The Microbe 10 (2026) 100657

Contents lists available at ScienceDirect

The Microbe

journal homepage: www.sciencedirect.com/journal/the-microbe

Association of *Entamoeba gingivalis* and *Trichomonas tenax* with Periodontitis in Hepatitis-C virus-Egyptian Infected Patients: A case-control study

Dina Sabry [a,b,*], Marwa Abdallah Shaker [c], Amira Hassouna [d], Sahar ElRefai [e], Nehal Diaa [f], Ghada A. Saad [g,h], Amany M. Tawfeik [i,j], Haneya A.A. Anani [i,j], Abdelrahman M. Attia [k], Asmaa Rashad Ali [j], Gena M. El-makromy [l], Hala E. Shams El-Din [g,h]

[a] *Medical Biochemistry and Molecular Biology, Faculty of Medicine, Badr University in Cairo (BUC), Egypt*
[b] *Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Egypt*
[c] *Hepatology, Gastroenterology, and infectious disease, Faculty of Medicine, Beni Suef University, Egypt*
[d] *Faculty of Health and Environmental Sciences, School of Science, Auckland University of Technology (AUT University), New Zealand*
[e] *Oral Pathology, Department of Basic Dental Sciences, College of DentistryCollege of Dentistry, Princess Nourah Bint Abdulrahman University, Riyadh, Kingdom of Saudi Arabia*
[f] *Clinical and Chemical Pathology Department, Consultant Clinical Pathology, Cairo University Hospitals, Egypt*
[g] *Medical Parasitology Department, Faculty of Medicine, Ain Shams University, Egypt*
[h] *Medical Parasitology Department, Faculty of Medicine, Badr University in Cairo (BUC), Egypt*
[i] *Medical Microbiology & Immunology, Faculty of Medicine, Badr University in Cairo (BUC), Egypt*
[j] *Medical Microbiology & Immunology Faculty of Medicine, Al-Azhar University in Cairo for girls, (AFMG), Egypt*
[k] *Karsh Division of Gastroenterology and Hepatology, Cedars-Sinai Medical Center, Los Angeles, CA, United States*
[l] *Internal medicine, internal medicine Department, Faculty of Medicine, Badr University in Cairo (BUC), Egypt*

ARTICLE INFO

Keywords:
Entamoeba gingivalis
Trichomonas tenax
HCV
Periodontitis
Oral dysbiosis, coinfection

ABSTRACT

An imbalance in the oral microbiota, known as oral dysbiosis, can lead to periodontitis. Hepatitis C virus (HCV) infections may impact oral health through immune function, diet, and lifestyle alterations, potentially contributing to periodontal disease development. The role of Protozoa in oral dysbiosis is still poorly understood. The contribution of oral Protozoa as *Entamoeba gingivalis* and *Trichomonas tenax* in periodontitis in HCV-infected patients has not been adequately invetigated. This research explored their prevalence and association with periodontitis in HCV-infected patients compared to healthy individuals without periodontitis (infected control). Venous blood samples were drawn to measure serum ALT, AST, albumin, urea, creatinine, iron, ferritin, transferrin, and sTfR. Serum and saliva samples were used to measure HCV RNA and detect the oral parasites *Entamoeba gingivalis* and *Trichomonas tenax* using polymerase chain reaction (PCR). A total of 242 participants were enrolled in the study, divided into the following groups: HCV patients with periodontitis (n = 73), HCV patients without periodontitis (n = 47), and healthy subjects (n = 122). AST, ALT, and urea were significantly elevated in HCV-infected patients compared to the control group($p < 0.05$). However, no significant differences were observed in albumin and creatinine levels between the two groups ($p > 0.05$). Iron-related parameters were significantly lower in HCV-infected patients compared to the control groups($p < 0.05$). The prevalence of *T. tenax* and *E. gingivalis* among the HCV patients with periodontitis was 34.2 % (n = 25) and 49.3 % (n = 36), respectively, with a high statistically significant difference compared to the control groups ($p < 0.001$). While *T. tenax* positivity only demonstrated a statistically significant positive correlation with HCV copy number in saliva ($p < 0.05$)., *E. gingivalis* positivity was significantly correlated with HCV copy number in both serum and saliva samples($p < 0.05$). Following HCV treatment, the prevalence of *E. gingivalis* and *T. tenax* decreased significantly, from 49.3 % and 34.2–11.0 % and 6.8 %, respectively ($p < 0.001$). The results showed that HCV patients with periodontitis had a considerably higher prevalence of *E. gingivalis* and *T. tenax*, which significantly decreased after HCV treatment. Therefore, the conventional perception of *E. gingivalis* and *T. tenax* as harmless

* Corresponding author at: Medical Biochemistry and Molecular Biology, Faculty of Medicine, Badr University in Cairo (BUC), Egypt.
E-mail address: dinasabry@kasralainy.edu.eg (D. Sabry).

https://doi.org/10.1016/j.microb.2026.100657
Received 27 May 2025; Received in revised form 17 December 2025; Accepted 5 January 2026
Available online 5 January 2026

symbionts warrants reassessment, as their role in the pathophysiology of periodontal diseases cannot be disregarded.

Introduction

The healthy state of the oral cavity, known as oral eubiosis, is maintained by the intricate interactions of many microorganisms that includes bacteria, viruses, fungi, and protozoa (Avila et al., 2009 and Lamont et al., 2018). Oral dysbiosis refers to the disturbance of the homeostatic state of the oral microbiota (Kinane et al., 2017) where different microbes may be involved directly or indirectly in the development of oral diseases, including mucosal diseases, gingivitis, and periodontal diseases (Li et al., 2017).

Gingivitis and periodontitis are the most typical manifestations of periodontal disease, a state of chronic inflammation in the gingiva, bone, and supporting ligaments (Könönen et al., 2019). Microorganisms can move from the oral cavity to other body regions, including the lungs and gastrointestinal tract. Inflammation and laceration of the oral mucosa are entry points for oral microorganisms to enter the bloodstream and heart, resulting in severe illnesses (Kitamoto et al., 2020).

Periodontitis is considered the most common inflammatory disease of the oral cavity, affecting all age groups (Kinane et al., 2017), with a prevalence of 47 % among adults over 30 years of age in Western countries (Marcenes et al., 2013). A recent study reported a periodontitis prevalence of 42 % among U.S. adults, with 7.8 % having severe forms of the disease.

