

Association Between the Amount of *Treponema Lecithinolyticum* in Subgingival Dental Plaque and the Severity of Periodontitis

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Abstract

The pathogenesis of periodontitis involves certain bacteria in the bacterial plaque, including *Treponema lecithinolyticum* (T1). The aim of this study was to evaluate the load of T1 in pocket periodontal disease and determine how it correlates with the severity of periodontitis.

We examined five subjects with pocket depths (PD) of 4 to 5 mm (group 1 (G1)) and ≥ 6 mm (group 2 (G2)) and subjects without periodontal disease (group 3 (G3), control). We collected 20 microbial samples (gingival crevicular fluids) from the deepest pockets (≥ 4 mm) by using a paper point. The bacterium load between the two groups was compared using a quantitative real-time polymerase chain reaction (qPCR). Statistical analysis was performed by using Mann-Whitney tests, Kruskal-Wallis test, and Spearman's correlation.

The qPCR results showed that the amount of T1 was higher in subjects with periodontitis than in healthy controls, but the difference was not statistically significant ($p \geq 0.05$). The Kruskal-Wallis test results of G1, G2, and G3 revealed that no significant differences existed. The amount of bacteria exhibited a moderately strong and positive correlation with PD and the Papilla Bleeding Index (PBI).

However, a weak correlation was found when recession and loss of attachment were analyzed. In subjects with periodontal disease, PD and PBI were associated with the amount of T1.

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Introduction

Periodontal disease is one of the two most common dental and oral diseases.¹ The prevalence of periodontitis among an Indonesian cohort aged 22 to 55 years was 83.6% (1.730 patients from 2.069).² Bacteria are the main causes of periodontal disease^{2,3}, and bacterial complexes are typically found in the subgingival space. Red complex bacteria are the most commonly associated bacterial species with periodontal disease, particularly in patients with chronic periodontitis. However, *Treponema lecithinolyticum* (T1) is also a periodontal pathogen that is associated with all stages of periodontal disease (gingivitis and periodontitis).⁴

T1 is an anaerobic gram-negative bacterium⁵⁻⁷ that is frequently detected in subgingival plaque.⁷ The cell size is $5.00 \mu\text{m} \times 0.15 \mu\text{m}$, and the organism contains two endoflagella and forms a white diffused subsurface colony with a diameter of approximately 3 mm seven days after incubation at 37°C .⁶ Therefore, this study aimed to evaluate the T1 load in periodontal pockets and determined how it correlates with the severity of periodontitis.

Materials and methods

This study received ethical approval from The Ethical Committee of Dental Research (KEPKG) of the Faculty of Dentistry of the University of Indonesia (protocol number 090220218) and was conducted in the Dental Teaching Hospital and Laboratory of Oral Biology of the Faculty of Dentistry of the University of Indonesia (RSKGM FKG UI). The clinical samples were collected from 20 of the deepest pockets of five patients (each patient had samples drawn from the four deepest pockets).

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We included subjects with periodontitis stage 2 or 3. The other inclusion criteria were pocket depth (PD) (≥ 4 mm), Papilla Bleeding Index (PBI), and loss of attachment (LoA) (≥ 2 mm).⁸⁻¹⁰ The exclusion criteria included mental disabilities, history of systemic diseases, history of bruxism or smoking, intake of drugs that can disrupt the periodontal tissue (such as cyclosporine and phenytoin)⁹, pregnancy or breastfeeding, scaling ≤ 6 months or consumption of antibiotics ≤ 3 months¹¹, and orthodontic treatment.^{12,13}

The diagnosis of periodontitis in this study was according to the American Academy of Periodontology (AAP; 2017) and included periodontitis stages 1 to 4. Stage 1 includes LoA 1 to 2 mm, radiographic bone loss in the coronal third ($<15\%$), and maximum probing depth ≤ 4 mm. Stage 2 includes interdental LoA 3 to 4 mm, radiographic bone loss in the coronal third ($15\%–33\%$), and maximum probing depth ≤ 5 mm. Stage 3 includes interdental LoA ≥ 5 mm, radiographic bone loss extending to the middle or apical third of the root, and probing depth ≥ 6 mm. Stage 4 includes interdental LoA ≥ 5 mm, radiographic bone loss extending to the middle or apical third of the root, and probing depth ≥ 6 mm; this stage is more complex than stage 3.¹⁴ In this study, only subjects with stage 2 and 3 diseases were the sources of microbiological samples. The sample category based on PD was divided into 1 to 3¹⁵, 4 to 5¹⁶, and ≥ 6 mm.¹⁴

