, 5] and several studies reported that *E. gingivalis* contributes to the initiation and progression of gingivitis and periodontitis. Moreover, the pathogenic perspective of *E. gingivalis* has been proven experimentally by the development of lesions in immunosuppressed animals [4]. However, there are controversies concerning its pathogenicity characteristic as this trophozoite is commonly found in oral cavity of healthy individuals and with gingivitis as well, and several authors consider *E. gingivalis* to be opportunistic [4, 6]. Accordingly, this data opens a scientific gate for more research to discover the pathophysiology of gingivitis and periodontitis. Data on the prevalence of this parasite remains limited in many developing countries including Yemen, and due to controversies on infectivity of these parasite, its correlation to periodontal diseases, and to its public health importance, this study was carried out to determine the prevalence of *E. gingivalis* and its association with some factors among patients with and without periodontal disease attending three dental clinics in Aden governorate, Yemen.

Materials and Methods

Study Design

A case control Study design was conducted for eight months from February to September 2022.

Study Setting

This study was carried out in three dental clinics in Aden governorate, Yemen; the first two were dental internship specialized clinic, oral surgical clinic, faculty of dentistry university of Aden and the last one dental clinic of Al-Gamhoria Modern General Hospital.

Study Population

Three hundred subjects in total who attended the above-mentioned clinics were enrolled in this study during the study period. The study participants were divided into three groups:100 individuals with healthy gingiva (healthy control group); 100 patients with gingivitis (gingivitis group); and 100 patients who presented with periodontitis (periodontitis group). The individuals with gingivitis and those with periodontitis represented the "case group". The control group was matched with the case group in terms of age and sex. A random selection of the patients was carried out. Subjects were categorized according to their clinical examination carried out by the help of dental specialist and according to the recently guidelines to classification of periodontal disease [7, 8,9].

Exclusion Criteria

The presence of one of the following criteria resulted in exclusion of the potential participant from the study: patients under 18 years' old, previous history of periodontal treatment (scaling or root planning) in the six months prior to enrollment, recent use of antimicrobial agents within the preceding three months, pregnancy, and immunocompromised.

Sample Size

Sample size for a Case-control study of 300 was determined on the basis of calculations done using Epitools - Epidemiological Calculators accessible without restraint online (https://epitools.ausvet.com.au/).

Data Collection

A pretested, structured questionnaire was used to collect data through a direct face to face interview of the participants. The first section of the questionnaire was designed to elicit the demographic characteristic such as age, gender, and educational level, and oral hygiene measures such as tooth and miswak tooth brushing, and social habits of the participants such as smoking, qat chewing, and diabetic mellitus were also included. The second section was utilized to record the laboratory investigations correlated to parasites identification.

Laboratory Methods

Specimen Collection and Processing

Under aseptic condition and by the help of dentist a specimen of dental plaque and/or calculus were collected according to [10]. Once collection was complete the specimens were transferred to the parasitology department at National Center of Public Health Laboratories /Aden, within one hour of collection and immediately processed to maintain protozoan viability and avoid the trophozoites lysis.

Microscopic Examination

The microscopic examination is carried out by an expert. Each specimen was divided in two parts one was used to prepare wet mount and the second to prepare smear for Giemsa staining.

Wet mount examination

Using sterile Pasteur pipette a drops of a diluted specimen were placed on clean microscopic slides (25.4x 76.2 mm) and a coverslip (24x 50 mm) was applied on the top, and the material spreads by pressure on the coverslip. This prepared a thin film which was then examined immediately with a light microscope at 10X and 40X. The identification of *E. gingivalis* was established by its shape depending on the expansion of pseudopodia formation, and sluggish movement [11]. Three smears of wet mount method for each samples used to strength the chance of detection of parasites.

Giemsa staining

Thick smear were prepared on clean microscopic slide and allowed to dry. It was then stained with commercially available Giemsa stain (Techno pharmchem, India) diluted (1:50, vol/vol); (For a 1:50, add 1ml Giemsa stock to 50 ml buffered water) for 50 min. Then the slide was gently washed under clean water and let air dried in vertical position, and scanned for *E. gingivalis* at 100X magnification. The characteristic morphologic features for *E. gingivalis* appear as irregular shaped with light red purple cytoplasm and an intracellular vacuoles visualized as darker red – purple [12]. Three smears stained with Giemsa staining used for each sample to strength the chance of detection of parasite.