An increased prevalence of periodontal disease has been linked to several risk factors, which can be categorized into modifiable factors, such as smoking, diabetes mellitus, psychological stress, alcohol intake, and poor oral hygiene, and non-modifiable factors, such as aging and genetic susceptibility (Masumoto et al., 2019).

There has been growing interest in the potential significance of *Entamoeba gingivalis* and *Trichomonas tenax*, two parasitic fractions of the oral microbiome, in developing periodontal disease (Eslahi et al., 2021).

E. gingivalis is an anaerobic protozoan parasite that widely colonizes the healthy oral cavity with a prevalence rate of 15 %, while in periodontitis patients, the infection rate may reach 70–80 % (Bao et al., 2020; Bonner et al., 2014). This opportunistic organism lives in the gingival fringes, dental tartar, necrotic mucosa around the teeth, gingival pockets, gums, and the region surrounding the teeth (Ghabanchi et al., 2010).

In 1849, a report on *E. gingivalis* used dental plaque samples, mentioning the existence of internal vesicles and amoebic motility (Gros. 1849). Its pathogenicity was first questioned by Kartulis, (1893) and the first comprehensive study linking it to periodontitis was reported in 1914, where amoebae were found in all 46 periodontitis patients recruited for the study (Barrett, 1914).

T. tenax has occasionally been identified in salivary glands, lymph nodes, and respiratory tract infections. Previously considered a harmless commensal (Hersh, 1985), *T. tenax* was later recognized as a zoonotic parasite. It has been identified in periodontal pockets at varying prevalence rates ranging from 0 % to 94.1 %, depending on the country and detection method (Ghabanchi et al., 2010). *T. tenax* has been isolated in cases involving the salivary glands and lymph nodes or respiratory tract infections.

Periodontal disease develops when the ecological balance of the oral cavity is disturbed, and periodontal microorganisms trigger an inflammatory response. Impaired immune systems, as seen in HCV patients, can alter the pathogenic mechanisms of the disease. The World Health Organization (WHO) reported that more than 70 million individuals are HCV-infected, with a prevalence of about 1 %, with the highest prevalence reported in the Eastern Mediterranean and European regions (Organization WHO, 2020).

Oral health deterioration in HCV-infected patients may result from liver dysfunction, immunocompromised status, and a lack of motivation to seek dental care (Coates et al., 2000; Carrozzo, 2001).

This study aimed to investigate the prevalence of *E. gingivalis* and *T. tenax* in HCV-infected patients with and without periodontitis, compared to healthy controls and to correlate their association with HCV viral load and HCV treatment.

Materials and methods

Ethical statement

All subjects in the study were informed of its purpose, and written consent was collected from each participant according to the norms of the Egyptian Ministry of Higher Education, following approval from the ethical committee of FM-BSU-REC, Approval No.: FMBSUREC/03102023/Shaker.

Study design and participants

All patients were recruited from the outpatient clinic at the Tropical Medicine Department, Beni Suef University, from April 2023 to October 2023. The study included 73 HCV patients diagnosed with periodontitis during their clinical examination at the Dental Clinic, Beni Suef University Hospital before the study. These patients were identified by gingival bleeding on probing, increased probing depth, and increased tooth mobility.

Patients who had received systemic antibiotics in the last 90 days, corticosteroids or any other medications that may lead to immunocompromised state were excluded. Also, smokers, despite their smoking habits were excluded for the present study.

The control groups were divided into two groups: 47 HCV patients with no symptoms or signs of periodontitis and 122 healthy participants (healthy controls) who were selected from a population attending clinics for routine check-ups, not HCV-infected, and without periodontitis or any systemic disorders.

Questionnaire

A predesigned questionnaire was completed for each patient, including demographic data and related risk factors (e.g., age, gender, residence, level of education, and behavioral characteristics such as smoking, use of toothbrush, and mouthwash). Additionally, a history of risk factors for HCV acquisition was established.

Samples collection and processing

Two samples were collected from all HCV- infected patients with and without periodontitis and from healthy control subjects: 5 mL of venous blood, collected in an EDTA vacuum tube and transported at low temperature with minimal vibration to the laboratory within two hours, and 5 mL of saliva, collected in a sterile container. All blood and saliva samples were preserved at −80°C for molecular testing of HCV and parasitic infections (*E. gingivalis* and *T. tenax*). The HVC infected patients had started initial treatment regimens with DAA treatment. On completion of HCV treatment, another venous blood and saliva samples were collected from all the patients.

Saliva sample standardization

We collected all saliva samples under standardized conditons. All subjects were instructed to refrain from eating, drinking, toothbrushing,

chewing gum for at least 90 min prior to sampling. Whole saliva samples were collected without stimulation in the morning between 9:00–10:00 a.m. to minimize circadian variation. Participants were asked to allow saliva to accumulate in the mouth and then drool into sterile collection tubes for 5 min. Samples were stored at −20 C for further DNA extraction.

Assessment of patients' biochemical parameter levels

AST, ALT, albumin, urea, and creatinine were measured using kits from Randox Laboratories Limited (Country Antrim, UK). Iron, ferritin, and transferrin were measured using kits from DRG International Inc. sTfR was measured using Quantikine™ IVD® ELISA kits (USA R&D Systems, Inc.).

Conventional PCR assay for parasitic infections

According to the manufacturer's instructions, DNA extraction from saliva samples was performed using a Qiagen extraction kit (United States). DNA purification (A260/A280 ratio) and concentration were measured using a spectrophotometer (dual wavelength Beckman, United States). The extracted and purified DNA samples were stored at −80°C. Primers were utilized to detect oral parasites using PCR. For *E. gingivalis*, the forward primer was 5′-AGGAATGAACGGAACGTACA-3′, and the reverse primer was 5′-CCATTTCCTTCTTCTATTGTTTMAC-3′, producing a 203 bp product in the 18S ribosomal RNA region (GenBank accession number: KX061779.1) **(**Bonner et al., 2014**).** For *T. tenax*, the forward primer was 5′-AGTTCCATCGATGCCATTC-3′, and the reverse primer was 5′-GCATCTAAGGACTTAGACG-3′, producing a 776 bp product in the 18S ribosomal RNA region (GenBank accession number: AY247748). The PCR mix included 5 μL DNA eluate, 5 μL 2X SuperFi™ PCR Master Mix (Thermo Fisher Scientific, USA), 1 μL of each primer, and 13 μL DNase/RNase-free water. Samples were processed in a PCR thermal cycler (Applied Biosystem, Foster City, USA). PCR conditions were as follows: initial denaturation at 94°C for 3.5 min, 40cycles of 45 s at 94°C for denaturation, 45 s at 60°C for annealing, 1 min at 72°C for elongation, followed by a final elongation at 72°C for 5 min **(**Bonner et al., 2014**)**. Nuclease-free water was used as a negative control to ensure DNA quality and exclude contamination. Positive controls were patient samples containing motile Trichomonas and *Entamoeba* that produced PCR results of the expected sizes. The PCR product size was 265 bp. The housekeeping gene β-actin (accession number: NG_007992) was used to verify PCR integrity, with forward primer 5′-GTCCTGTGGCATCCACGAAA-3′ and reverse primer 5′-AGTGAGGACCCTGGATGTGAC-3′. Amplified DNA was analyzed using 2 % agarose gel electrophoresis, visualized under a UV trans-illuminator, photographed, and compared with a molecular weight DNA ladder for interpretation.