Subjects who met the inclusion criteria were provided with study information, written informed consent, and anamnesis. We further examined the plaque index, PBI, PD, and LoA. Clinical photos and dental radiographs were also obtained. The diagnosis decision involved one periodontal consultant. In this study, microbiological samples were collected from gingival crevicular fluid (GCF) following the removal of supragingival plaque by using curettes. We then dried and isolated the tooth surface by using a cotton roll.^{10,17} Subsequently, three paper points (no. 30) were inserted into the pockets, and they were maintained in the pockets for 20 to 30 seconds.^{17,18} After the points were removed from the socket, they were placed into a sterile Eppendorf tube containing 200 μ l of tris-EDTA buffer, refrigerated, and then immediately transferred to the oral biology laboratory for further analysis. DNA extraction was performed using TRIzol™ Reagent (ThermoFisher Scientific, USA) according to the instructions

provided by the company. The extracted DNA was stored in a freezer (-20 °C) until it was subjected to the quantitative real-time polymerase chain reaction (qPCR) method.

All DNA samples were checked for purity by using spectrometry (Metertech SP-8001, Metertech Inc., Taipei, Taiwan, R.O.C). For qPCR, the PCR mix consisted of 5.0 μ l fluorescence dye (PowerUp™ SYBR™ Green Master Mix, ThermoFisher Scientific, USA), 0.5 μ l primer forward TI 0.3 μ M, 0.5 μ l primer reverse TI 0.3 μ M, 1 μ l NFW, and 3 μ l DNA pellet sample or NFW for negative control. In this study, the primer sequences used were generated from Primer-BLAST NCBI. Absolute quantification was performed by creating a standard curve by using plasmid (derived from strain OMZ 684, Pi ATCC 700332, GenScript®, USA) and by dilution to obtain a DNA concentration of 10^{-6} to 10^{-10} . Quantification assays were performed using Applied Biosystem (ThermoFisher Scientific, USA) with a thermal cycle and 10 minutes incubation at 95 °C to active the polymerase. Thereafter, 2-step amplification was performed for 15 seconds at 95 °C and 60 seconds at 60 °C for 40 cycles.⁴ Data analysis was performed by SPSS software program (SPSS, Chicago, USA).

Results

Our subjects consisted of 60% males and 40% females, with an age range of 34 to 61 years old. GCF retrieval was performed at 4 to 7 mm for subjects with periodontitis (20 periodontal pockets) and at 1 to 3 mm in healthy subjects (2 periodontal sulci). The categories for PD were 1, 2, and 3 mm, which signify periodontal health¹⁹ (total sample (s) = 2), periodontitis stage 2 (s = 13), and periodontitis stage 3 (s = 7), respectively. Figure 1 shows the standard curve of bacteria TI.

The mean quantities of TI in healthy subjects and subjects with periodontitis were 1.24×10^1 and 6.35×10^3 CFU/ml, respectively.

The Shapiro–Wilk test showed that the sample data were not normally distributed ($p \leq 0.05$); thus, non-parametric comparison tests were used. On the basis of the Mann-Whitney test, there was no significant difference between the amount of TI in the healthy sample and the sample with periodontitis (Table 1).