Ethical considerations

The study protocol was approved by "the Committee of Research and Postgraduate Studies, at the Biology department (Animal specialty) faculty of Education, Aden University. Permissions from the faculty of dental medicine, Al-Gamhoria Hospital Authority and national center of public health laboratories authorities, where the study was conducted was obtained. After providing a clear explanation, each participant's verbal consent was obtained before participation. Confidentiality of the study participants was maintained.

Data analysis

The collected data were analyzed and processed by using Statistical Package for the Social Sciences (SPSS) software version of 20. For variables difference, chi-square tests, and P values were calculated, with differences at less than 0.05% being regarded as significant.

Results

The total number of study participants enrolled in this study was 300 distributed as follows: healthy group (n=100), gingivitis group (n=100), and periodontitis group (n=100). Table 1 shows the participant's demographic characteristics.

The participant's ages ranged from ≤29 to ≥ 60 years. 21.7% (65/300) of the participants in this investigation were between 50-59 years old and about 52.0% were males and the about 44.3% of participants were illiterate and completed the primary education, and only 31.3% were completed university education.

Table 1: Demographic characteristics of the study participants (n=300).

Variables	Categories	No.	%*
Mean, SD	44.43±15.2		
Age groups (years)	≤ 29	59	19.7
	30-39	56	18.7
	40-49	59	19.7
	50-59	65	21.7
	≥ 60	61	20.3
Gender	Male	156	52.0
	Female	144	48.0
Educational level	Illiterate	46	15.3
	Primary	87	29.0
	Secondary	73	24.3
	University	94	31.3

* Percentages were taken from the total sample size (300).

Regarding risk factors such as oral hygiene and social habits like tooth and miswak brushing, smoking, qat chewing, and history of diabetes mellitus, table 2 shows that, more than third (36.7%;110/300) of participants were irregular tooth brush users, only 54 (18.0%) use tooth brush twice daily, and 66 (22.0%) were non- tooth brush users. About two third (65.3%; 196/300) were non-miswak users, 90 (30%) were sporadically miswak users, and only 9 and 5 were twice and once per day miswak users, respectively. Majority of participants (81.3%; 244/300) were non-smokers and only 56 (18.7%) were smokers. On the other hand, two third of participants (66.7%; 200/300) were non-qat chewers and one third of participants (33.3%; 100/300) were qat chewers. 269 (89.7%) were non-diabetic and 31 (10.3%) were diabetics (Table 2).

Table 2: Risk factors among study participants (n=300).

Variables	Categories	No.	%*
Frequency of tooth brushing (daily)	Twice	54	18.0
	Once	70	23.3
	Sporadic	110	36.7
	Never	66	22.0
Frequency of miswak brushing (daily)	Twice	9	3.0
	Once	5	1.7
	Sporadic	90	30.0
	Never	196	65.3
Smoker	No	244	81.3
	Yes	56	18.7
Qat chewing	NO	200	66.7
	Yes	100	33.3
DM	No	269	89.7
	Yes	31	10.3

* Percentages were taken from the total sample size (300)
DM; Diabetic mellitus

As seen in table 3, the overall prevalence of *E. gingivalis* was 18.3% (55 out of 300). The highest prevalence rate of 29% (29 out of 100) was found among the periodontitis group followed by the gingivitis group with prevalence rate of 18% (18 out of 100), and the healthy group with lowest prevalence rate of 8% (8 out of 100). Statistically significant difference in *E. gingivalis* prevalence was reported upon comparing the healthy group to the gingivitis and periodontitis groups (p=0.001); (Table 3).

Table 3: Frequency of *Entamoeba gingivalis* among the study groups.

Variables	Entamoeba gingivalis				p-*** value
	Positive		Negative		
	No.	%**	No.	%**	
Healthy (n=100)	8	8	92	92	0.001
Gingivitis (n=100)	18	18	82	82	
Periodontitis (n=100)	29	29	71	71	
Total (n=300) NO.%*	55	18.3	245	81.7	

* Percentages were taken from the total sample size (300)
** Percentages were taken from the rows sample (100)
*** Chi-square test. statistically significant p < 0.05

Table 4. shows the prevalence of *Entamoeba gingivalis* according to demographic characteristics. The highest prevalence rate of 23.7% was reported among participants in the age group of 40-49 years old. The prevalence of *E. gingivalis* was higher in males (23.7%) than in females (12.5%), and the illiterate participants represented 17.4%, the primary 20.7 %, the secondary 23.3% and the university participants 12.8%. Statistically significant differences was found between gender and the presence of the parasite (*p*= 0.012). However, no significant association was found between the age and educational level, and the prevalence of *E. gingivalis*.

Table 4: Prevalence of *Entamoeba gingivalis* according to demographic characteristics.