HCV-RNA extraction and quantitative PCR detection

HCV RNA was isolated from 140 μL of serum and equal saliva volume using the QIAamp Viral RNA mini kit (Qiagen, Hilden, Germany). RNA concentration was quantified using an external standard curve (HCV Standards IU/mL) and an internal positive control. Quantitative reverse transcription PCR was performed using the AgPath-ID™ One-Step TaqMan Universal RT-PCR kit (Applied Biosystems, Foster City, CA, USA), duplicating each sample run. The RT-PCR mixture was incubated for 2 min at 55°C, followed by 10 min at 45°C to facilitate reverse transcriptase-mediated cDNA synthesis, and 10 min at 95°C to activate AmpliTaq Gold. The PCR cycling program consisted of 45 cycles of 15 s at 95°C and 45 s at 60°C (universal conditions).

Statistical analysis

Data were analyzed using R version 3.6.3. Categorical data were presented as frequencies and proportions and compared using the chi-square test. Continuous data were presented as medians with inter-quartile ranges and compared using the Mann–Whitney *U* test. The Wilcoxon signed-rank and McNemar tests were used to compare conditions before and after treatment. Spearman's correlation was employed to assess the correlation of *E. gingivalis* and *T. tenax* with HCV copy numbers in serum and saliva. A p-value of < 0.05 was considered statistically significant, with $p < 0.001$ considered highly significant **(**Team RDC, 2010**).**

Results

Demographic and clinical characteristics

The study included 242 participants divided into three groups: 73 HCV-infected patients with periodontitis (cases), 47 HCV-infected patients without periodontitis (HCV-infected control group), and 122 healthy controls. The median age differed significantly across the groups, with the HCV-infected group without periodontitis being the oldest (47 years), compared to the HCV-infected with periodontitis (44 years) and healthy controls (32.5 years) ($p < 0.001$). Key biochemical markers, including AST, ALT, urea, serum iron, TIBC, serum ferritin, transferrin, and sTfR, showed significant differences among the groups ($p < 0.001$), whereas albumin and creatinine levels showed no significant differences ($p > 0.05$) (Table 1).

Liver function markers

HCV-infected patients with periodontitis had significantly elevated levels of AST and ALT compared to HCV-infected patients without periodontitis and healthy controls ($p < 0.001$) **(**Table 1**).**

Iron-related parameters

HCV-infected patients with periodontitis had significantly lower median serum iron levels (42 μg/dL) compared to the HCV-infected without periodontitis group (45 μg/dL) and healthy controls (130.5 μg/dL) ($p < 0.001$). TIBC median levels were significantly lower in HCV-infected patients with periodontitis (230 μg/dL) than in healthy controls (256 μg/dL) ($p < 0.001$), though higher than in HCV-infected patients without periodontitis (200 μg/dL) ($p < 0.001$). sTfR median levels were significantly higher in the HCV-infected without periodontitis group (22 nmol/L) compared to HCV-infected patients with periodontitis (14 nmol/L) and healthy controls (11.5 nmol/L) ($p < 0.001$). Serum ferritin levels were higher in the HCV-infected without periodontitis group (210 ng/mL) compared to HCV-infected patients with periodontitis (160 ng/mL) and healthy controls (197 ng/mL) ($p < 0.001$) **(**Table 1**).**

HCV RNA levels

The median HCV RNA levels in HCV-infected patients with periodontitis (760,000 [300,000, 2411,589.00]) and the HCV-infected control group without periodontitis (1100,000 [122,936.50, 2000,000]) showed no statistically significant difference ($p = 0.733$). However, salivary HCV RNA levels were significantly lower in the HCV-infected control group without periodontitis (16,000 [3288.50, 63,126.00]) compared to HCV-infected patients with periodontitis (100,000 [33,118.00, 500,000]) ($p < 0.001$) (Table 1). HCV RNA was undetectable in the healthy control group.

Detection of E. gingivalis and T. tenax among the studied groups

PCR testing was conducted for all participants ($n = 242$). The prevalence of *T. tenax* among HCV patients with periodontitis was 34.2 % (25 out of 73 patients), with a highly significant difference compared to

Table 1
Characteristics of the study population.

Variable	Level	Overall (n = 242)	Case (n = 73)	HCV infected with no Periodontitis control group (n = 47)	Control (n = 122)	P-value
Group (%)	Case	73 (30.2)				
	HCV infected with no Periodontitis control group	47 (19.4)				
	Healthy control	122 (50.4)				
AGE (median [IQR])		40 [30.00, 49.00]	44 [36.00, 50.00]	47 [40.00, 53.00]	32.50 [26.00, 40.75]	< 0.001
AST 40 (median [IQR])		33 [25.00, 44.75]	52 [39.00, 73.00]	41 [33.00, 49.00]	28.00 [23.00, 32.00]	< 0.001
ALT 40 (median [IQR])		33 [26.00, 45.75]	58 [43.00, 83.00]	40 [31.50, 48.00]	28.00 [24.00, 32.00]	< 0.001
Albumin (median [IQR])		4.2 [3.80, 4.50]	4.2 [3.90, 4.40]	4.2 [3.70, 4.70]	4.15 [3.80, 4.50]	0.968
Urea (median [IQR])		34 [26.00, 40.00]	40 [37.00, 42.00]	40 [35.00, 42.00]	26.00 [23.00, 31.00]	< 0.001
Creatinine (median [IQR])		0.9 [0.70, 1.20]	0.9 [0.75, 1.10]	0.9 [0.70, 1.15]	0.90 [0.60, 1.20]	0.969
S iron µg dl (median [IQR])		67 [42.25, 130.75]	42 [37.00, 49.00]	45 [39.00, 52.50]	130.50 [110.00, 154.50]	< 0.001
TIBC µg dl (median [IQR])		240 [210.00, 285.00]	230 [202.00, 284.00]	200 [152.50, 242.50]	256.00 [234.00, 287.75]	< 0.001
S ferritin ng mL (median [IQR])		180 [155.00, 210.00]	160 [126.00, 177.00]	210 [165.00, 293.50]	197.00 [170.00, 215.00]	< 0.001
Transferrin mg dl (median [IQR])		270 [220.25, 324.00]	250 [197.00, 310.00]	250 [225.00, 266.00]	295.00 [251.50, 353.00]	< 0.001
sTfR nmol L (median [IQR])		13 [11.50, 20.30]	14 [13.50, 23.50]	22 [19.80, 28.00]	11.50 [10.50, 12.10]	< 0.001
HCV RNA (median [IQR])		880000 [2e+ 05, 2277,960.00]	760000 [300000, 2411,589.00]	1100000 [122,936.50, 2000000]	NA [NA, NA]	0.733
HCV RNA saliva (median [IQR])		63000 [8387.75, 238,734.25]	100000 [33,118.00, 500000]	16000 [3288.50, 63,126.00]	NA [NA, NA]	< 0.001