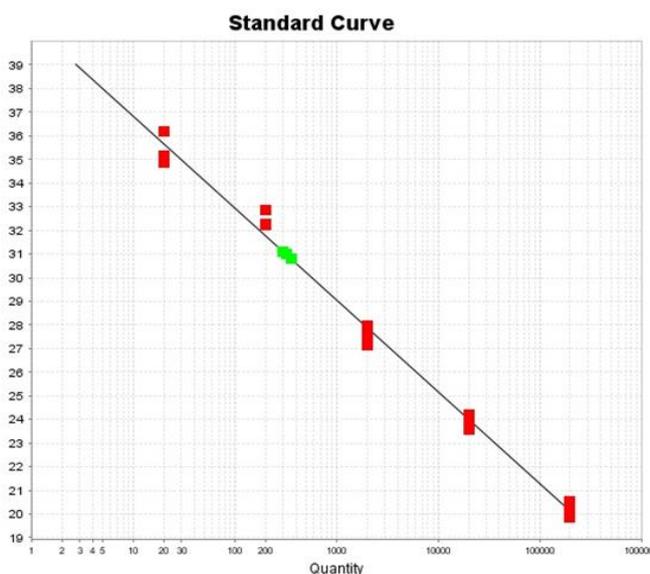


Figure 1. Standard curve used in this study (slope=-3.8; Y-intercept=40.7; $R^2=0.991$; eff=80.7%): red dots represent plasmid concentrations and green dots represent sample concentrations.

Group samples	Mean (SD) in CFU/ml	P-value
Healthy (s = 2)	1.24×10^1 (1.26×10^1)	0.110
Periodontitis (s =20)	6.35×10^3 (1.71×10^4)	

*Mann-Whitney test; SD = standard deviation; s = total sample

Table 1. Comparison of *Treponema lecithinolyticum* quantity between healthy controls and subjects with periodontitis.

Group samples	Mean (SD) in CFU/ml	P-value
G1 (s=13)	5.54×10^3 (1.87×10^3)	0.148
G2 (s = 7)	7.85×10^3 (1.48×10^4)	
G3 (s = 2)	1.24×10^1 (1.26×10^1)	

*Kruskal-Wallis test; SD = standard deviation; s = total sample; G1 = pocket depth 4 to 5 mm; G2 = pocket depth \geq 6 mm; G3 = healthy control

Table 2. Comparison of *Treponema lecithinolyticum* quantity between G1, G2, and G3.

The Kruskal-Wallis test between PD 4 to 5 mm (group (G1)), PD \geq 6 mm (group 2 (G2)), and healthy controls (group (G3)) revealed no significant differences (Table 2).

The Spearman's correlation test (Table 3) showed the following: a moderately strong and positive linear correlation existed between the quantity of TI and PD ($r = 0.466$), a weak and positive linear correlation existed between the quantity of TI and recession ($r = -0.029$), a weak correlation existed between the quantity of TI and CAL ($r = -0.170$), and a moderate linear correlation existed between PBI and the quantity of TI ($r = 0.800$).

Clinical parameters	r value	P-value
PD	0.466	0.029
Recession	-0.029	0.896
LoA	-0.170	0.448
PBI	0.800	0.104

*Spearman's correlation test; PD = pocket depth; LoA = loss of attachment; PBI = papilla bleeding index

Table 3. Correlation between *Treponema lecithinolyticum* quantity and clinical parameters.

Discussion

Periodontal disease can worsen with the addition of a periodontal pathogen in the subgingival area. Therefore, microbiological examinations are important, particularly when the potential treatment requires the administration of an antibiotic; however, whether this microbiological examination is necessary is still unknown. Microbiological examinations reveal bacteria in the subgingiva, but the major bacterial etiology of periodontal disease has not been investigated. Current microbiological studies primarily focus on classic bacterial pathogens only. Furthermore, most commercial tests for bacterial pathogens such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Fusobacterium* spp., and *Prevotella intermedia* cannot be used as diagnostic tools, and are only used to view PD.¹⁶

This study focused on the microbiological load comparisons between subjects with periodontitis and healthy samples by using TI as the targeted bacterium. The results show that subjects with periodontitis had more TI than healthy subjects. These results were similar to those of Riep et al.¹⁶ and Kumar et al.²⁰, who showed higher concentrations of TI in subjects with periodontitis compared with healthy controls. According to Scapoli et al.^{4,20}, the amount of TI was related to the occurrence of gingivitis. However, this does not mean that there should be more TI at diseased sites than at healthy sites.^{5,21} Periodontal pathogens usually only represent 1% to 5% of the colony-forming units at sites with periodontitis.²²