Variables	Categories	Entamoeba gingivalis				Total (n=300) No.(%)*	P-value***
		Positive (n=55)		Negative (n=245)			
		No.	%**	No.	%**		
Age groups (years)	≤ 29	7	11.9	52	88.1	59(19.7)	0.300
	30-39	7	12.5	49	87.5	56(18.7)	
	40-49	14	23.7	45	76.3	59(19.7)	
	50-59	14	21.5	51	78.5	65(21.6)	
	≥60	13	21.3	48	78.7	61(20.3)	
Gender	Male	37	23.7	119	76.3	156(52)	0.012
	Female	18	12.5	126	87.5	144(48)	
Educational level	Illiterate	8	17.4	38	82.6	46(15.3)	0.322
	Primary	18	20.7	69	73.3	87(29)	
	Secondary	17	23.3	56	76.7	73(24.3)	
	University	12	12.8	82	87.2	94(31.4)	

* Percentages were taken from the total sample size (300)
** Percentages were taken from the rows total
*** Chi-square test. statistically significant p < 0.05

Regarding oral hygiene, social habits and history of diabetes mellitus, table 5 shows that, the highest prevalence rates of 31.8% and 18.2% were found among individuals who never used tooth brush and sporadically use tooth brush, respectively. While, low rates of 9.3% and 12.9% were found among those who use tooth brush twice and once per day, respectively. The difference between values was significant (p= 0.006).

Among twice per day Miswak users, 3 were found to be positive (33.3%), and among those sporadically and never use Miswak, the prevalence rates were 23.3% and 15.8%, respectively, as shown in table 5; there was no statistical significant (p > 0.05). Smokers revealed high prevalence rate (41.1%) as compare to non-smokers (32.1%). There was significant difference between smokers and non-smokers in the prevalence of *E. gingivalis* (p<0.001). In Qat chewers, 29% were found to be positive with *E. gingivalis*, while 13% of non-qat chewers were found to be positive. Also, the difference between values was significant (p= 0.001).

Higher prevalence rate (35.5%) was found among diabetics as compare to non-diabetics (16.4%). Also, significant difference between diabetics and non-diabetics was found in prevalence of *E. gingivalis* (p= 0.009) as shown in table 5.

Table 5: Prevalence of *Entamoeba gingivalis* according to risk factors

Variables	Categories	Entamoeba gingivalis				Total (n=300) No.(%)*	P-***value
		Positive (n=55)		Negative (n=245)			
		No.	%**	No.	%**		
Frequency of tooth brushing (daily)	Twice	5	9.3	49	90.7	54(18)	0.006
	Once	9	12.9	61	87.1	70(23.3)	
	Sporadic	20	18.2	90	81.8	110(36.7)	
	Never	21	31.8	45	68.2	66(22)	
Frequency of miswak brushing (daily)	Twice	3	33.3	6	66.7	9(3)	0.186
	Once	0	0	5	100	5(1.7)	
	Sporadic	21	23.3	69	76.7	90(30)	
	Never	31	15.8	165	84.2	196 (65.3)	
Smoking	No	32	32.1	212	86.9	244(81.3)	<0.001
	Yes	23	41.1	33	58.9	56(18.7)	
Qat chewing	NO	26	13	174	87	200(66.7)	0.001
	Yes	29	29	71	71	100(33.3)	
D M	No	44	16.4	225	83.6	269(89.7)	0.009
	Yes	11	35.5	20	64.5	31(10.3)	

* Percentages were taken from the total sample size (300)
** Percentages were taken from the rows total
*** Chi-square test. statistically significant p < 0.05
DM. Diabetic mellitus

Discussion

The results of this study showed that fifty five (18.3%) out of the 300 total precipitants were evidenced to be positive for *E. gingivalis* occurrence. Compared with the previous studies from different countries in which the microscopic method was used to identify *E. gingivalis*, more or less similar results were reported. Younis and Edamame [13] from Libya detected *E. gingivalis* in 17% out of 100 examined samples; Al- Najar and Adnan [14] from Iraq reported a prevalence rate of 28% for *E. gingivalis* among their participants; in addition, Hassan *et al*. [15] from Egypt, reported a prevalence rate of 25.8% among120 participants. On the other hand, in DNA-based studies, more higher prevalence rates were observed. As a study from Jordan, Yaseen *et al.* [16], found a higher prevalence of 71.7% among 237 participants. These differences in prevalence rate could be explained by differences in diagnostic sensitivity among the methods used, small sample sizes, and possible bias in selection of study subjects [15, 16, 17]. Although, the prevalence of *E. gingivalis* varies in different countries but remains higher in those patients with periodontal disease as this study showed [18].