HCV-infected patients without periodontitis and healthy controls ($p < 0.001$). *E. gingivalis* was detected in 36 HCV-infected patients with periodontitis, with a prevalence rate of 49.3 %, showing a highly significant difference compared to HCV-infected patients without periodontitis and healthy controls ($p < 0.001$) **(**Table 2 **and** Fig. 1**).**

Correlation of T. tenax and E. gingivalis with HCV copy number in serum and saliva

A statistically significant positive correlation was found between the prevalence of *T. tenax* and HCV copy number in saliva (correlation coefficient = 0.214, $p = 0.009$). However, no significant correlation was observed between *T. tenax* and HCV copy number in serum samples (correlation coefficient = 0.076, $p = 0.363$). For *E. gingivalis*, there was a statistically significant positive correlation with HCV copy numbers in both serum and saliva samples, with correlation coefficients of 0.216 and 0.200, respectively ($p < 0.05$) **(**Table 3**).**

Impact of HCV treatment on clinical and biochemical parameters

The study evaluated the effect of HCV treatment with DAA on clinical and biochemical parameters in 73 HCV-infected patients with periodontitis. AST and ALT levels were significantly reduced after treatment. Median AST levels decreased from 52.00 [39.00, 73.00] before treatment to 42.00 [32.00, 57.00] after treatment ($p = 0.001$), and median ALT levels decreased from 58.00 [43.00, 83.00] to 55.00 [32.00, 68.00] ($p = 0.033$), indicating improved liver function (Table 4). No significant differences were observed in albumin, urea, creatinine, serum iron, serum ferritin, and sTfR levels ($p > 0.05$). TIBC levels significantly decreased from 230.00 [202.00, 284.00] before treatment to 210.00 [187.00, 245.00] after treatment ($p = 0.007$). Transferrin levels significantly decreased from 250.00 [197.00, 310.00] to 200.00 [175.00, 285.00] ($p = 0.012$) (Table 4), suggesting changes in iron metabolism due to HCV treatment. HCV RNA levels decreased significantly after one month treatment, with median levels dropping from 760,000 [300,000, 2411,589.00] to 170,000 [22,456.00, 442,218.00] ($p < 0.001$), and a similar reduction was observed in salivary HCV RNA levels from 100,000 [33,118.00, 500,000] to 18,000 [4800.00, 65,000.00] ($p < 0.001$) **(**Table 4**).**

Impact of HCV treatment on the prevalence of T. tenax and E. gingivalis in saliva

After HCV interferon treatment, a significant reduction in HCV copy numbers in both serum and saliva samples was observed. The prevalence of *T. tenax* decreased from 34.2 % to 6.8 %, and *E. gingivalis* decreased from 49.3 % to 11.0 %, both showing statistically significant reductions ($p < 0.001$) after treatment (Table 5).

Discussion

Hepatitis C virus (HCV) infection remains a significant global health

Table 2
Prevalence of *E. gingivalis* and *T. tenax* among the studied groups by PCR.

Oral Parasite	HCV patients with periodontitis (Cases) N (%)	HCV patients without periodontitis (infected control) N (%)	Healthy subjects (Controls) N (%)	P-value
E. gingivalis	36 (49.3)	0 (100)	0 (100)	**< 0.001**
T. tenax	25 (34.2)	0 (100)	0 (100)	**< 0.001**

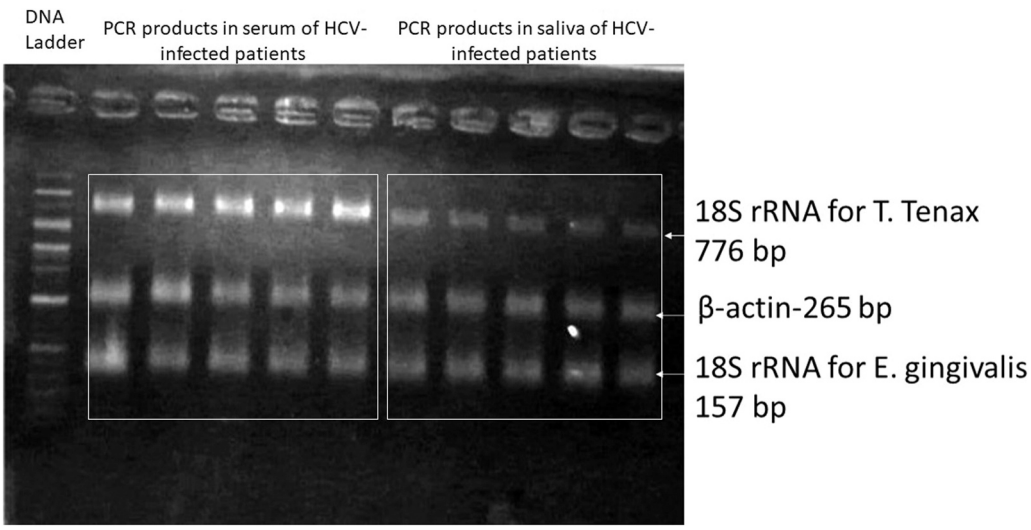

Fig. 1. Agarose gel electrophoresis showed PCR product of (lanes 1–6) of all studied genes (18S rRNA for T. Tenax −776 bp, 18S rRNA for E. gingivalis-157 bp parasitic infections detected) in HCV-infected patients' were normalized versus β-actin-265 bp as house keeping gene. These quantitative PCR products were present in both serum (lanes 1–5) and saliva (lanes6–10) of HCV infected patients.