In this study, there were comparable amounts of TI in subjects with periodontitis and healthy subjects. This result was contrary to that of Scapoli et al.⁴ and Kumar et al.²⁰ This discrepancy could be due to the detection system used in this study. The qPCR detection system used by Scapoli et al.⁴ was the TaqMan probe; SYBR green was used in the current study. The

TaqMan probe has better specificity than SYBR green²³ but requires additional manipulation and cost.²⁴

There were no significant differences in TI between G1, G2, and G3. This result is contrary to Riep et al.¹⁶ because the samples with periodontal disease included those from subjects with chronic periodontitis and aggressive periodontitis, which is an old classification system. In the current study, we classified periodontitis as stages 2 and 3 according to the latest AAP classification scheme. Furthermore, we did not include subjects with aggressive periodontitis according to earlier classifications; however, the mean value of TI was greater in PD \geq 6 mm (G2) than in PD 4 to 5 mm (G1) and G3 because there was reduced oxygen pressure (pO₂) in the deepest pockets, thus allowing anaerobic bacteria to grow.²⁵

In this study, we found a moderate correlation between the quantity of TI and PD or PBI because bacterial invasion into periodontal tissues may lead to an inflammatory response and to the damage of periodontal tissue. Clinically, this is detected as a periodontal pocket.²⁶ A weak positive correlation between TI quantity, recession, and LoA was also seen in this study. This finding may be due to the anaerobic nature of TI bacteria: a recession of the tooth caused root surface exposure to the oral cavity, thus rendering TI unable to survive within the changed atmosphere. However, some limitations (stated above) may also have influenced the results found in this study.

Conclusions

More TI was found in subjects with periodontitis than in the healthy sample, but the difference was not statistically significant. The amount of TI was associated with PD and PBI; however, further research is necessary to confirm these results.

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Declaration of Interest

The authors declare that there are no conflicts of interest.

References

1. Petersen PE, Ogawa H. Strengthening the Prevention of Periodontal Disease: the WHO Approach. *J Periodontol* 2005;76(12):2187-93.
2. Tadjoeidin FM, Fitri AH, Kuswandani SO, Sulijaya B, Soeroso Y. The Correlation Between Age and Periodontal Diseases. *J Int Dent Med Res* 2017; 10(2):327-32.
3. Teughels W, Godts C, Quirynen, Jakubovics N. Biofilm and Periodontal Microbiology. In: Carranza FA, Forrest JL, Jepsen S, Klokkevold PR, Newman MG, Preshaw P, et al, eds. *Carranza's Clinical Periodontology*. 12th ed. St. Louis: Elsevier Inc; 2015:132-69.
4. Scapoli L, Girardi A, Palmieri A, et al. Quantitative Analysis Of Periodontal Pathogens In Periodontitis And Gingivitis. *J Biol Regul Homeost Agents* 2015; 29(3 Suppl 1):101-10.
5. Pérez-Chaparro PJ, Gonçalves C, Figueiredo LC, et al. Newly Identified Pathogens Associated with Periodontitis: A Systematic Review. *J Dent Res* 2014; 93(9):846-58.
6. Hiranmayi KV, Sirisha K, Rao MVR, Sudhakar P. Novel Pathogens in Periodontal Microbiology. *J Pharm Bioallied Sci* 2017; 9(3):155-63.
7. Wyss C, Choi BK, Schupbach P, Moter A, Guggenheim B, Gobel UB. *Treponema lecithinolyticum* sp. nov., a Small Saccharolytic Spirochete with Phospholipase A and C Activities Associated with Periodontal Diseases. *Int J Syst Bacteriol* 1999; 49:1329-39.
8. Page RC, Eke PI. Case Definitions for Use in Population-Based Surveillance of Periodontitis. *J Periodontol* 2007; 78(& Suppl):1387-99.
9. Burgess D, Huang H, Harrison P, Aukhil I, Shaddox L. *Aggregatibacter actinomycetemcomitans* in African Americans with Localized Aggressive Periodontitis. *JDR Clin Trans Res* 2017; 2(3):249-57.
10. Moter A, Riep B, Haban V, Heuner K, Siebert G, Berning M, et al. Molecular Epidemiology of Oral Treponemes in Patients with Periodontitis and in Periodontitis-Resistant Subjects. *J Clin Microbiol* 2006; 44(9):3078-85.
11. Soeroso Y, Akase T, Sunarto H, et al. The Risk Reduction of Recurrent Periodontal Pathogens of Local Application Minocycline HCL 2% Gel, Used as an Adjunct to Scaling and Root Planing for Chronic Periodontitis Treatment. *Ther Clin Risk Manag* 2017;13:307-14.
12. Chahboun H, Arnau MM, Herrera D, Sanz M, Ennibi OK. Bacterial Profile of Aggressive Periodontitis in Morocco: A Cross-Sectional Study. *BMC Oral Health* 2015;15(25):1-8.
13. Teodorescu A, Zetu L, Teslaru S, et al. Bacterial Profiles for Chronic and Aggressive Periodontitis in A Sample Population Group A Cross-Sectional Study. *Int J Med Dent* 2017;7(2):135-9.
14. Tonetti MS, Greenwell H, Kornman KS. Staging and Grading of Periodontitis: Framework and Proposal of A New Classification and Case Definition. *J Periodontol* 2018;89 Suppl 1:159-72.
15. Lang NP, Bartold PM. Periodontal Health. *J Periodontol* 2018;89 Suppl 1:9-16.
16. Riep B, Edesi-Neuß L, Claessen F, et al. Are Putative Periodontal Pathogens Reliable Diagnostic Markers? *J Clin Microbiol* 2009; 47(6):1705-11.
17. Guentsch A, Kramesberger M, Sroka A, Pfister W, Potempa J, Erick S. Comparison of Gingival Crevicular Fluid Sampling Methods in Patients with Severe Chronic Periodontitis. *J Periodontol* 2011;82(7):1051-60.