According to the three study groups, in this study the distribution of positive cases with *E. gingivalis* was as follows: the highest prevalence rate of 29% was among the periodontitis group (n=29/100), followed by the gingivitis group (18% & n=18/100), and the healthy group had the lowest prevalence rate (8% & n=8/100). In accordance to our results, Hassan *et al.* [15] and Garcia *et al.* [17] reported that the prevalence of *E. gingivalis* differs among patients with periodontitis, gingivitis and healthy individuals but remains higher in those patients with periodontal disease compared with healthy control group, as this study reported**.** Therefore, it was hypothesized that this protozoa might be involved in the pathogenesis and progression of periodontal disease (periodontitis and gingivitis) [16, 11]. Consequently, the results of this study were compatible with these results, as the patients with periodontal disease in this study were more susceptible to *E. gingivalis*. This was emphasized by the fact that 23.5% of participants in periodontitis group and gingivitis group had *E. gingivalis*, and only 8% of healthy group infected with *E. gingivalis*. This could be indicate the role of *E. gingivalis* in pathogenesis and progression of periodontal disease. However, some studies showed no significant correlation between periodontal disease and the presence of *E. gingivalis* [3].

In the present study, the occurrence *E. gingivalis* infection was reported in only 8% of the healthy individuals group. This results were compatible with the results from Iraq where the prevalence of *E. gingivalis* was 8% among healthy individuals [19], and India, with a rate of 4% among healthy controls [20]. Since, *E. gingivalis* detected in the buccal cavity of healthy individuals, some researchers suppose that this parasite could be opportunistic, that is, capable of proliferating in a gingival environment modified by periodontal disease [9, 13]. In addition, Hassan *et al*. [15] concluded that the high occurrence of *E. gingivalis* among diseased groups compared to the healthy group could be related to the presence of genetic variants for *E. gingivalis* rather than the immune status of the individuals. Accordingly, the result of this study could be explained by the presence of genetic proliferating variant of parasite in individuals with periodontal disease (periodontitis and gingivitis) in contrast to slowly multiplying variant in the healthy individuals group [21]. However, further studies using molecular approaches are needed to assess the molecular epidemiology of this oral parasite and to investigate whether variations in strains, have a significant contribution in health and disease [22].

Findings of this study indicated that the highest rate (23.7%) of *E. gingivalis* was found among participants aged between 40-49 years old, and the lowest rate (11.9%) was among participants aged between18-29 years old. These results are in line with the findings that *E. gingivalis* do not occur in small children and rarely found in older ones [23]. In addition, Gharavi *et al.* reported that the prevalence of *E. gingivalis* was related to an age higher than 20 years [24]. On the other hand, in agreement with our findings, Albuquerque *et al*. and Maybodi *et al.* [25] not found relationship between the age and colonization of *E. gingivalis.* The difference might be related to geographical distribution and period of study.

Regarding the gender of participants, this study showed high significant rate among males than in females for *E. gingivalis* prevalence (23.7% vs 12.5% respectively). This results agreed with [25] and differ from another study, in which both sexes were equally infected with *E. gingivalis* [24].

Although, a high prevalence rate (20.9%) was found in individuals with low-education (illiterate, primary , secondary educational level) compared with low prevalence rate (12.8%) in well-educated individuals (with university education), there was no statistically significant association between the low level of education and prevalence of the parasite(P= > 0.05). In contrast, Hamad *et al.* [26] found a positive relationship between the presence of the parasite in the mouth of persons with low level of education. Majority of the participants (68.7%) in this study were with low level of education, therefore, the slightly high prevalence of *E. gingivalis* (18.3%) reported in this study might be due to the differences in the studied population's education level.

Regarding social habits such as tooth and miswak brushing, smoking, and qat chewing, in the current study, high prevalence rate was found among those who never use tooth brush (31.8%) and the lowest was among those who twice tooth brush users (9.3%). There were

significant difference between twice tooth brush users and those who, once, sporadic and never tooth brush users (p=0.006). This could be explained by that bad oral hygiene encourages inflammation of the mucous membrane and periodontal diseases. In addition, bad oral hygiene favors the accumulation of food debris and the development of dental plaque, which is the basis for the growth of *E. gingivalis* [27].

For more than 1,000 years, Moslems all over the world were using chewing sticks (Miswak) for cleaning teeth, made from the plant Salvadora persica. For religious and cultural reasons, miswak use is firmly established and widespread in Yemen, Saudi Arabia and most other Muslim countries. It has been shown that miswak use was at least as effective as tooth brushing for reducing plaque and gingivitis, it is beneficial for prevention/treatment of periodontal disease [28].