Table 3
Correlation of *E. gingivalis* and *T. tenax* with HCV copy number in serum and saliva.

		HCV copy No. in **serum**	HCV copy No. in **saliva**
T. tenax	Correlation Coefficient	0.075	0.214
	P-value	**0.36**	**0.009**
E. gingivalis	Correlation Coefficient	0.216	0.2
	P-value	**0.008**	**0.01**

concern despite recent efforts to raise awareness about transmission and treatment options. Severe periodontitis, the sixth most prevalent disease worldwide, affects millions and requires public health attention due to its considerable dental and systemic repercussions (Marcenes et al., 2013). The impact of HCV infection on oral health may stem from immune dysfunction, liver disease, or reduced motivation to seek dental care (Carrozzo, 2001).

While extensive research has explored the bacterial component of the oral microbiome and its involvement in the etiology of periodontitis (Teles et al., 2013), the protozoan fraction has not been studied as extensively (Eslahi et al., 2021; Santi-Rocca, 2020; Bisson et al., 2019). Nonetheless, this protozoan presence appears significant in specific clinical settings, as evidenced by *E. gingivalis* RNA constituting up to 9 % of total RNA in periodontal pockets (Deng et al., 2017). Thus, further research is warranted to assess the human oral "protozoome" role in periodontitis, particularly when it is associated with chronic diseases such as HCV infection. Divergent views exist regarding the effect of HCV infection on oral health, with immune system weakening, liver dysfunction, and reduced motivation for dental care being potential causes of oral deterioration in infected patients (Carrozzo, 2008).

Numerous studies have discussed the impact of HCV infection on the oral cavity, focusing on pathological alterations to teeth, oral microbiota dysbiosis, and other extrahepatic manifestations (EHMs) with oral components (Alavian et al., 2013).

In this study, we investigated 242 individuals using HCV real-time PCR. Seventy-three HCV-infected patients with clinically diagnosed periodontitis, characterized by gingival bleeding on probing, increased probing depth, and increased tooth mobility, were included. Immunocompromised individuals and patients who had received systemic antibiotics within the last 90 days were excluded. Forty-seven HCV-infected patients without periodontitis were included as the infected control group, alongside 122 healthy individuals recruited from a population undergoing routine check-ups. HCV RNA was detected in HCV-infected patients and their saliva, with a median viral load of 760,000 [300,000, 2411,589.00], while no HCV RNA was detected in the healthy control group.

Han et al. highlighted the synergy between periodontal and liver

Table 4
Impact of HCV treatment on clinical and biochemical parameters of HCV infected with periodontitis patients (cases).

Variable	Level	Before HCV Treatment (n = 73)	After HCV treatment (n = 73)	P-value
AST 40 (median [IQR])	52.00	52 [39.00, 73.00]	42 [32.00, 57.00]	0.001
ALT 40 (median [IQR])	58.00	58 [43.00, 83.00]	55 [32.00, 68.00]	0.033
Albumin (median [IQR])	4.20	4.2 [3.90, 4.40]	4.1 [4.00, 4.50]	0.72
Urea (median [IQR])	40.00	40 [37.00, 42.00]	40 [35.00, 40.00]	0.199
Creatinine (median [IQR])	0.90	0.9 [0.75, 1.10]	0.89 [0.80, 1.00]	0.895
S iron μg dl (median [IQR])	42.00	42 [37.00, 49.00]	41 [37.00, 48.00]	0.499
TIBC μg dl (median [IQR])	230.00	230 [202.00, 284.00]	210 [187.00, 245.00]	0.007
S ferritin ng mL (median [IQR])	160.00	160 [126.00, 177.00]	160 [121.00, 177.00]	0.651
Transferrin mg dl (median [IQR])	250.00	250 [197.00, 310.00]	200 [175.00, 285.00]	0.012
sTfR nmol L (median [IQR])	14.00	14 [13.50, 23.50]	14 [13.00, 22.40]	0.107
HCV RNA (median [IQR])	760000.00	760000 [300000, 2411,589.00]	170000 [22,456.00, 442,218.00]	< 0.001
HCV RNA saliva (median [IQR])	100000.00	1000005 [33,118.00, 500000]	18000 [4800.00, 65,000.00]	< 0.001

Table 5
Prevalence of *T. tenax* and *E. gingivalis* in saliva post HCV treatment.

		Before HCV treatment (n = 73)	After HCV treatment (n = 73)	P-value
HCV RNA (median [IQR]) in serum		760000	170000	**< 0.001**
HCV RNA (median [IQR]) in saliva		1000005	18000	**< 0.001**
***T. tenax* N (%)**	Positive	25 (34.2)	5 (6.8)	**< 0.001**
	Negative	48 (65.8)	68 (93.2)	
***E. gingivalis* N (%)**	Positive	36 (49.3)	8 (11.0)	**< 0.001**
	Negative	37 (50.7)	65 (89.0)	

disorders, aside from viral hepatitis, underscoring the potential combined impact of periodontal and hepatic inflammation (Han et al., 2016).

In a study involving 87 patients aged 35–44, oral health issues in HCV-positive individuals were investigated. The DMFT (Decayed, Missing, Filled Teeth) index indicated that the dental pathological alterations were three times more significant in HCV-infected individuals than in the control group. HCV-positive patients reported more missing teeth than the control group, although they had fewer dental fillings (Coates et al., 2000).

Studies suggest that metabolic syndrome and chronic inflammatory processes may contribute to insulin resistance in chronic HCV. These pathogenic pathways, particularly the production of pro-inflammatory cytokines such as TNF-alpha, adiponectin, and IL-6, are involved in the initiation and progression of both periodontal disease and insulin resistance (Martinez-Herrera et al., 2017; Serfaty and Capeau, 2009).

The liver plays a central role in regulating various aspects of immune function, including the generation of protective cells and the non-specific immune response that leads to persistent inflammation. Chronic liver inflammation impairs immune defenses, affecting neutrophil phagocytosis, adhesion, mobility, and complement system activity, which supports the antibody-cell defense mechanism. The complement system's interaction with neutrophils is crucial for defending against periodontal pathogens (Hajishengallis et al., 2015).