18. Rassameemasmaung S, Sirikulsathean A, Amornchat C, Maungmingsook P, Rojanapanthu P, Gritsanaphan W. Topical Application of *Garcinia Mangostana* L. Pericarp Gel as an Adjunct to Periodontal Treatment. *Complement Ther Med* 2008; 16(5):262-7.
19. Chapple ILC, Mealey BL, VanDyke TE, et al. Periodontal Health and Gingival Diseases and Conditions on an Intact and a Reduced Periodontium: Consensus Report of Workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri - Implant Diseases and Conditions. *J Periodontol* 2018;89 Suppl 1:74-84.
20. Kumar PS, Griffen AL, Barton JA, Paster BJ, Moeschberger ML, Leys EJ. New Bacterial Species Associated with Chronic Periodontitis. *J Dent Res* 2003;82(5):338-44.
21. Armitage GC, Cullinan MP. Comparison of the Clinical Features of Chronic Andaggressive Periodontitis. *Periodontol* 2000 2010;53:12-27.
22. Armitage GC. Comparison of the Microbiological Features of Chronic and Aggressive Periodontitis. *Periodontol* 2000 2010;53:70-88.
23. Tajadini M, Panjehpour M, Javanmard S. Comparison of SYBR Green and TaqMan Methods in Quantitative Real-Time Polymerase Chain Reaction Analysis of Four Adenosine Receptor Subtypes. *Adv Biomed Res* 2014;3(1):85.
24. Maeda H, Fujimoto C, Haruki Y, et al. Quantitative Real-Time PCR using TaqMan and SYBR Green for *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *tetQ* Gene and Total Bacteria. *FEMS Immunol Med Microbiol* 2003; 39(1):81-6.
25. Loesche WJ, Grossman NS. Diagnosis and Treatment Periodontal Disease as A Specific, Albeit Chronic Infection. *Clin Microbiol Rev* 2001;14(4):727-52.
26. Mombelli A. Microbial Colonization of the Periodontal Pocket and its Significance for Periodontal Therapy. *Periodontol* 2000 2018;76(1):85-96.