However, there was no significant difference in the prevalence of *E. gingivalis* between miswak users and non- miswak users, which may be due to small number of miswak users (n=9) compared with non- miswak users (n=196) in this study.

The present study revealed highest prevalence rate among smokers (41.1%) as compare to non-smokers (32.1%). There were significant difference between smokers and non-smokers and occurrence of the parasite (p=<0.001). Tobacco reduce the synthesis of IgG and IgM by plasma cells, as well as the phagocytic activity and chemotactic response of gingival neutrophils, so the host's defense against bacteria in the gingival pocket is substantially impaired [29]. The use of tobacco increase the risk of periodontitis and gingivitis [30].

Qat is known also as khat or Catha edulis Forsk. Qat leaves contain more than 40 alkaloids, glycosides, tannis, terpenoids and others. Qat leaves contain alkaloids such as cathine and cathinone which have psychostimulation effect in the form of euphoria and excitement [31]. Millions of men and women practice this habit in Yemen society. The effects include periodontal tissue, keratotic white lesions, keratinization of nonkeratinized oral mucosa, epithelial dysplasia and might be a potential cause of oral malignancy especially when accompanied by alcohol and tobacco consumption [32]. Furthermore, qat consumption may affect the periodontal tissue and can cause damage to the periodontal ligament as pocketing and gum recession [33]. The result of the present study in accordance with previous works that implicated qat to enhanced periodontal disease, where qat chewers patients show higher prevalence (29%) than non-qat chewers (26%). There was significant difference between qat chewers and non-qat chewers and occurrence of diseases.

Many mechanisms exclusive to diabetes may contribute to the increased risk of infection in diabetic patients. Higher glucose level in blood and urine encourage and promote the growth and multiplication of pathogenic microorganisms [34]. There was significant difference (p=0.009) in the occurrence of *Entamoeba gingivalis* between diabetic and non-diabetic patients. This result was in agreement with Ibrahim and Abbas [35] who found a significant high rate of *E. gingivalis* in diabetic patients. On the contrary, other study found no significant difference in the occurrence of *Entamoeba gingivalis* between diabetic and non-diabetic patients, which may be due to the small sample size [16].

Conclusions:

We conclude that the higher prevalence of *E. gingivalis* in periodontal disease compared to healthy individuals may be an indicator of the potential role *E. gingivalis* in the pathogenesis of periodontal diseases.

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مقالة بحثية

مدى انتشار المتحولة اللثوية والعوامل المرتبطة بها بين مرضى التهاب اللثة والتهاب دواعم السن والأشخاص الأصحاء

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المُلخّص

هذه الدراسة أجريت لتحديد مدى انتشار طفيل المتحولة اللثوية (*Entamoeba gingivalis*) بين 300 مشارك (156 ذكور و 144 إناث) موزعون على النحو التالي: 100 مريض يعانون من التهاب ما حول السن (مجموعة التهاب ما حول السن) و 100 مريض يعانون من التهاب اللثة (مجموعة التهاب اللثة) و 100 مشارك لا يعانون من امراض اللثة أو مشاكل صحية مرتبطة بالتهاب ما حول السن (مجموعة الأصحاء). بأستعمال طريقة المسح المباشر وصبغة جمسا، بينت النتائج ان نسبة مدى الأنتشار للمتحولة اللثوية 18,3% وكانت أعلى نسبة أنتشار للطفيل هي 29% لدى المرضى المصابين بالتهاب ما حول السن يعقبها المرضى المصابين بالتهاب اللثة ب 18% ثم مجموعة الأصحاء ب 8%. أعلى نسبة أصابة (23,7%) كانت عند الفئة العمرية من 40 الى 49 سنة. وكانت الأصابة أعلى في الذكور مقارنة بالأناث مع وجود فارق معنوي. وكان مدى انتشار الطفيل شائعا لدى غير مستخدمي فرشاة الأسنان، المدخنين، الماضغون للقات والمصابون بداء السكري. وكانت هناك فروقا معنوية بين مستخدمي فرشاة الأسنان وغير المستخدمين لفرشاة الأسنان وبين المدخنين وغير المدخنين والماضغون للقات وغير الماضغون والمصابون بداء السكري والغير مصابون وبين حدوث الأصابة.

الكلمات المفتاحية: مدى الأنتشار، المتحولة اللثوية، امراض ما حول السن، عدن.

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