Periodontal disease arises when the ecological balance of the oral cavity is disrupted, leading periodontal microorganisms to trigger an inflammatory response. This inflammation becomes chronic, resulting in the degradation of periodontal tissues. An impaired immune system can alter the disease's pathogenic process, shifting the oral microbiota and producing an inadequate inflammatory response (Silva et al., 2015). Increasing evidence suggests that imbalances in microbiota-immunity interactions may contribute to a wide range of immune-mediated illnesses across the body. However, the onset and progression of this dysbiosis in different organs remain unclear.

Chronic HCV infection is linked to a dysbiotic oral microbiome characterized by an abundance of certain organisms. Following HCV clearance through direct-acting antivirals (DAAs), the oral microbiome shifts toward a healthier composition (Gamal-AbdelNaser et al., 2023).

Bajaj et al. (2016) demonstrated that gut dysbiosis and a pro-inflammatory systemic environment persist in HCV cirrhosis, even after sustained virologic response (SVR), potentially affecting recovery rates. Heidrich et al. (2018) found that both liver disease stage and HCV infection are associated with reduced alpha diversity and distinct microbial community patterns. These differences may result from direct interactions between HCV and the microbiota or indirect effects via the immune system.

In the present study, we investigated the prevalence of two oral protozoa, *E. gingivalis* and *T. tenax*, and their potential relation to periodontitis in HCV patients before and after antiviral treatment, in comparison to HCV-infected individuals without periodontitis and healthy controls. We utilized molecular detection of the parasites in both saliva and dental samples, similar to the method used by Bao et al. (2020) to increase sensitivity. Previous studies typically used only dental samples for molecular detection (Bonner et al., 2014; Santi-Rocca, 2020), while others relied on microscopy of fresh wet mounts or stained smears (Ghabanchi et al., 2010; Hassan et al., 2019). In this study, we implemented highly sensitive PCR protocols, enhanced by a silica column-based DNA extraction method, eliminating PCR inhibitors for improved detection sensitivity.

Our study showed a relatively high prevalence of *E. gingivalis* (49.3 %) in HCV patients with periodontitis, with a significant difference compared to HCV-infected patients without periodontitis and healthy controls. This prevalence aligns with Gharavi et al. (2006), who reported a prevalence rate of 41.7 % among patients referred to the Faculty of Dentistry in Tehran. Yaseen et al. (2021) observed an even higher prevalence (87.4 %) (Badri et al., 2021) among individuals with periodontal disease, while other studies report rates ranging from 30 % to 80 % (Garcia et al., 2018). However, lower prevalence rates have been documented, from 0.5 % (Maraghi et al., 2012) to 18 % in adolescents with periodontal diseases (Ghabanchi et al., 2010).

Regarding *T. tenax*, the prevalence (34.2 %) was lower than that of *E. gingivalis* in HCV patients, though still significantly higher than in HCV-infected individuals without periodontitis and healthy controls. Numerous studies, including Meabed and Henin (2022), have similarly documented higher prevalence rates for *E. gingivalis* than for *T. tenax*. Ozumba et al. (2004) found *E. gingivalis* more common than *T. tenax* in Nigeria. In a recent study conducted in Iraq, the prevalence of *E. gingivalis* and *T. tenax* was reported as 20 % and 18.9 %, respectively, among diabetic patients and 3.3 % and 5.4 % among those with renal disease (Malaa et al., 2022).

The variable reports on the prevalence of these oral parasites may be attributed to study location, sample size, and selection criteria. Moreover, many prior studies relied on microscopy for parasite detection, with results influenced by examiner skill, microscopy type (light vs. phase-contrast), staining techniques, mounting media, and the delay between sample collection and examination, all affecting parasite motility. These factors were previously cited as potential explanations for result inconsistencies (Bonner et al., 2014).

No significant differences were observed in albumin, urea, creatinine, serum iron, serum ferritin, or soluble transferrin receptor levels after HCV treatment ($p > 0.05$). However, total iron-binding capacity (TIBC) decreased significantly from 230.00 [202.00, 284.00] before treatment to 210.00 [187.00, 245.00] after treatment ($p = 0.007$). Similarly, transferrin levels showed a significant reduction from 250.00 [197.00, 310.00] to 200.00 [175.00, 285.00] post-treatment ($p = 0.012$). These findings may indicate alterations in iron metabolism associated with HCV treatment.

After HCV treatment, the prevalence of *E. gingivalis* and *T. tenax* decreased markedly from 49.3 % and 34.2–11 % and 6.8 %, respectively. To the best of the authors' knowledge, this is the first study investigating the prevalence of these oral parasites in HCV patients in Egypt. However, other studies have linked these parasites to chronic diseases such as diabetes, with 80 % and 70 % of diabetics in one study testing positive for *E. gingivalis* and *T. tenax*, respectively. The parasitic infection was significantly associated with HbA1c levels (Meabed and Henin, 2022).

In the same context, a study on patients with chronic renal disease found that 66.7 % and 24.2 % of hemodialysis participants were infected with *T. tenax* and *E. gingivalis*, respectively (Azadbakht et al., 2023). Conversely, another study found no significant difference in the prevalence of *T. tenax* among kidney transplant patients, diabetics, and rheumatoid arthritis patients compared to healthy individuals with properly functioning immune systems (Dybicz et al., 2018). The authors attributed this to medications that may inhibit *T. tenax* infections, preventing an increased risk of oral trichomoniasis.

The elevated prevalence of these oral parasites in individuals with periodontal disease suggests that they may be more than mere markers

of the condition, potentially correlating with disease severity and progression. The conventional perception of *E. gingivalis* and *T. tenax* as harmless symbionts requires reassessment, as their role in periodontal disease pathophysiology cannot be overlooked.

Limitations of the study

-The cross-sectional design of this study limits its ability to allow conclusions about causality between HCV infection, periodontitis, and oral parasitic colonization. Also, the study's limitations included omitting inflammatory marker measurements, such as TNF-alpha. The absence of a non-HCV periodontitis group made it difficult to isolate the individual risk factors associated with HCV infection and periodontitis and parasitic infection.

-Although antiviral treatment may modulate systemic immune function, we cannot exclude the possibility that improved oral hygiene practices may have contributed to the reduction in parasite positivity.

Conclusion

HCV infection contributes to oral health deterioration through various mechanisms. *E. gingivalis* and *T. tenax* were significantly more prevalent in HCV patients compared to healthy controls, with a notable reduction in prevalence following HCV treatment.

CRediT authorship contribution statement

Nehal Diaa: Formal analysis. **Sahar ElRefai:** Formal analysis, Data curation. **Tawfeik Amany:** Validation, Supervision, Project administration, Investigation. **Saad Ghada:** Conceptualization. **Attia Abdelrahman:** Software, Resources, Formal analysis. **Anani Haneya:** Validation, Resources, Data curation. **Ali Asmaa:** Data curation. **Shams El-Din Hala:** Methodology, Investigation, Data curation, Conceptualization. **Dina Sabry:** Resources, Methodology, Investigation, Conceptualization. **El-makromy Gena:** Data curation, Conceptualization. **Amira Hassouna:** Writing – original draft, Supervision, Resources, Formal analysis, Conceptualization. **Shaker Marwa:** Writing – original draft, Visualization, Formal analysis, Data curation.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Alavian, S.-M., Mahboobi, N., Mahboobi, N., Karayiannis, P., 2013. Oral conditions associated with hepatitis C virus infection. Saudi J. Gastroenterol. Off. J. Saudi Gastroenterol. Assoc. 19 (6), 245. https://doi.org/10.4103/1319-3767.121032.

Avila, M., Ojcius, D.M., Yilmaz, Ö., 2009. The oral microbiota: living with a permanent guest. DNA Cell Biol. 28 (8), 405–411. https://doi.org/10.1089/dna.2009.0874.

Azadbakht, K., Baharvand, P., Al-Abodi, H.R., Yari, Y., Hadian, B., Fani, M., et al., 2023. Molecular epidemiology and associated risk factors of oral cavity parasites in hemodialysis patients in western Iran. J. Parasit. Dis. 47 (1), 146–151. https://doi.org/10.1007/s12639-022-01551-w.

Badri, M., Olfatifar, M., Abdoli, A., Houshmand, E., Zarabadipour, M., Abadi, P.A., et al., 2021. Current global status and the epidemiology of *Entamoeba gingivalis* in humans: a systematic review and meta-analysis. Acta Parasitol. 66 (4), 1102–1113. https://doi.org/10.1007/s11686-021-00423-2.

Bajaj, J.S., Sterling, R., Betrapally, N., Nixon, D., Fuchs, M., Daita, K., et al., 2016. HCV eradication does not impact gut dysbiosis or systemic inflammation in cirrhotic patients. Aliment. Pharmacol. Ther. 44 (6), 638–643. https://doi.org/10.1111/apt.13732.

Bao, X., Wiehe, R., Dommisch, H., Schaefer, A., 2020. *Entamoeba gingivalis* causes oral inflammation and tissue destruction. J. Dent. Res. 99 (5), 561–567. https://doi.org/10.1177/0022034520901738.

Barrett, M., 1914. The protozoa of the mouth in relation to pyorrhea alveolaris. Dent. Cosm. 56, 948–953.

Bisson, C., Dridi, S.-M., Machouart, M., 2019. Assessment of the role of *Trichomonas tenax* in the etiopathogenesis of human periodontitis: A systematic review. PloS One 14 (12), e0226266. https://doi.org/10.1371/journal.pone.0226266.

Bonner, M., Amard, V., Bar-Pinatel, C., Charpentier, F., Chatard, J.-M., Desmuyck, Y., et al., 2014. Detection of the amoeba *Entamoeba gingivalis* in periodontal pockets. Parasite 21. https://doi.org/10.1051/parasite/2014029.

Carrozzo, M., 2001. Oral health in patients with hepatitis C virus infection: an underestimated problem? Oral. Dis. 7 (5), 267–270. https://doi.org/10.1034/j.1601-0825.2001.00782.x.

Carrozzo, M., 2008. Oral diseases associated with hepatitis C virus infection. Part 1. sialadenitis and salivary glands lymphoma. Oral. Dis. 14 (2), 123–130. https://doi.org/10.1111/j.1601-0825.2007.01436.x.

Coates, E., Brennan, D., Logan, R., Goss, A., Scopacasa, B., Spencer, A., et al., 2000. Hepatitis C infection and associated oral health problems. Aust. Dent. J. 45 (2), 108–114. https://doi.org/10.1111/j.1834-7819.2000.tb00249.x.

Deng, Z.-L., Szafrański, S.P., Jarek, M., Bhuju, S., Wagner-Döbler, I., 2017. Dysbiosis in chronic periodontitis: key microbial players and interactions with the human host. Sci. Rep. 7 (1), 3703. https://doi.org/10.1038/s41598-017-03804-8.

Dybicz, M., Perkowski, K., Sedzikowska, A., Baltaza, W., Chomicz, L., 2018. Studies on prevalence of infection with *Trichomonas tenax* identified by molecular techniques-in respect to oral health of patients with various systemic disease requiring immunosuppressive therapy. Ann. Parasitol. 64 (3). https://doi.org/10.17420/ap6403.151.

Eslahi, A.V., Olfatifar, M., Abdoli, A., Houshmand, E., Johkool, M.G., Zarabadipour, M., et al., 2021. The neglected role of *Trichomonas tenax* in oral diseases: a systematic review and meta-analysis. Acta Parasitol. 1–18.

Gamal-AbdelNaser, A., Mohammed, W.S., ElHefnawi, M., AbdAllah, M., Elsharkawy, A., Zahran, Fh.M., 2023. The oral microbiome of treated and untreated chronic HCV infection: A preliminary study. Oral. Dis. 29 (2), 843–852. https://doi.org/10.1111/odi.14007.

Garcia, G., Ramos, F., Maldonado, J., Fernandez, A., Yáñez, J., Hernandez, L., et al., 2018. Prevalence of two *Entamoeba gingivalis* ST1 and ST2-kamaktli subtypes in the human oral cavity under various conditions. Parasitol. Res. 117, 2941–2948. https://doi.org/10.1007/s00436-018-5990-8.

Ghabanchi, J., Zibaei, M., Afkar, M.D., Sarbazie, A., 2010. Prevalence of oral *Entamoeba gingivalis* and *Trichomonas tenax* in patients with periodontal disease and healthy population in Shiraz, southern Iran. Indian J. Dent. Res. 21 (1), 89–91. https://doi.org/10.4103/0970-9290.62821.

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. Iran. J. Parasitol. 1 (1), 43–46.

Gros G. Fragments d'helminthologie et de physiologie microscopique1849.

Hajishengallis, G., Chavakis, T., Hajishengallis, E., Lambris, J.D., 2015. Neutrophil homeostasis and inflammation: novel paradigms from studying periodontitis. J. Leukoc. Biol. 98 (4), 539–548. https://doi.org/10.1189/jlb.3VMR1014-468R.

Han, P., Sun, D., Yang, J., 2016. Interaction between periodontitis and liver diseases. Biomed. Rep. 5 (3), 267–276. https://doi.org/10.3892/br.2016.718.

Hassan, S.S., Madkour, G.G., Henin, R.W., Gad, S.W.F., El-Aal, A., 2019. Is *Entamoeba gingivalis* a risk factor for periodontal diseases? A case-control study. Perio J. 3 (1), 18–28. https://doi.org/10.26810/perioj.2019.a3.

Heidrich, B., Vital, M., Plumeier, I., Döscher, N., Kahl, S., Kirschner, J., et al., 2018. Intestinal microbiota in patients with chronic hepatitis C with and without cirrhosis compared with healthy controls. Liver Int. 38 (1), 50–58. https://doi.org/10.1111/liv.13485.

Hersh, S., 1985. Pulmonary trichomoniasis and Trichomonas tenax. J. Med. Microbiol 20 (1), 1–10. https://doi.org/10.1099/00222615-20-1-1.

Kartulis, S., 1893. Muskelparasiten beim Menschen. Z. Hyg. Infekt. 13 (2).

Kinane, D.F., Stathopoulou, P.G., Papapanou, P.N., 2017. Periodontal diseases. Nat. Rev. Dis. Prim. 3 (1), 1–14. https://doi.org/10.1038/nrdp.2017.38.

Kitamoto, S., Nagao-Kitamoto, H., Hein, R., Schmidt, T., Kamada, N., 2020. The bacterial connection between the oral cavity and the gut diseases. J. Dent. Res. 99 (9), 1021–1029. https://doi.org/10.1177/0022034520924633.

Könönen, E., Gursoy, M., Gursoy, U.K., 2019. Periodontitis: a multifaceted disease of tooth-supporting tissues. J. Clin. Med. 8 (8), 1135. https://doi.org/10.3390/jcm8081135.

Lamont, R.J., Koo, H., Hajishengallis, G., 2018. The oral microbiota: dynamic communities and host interactions. Nat. Rev. Microbiol 16 (12), 745–759. https://doi.org/10.1038/s41579-018-0089-x.

Li, F., Zhu, C., Deng, F.-Y., Wong, M.C.M., Lu, H.-X., Feng, X.-P., 2017. *Herpesviruses* in etiopathogenesis of aggressive periodontitis: A meta-analysis based on case-control studies. PloS One 12 (10), e0186373. https://doi.org/10.1371/journal.pone.0186373.

Malaa, S., Abd Aun Jwad, B., Al-Masoudi, H.K., 2022. Assessment of *Entamoeba gingivalis* and *Trichomonas Tenax* in Patients with Chronic Diseases and its Correlation with Some Risk Factors. Arch. Razi. Inst. 71 (1). https://doi.org/10.22092/ari.2021.356549.1868.

Maraghi, S., Azizi, A., Rahdar, M., Vazirianzadeh, B., 2012. A study on the frequency of buccal cavity protozoa in patients with periodontitis and gingivitis in Ahvaz, southwest of Iran in 2009. Jundishapur J. Health Sci. 4 (4), 85–89.

Marcenes, W., Kassebaum, N.J., Bernabé, E., Flaxman, A., Naghavi, M., Lopez, A., et al., 2013. Global burden of oral conditions in 1990-2010: a systematic analysis. J. Dent. Res. 92 (7), 592–597. https://doi.org/10.1177/0022034513490168.

Martinez-Herrera, M., Silvestre-Rangil, J., Silvestre, F.-J., 2017. Association between obesity and periodontal disease. A systematic review of epidemiological studies and controlled clinical trials. Med. Oral. Patol. Oral. Cir. Bucal 22 (6), e708. https://doi.org/10.4317/medoral.21786.

Masumoto, R., Kitagaki, J., Fujihara, C., Matsumoto, M., Miyauchi, S., Asano, Y., et al., 2019. Identification of genetic risk factors of aggressive periodontitis using genomewide association studies in association with those of chronic periodontitis. J. Periodontal Res. 54 (3), 199–206. https://doi.org/10.1111/jre.12620.

Meabed, E.M., Henin, R.W., 2022. Prevalence of *Entamoeba gingivalis* and *Trichomonas tenax* among patients suffering from chronic systemic diseases in Egypt. AfroEgypt. J. Infect. Endem. Dis. 12 (4), 390–401. https://doi.org/10.21608/aeji.2022.171351.1264.

Organization WHO (2020) Epidemiology of hepatitis C virus in the WHO Eastern Mediterranean Region: implications for strategic action.

Ozumba, U., Ozumba, N., Ndiokwelu, E., 2004. Oral protozoa in a Nigeria population. Afr. J. Clin. Exp. Microbiol 5 (1), 15–19. https://doi.org/10.4314/AJCEM.V5I1.7355.

Santi-Rocca, J., 2020. The Protozoome of the Periodontal Sulcus: from health to disease. October 16–18, 2019 Eukaryome Impact on Human Intestine Homeostasis and Mucosal Immunology: Overview of the First Eukaryome Congress at Institut Pasteur Paris. Springer. https://doi.org/10.1007/978-3-030-44826-4_9.

Serfaty, L., Capeau, J., 2009. Hepatitis C, insulin resistance and diabetes: clinical and pathogenic data. Liver Int. 29, 13–25. https://doi.org/10.1111/j.1478-3231.2008.01952.x.

Silva, N., Abusleme, L., Bravo, D., Dutzan, N., Garcia-Sesnich, J., Vernal, R., et al., 2015. Host response mechanisms in periodontal diseases. J. Appl. Oral. Sci. 23, 329–355. https://doi.org/10.1590/1678-775720140259.

Team RDC (2010) R: A language and environment for statistical computing. (No Title).

Teles, R., Teles, F., Frias-Lopez, J., Paster, B., Haffajee, A., 2013. Lessons learned and unlearned in periodontal microbiology, 2000 Periodontol 62 (1), 95–162. https://doi.org/10.1111/prd.12010.

Yaseen, A., Mahafzah, A., Dababseh, D., Taim, D., Hamdan, A.A., Al-Fraihat, E., et al., 2021. Oral colonization by *Entamoeba gingivalis* and *Trichomonas tenax*: A PCR-based study in health, gingivitis, and periodontitis. Front. Cell. Infect. Microbiol. 11, 782805. https://doi.org/10.3389/fcimb.2021.782805.