International Journal of Scientific Reports
Paudel S et al. Int J Sci Rep. 2020 Aug;6(8):286-291
http://www.sci-rep.com
pISSN 2454-2156 | eISSN 2454-2164

DOI: http://dx.doi.org/10.18203/issn.2454-2156.IntJSciRep20203110

Original Research Article

Detection of oral spirochete (*Treponema denticola*) by modified Fontana staining technique from sub-gingival plaques of patients suffering from periodontal disease and to analyse the morphology of spirochetes with respect to the disease's status

Sunil Paudel[1]*, Jyoti M. Nagamoti[2], Ved Prakash Mishra[3], Suraj Twanabasu[4], Susan Kusma[5]

[1]Department of Microbiology, Nobel College, Pokhara University, Sinamangal, Kathmandu, Nepal
[2]Department of Microbiology, Jawaharlal Nehru Medical College, KLE University, Belagavi, Karnataka, India
[3]Department of Public Health, Nobel College, Pokhara University, Sinamangal, Kathmandu, Nepal
[4]Department of Pathology, Vayodha Hospital, Balkhu, Kathmandu, Nepal
[5]Department of Pathology, Lalupate Medical Center Pvt Ltd, Gaushala, Kathmandu, Nepal

Received: 07 April 2020
Revised: 10 May 2020
Accepted: 12 May 2020

***Correspondence:**
Sunil Paudel,
E-mail: oryopdl04@gmail.com

ABSTRACT

Background: Infections pertaining to mankind particularly those affecting the periodontal tissues are of serious concerns worldwide and are increasing on a global scale particularly in the tropical and subtropical countries like India. The clinical presentation, though very typical of periodontal infection, is often confused with other oral infection, making laboratory diagnosis and confirmation necessary. The management of periodontal infections needs personal hygiene, awareness of infection, proper diagnosis and medication. The present study was undertaken to demonstrate the oral spirochete (*Treponema denticola*) from periodontal pathogens suffering from periodontal disease.
Methods: A total of 55 clinically diagnosed selected patients of periodontal infection attending the outpatient Department of Periodontics, V. K. Institute of Dental Science, KLE University, Belgaum were studied. Dental plaque was taken as specimens from the patients were processed by modified Fontana staining and observed in microscope.
Results: A total number of 55 plaque samples were stained by modified Fontana staining technique. 30 cases were positive and 25 cases were negative. Males were affected more as compared to the females and the age group ranging from 15 to 65. Farmers were more affected compared to other occupation.
Conclusions: Microscopic method by modified silver nitrate staining can be a very useful screening method for evaluation of oral spirochetes in a clinical setting if used judiciously keeping in mind the variables that can affect the results.

Keywords: Spirochetes, Modified Fontana stain, *Treponema denticola*, Periodontal disease

INTRODUCTION

Oral health is an integral component of general health and is essential for wellbeing. There is evidence to prove the interrelationship between oral and general health.[1] The oral spirochetes are often the dominant bacterial types observed in sub-gingival plaque removed from diseased periodontal sites, and yet they are one of the least-studied and understood members of the plaque flora.[2] Periodontitis is defined as an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or group of specific microorganisms

resulting in progressive destruction of periodontal ligament and alveolar bone with pocket formation, recession or both.[3] The disease prevalence is 13-57% worldwide and 65-80% in India accounting to a major health problem.[4,5] Periodontal disease is an infectious disease caused by oral bacteria and that it has complex associations with immunological, genetic, and environmental factors.[6] It is also associated closely with dental plaque, which has been recognized as a biofilm contributing to representative oral diseases such as dental caries and periodontal disease.[7] Even though more than 60 different phylotypes of oral spirochetes are known to exist, only 10 have been cultivated so far.[8] *Treponema denticola* was one of the spirochetes most frequently isolated from severely diseased sites in young adults, and the gingival crevice area is the principal ecological niche inhabited by treponemes.[9] The classical method of Fontana is used to demonstrate the spirochete, but the modified Fontana staining technique is best method to demonstrate the smaller spirochetes like *Treponema denticola* (where Spirochaetes are stained brownish-black on a brownish-yellow background).

Objectives

The objectives of this were to detect oral spirochete (*Treponema denticola*) by modified Fontana staining technique from sub-gingival plaques of patients suffering from periodontal diseases and to analyze the morphology of spirochetes with respect to the disease's status.

METHODS

This study was conducted in out-patient department of KLE's V. K. Institute of Dental Sciences, Belgaum. Samples were collected from sub-gingival pockets in patients with chronic periodontitis attending the periodontology outpatient department at our Institute of Dental Sciences, over a period of 6 months from (August 2013 to January 2014). The study comprised of 55 cases (sample size was calculated using formula $4pq/d^2$, where p is prevalence; q is 100-p and d is 10% of the error).[10]

Clinically diagnosed all new cases of chronic periodontitis were included in the study and patients with history of systemic conditions such as diabetes mellitus, nutritional deficiencies, pregnant woman, antibiotic usage in the last 3 months and patients with history of undergoing any dental procedures in the last 3 months were excluded.[10]

Sample collection

Tooth surfaces were dried with sterile gauze to avoid contamination by saliva.[10] Supra-gingival plaque was removed with gauze and discarded, and sub-gingival plaque was then immediately taken and placed in sterile microscopic glass slide by using sterile periodontal Gracy Curette. Detailed clinical history regarding age, sex, occupation, chief complaints, past history was obtained from each patient. Oral examination was done to record periodontal pocket depth.

Laboratory methods

The smear was stained by modified Fontana staining technique for presumptive type of microorganisms present in the sample. Numbers of positive and negative cases of samples are recorded according to age, sex and occupation.

Preparations of modified Fontana stain solution

Reagent A - fixative

Glacial acetic acid – 1 ml, formalin (40% formaldehyde) – 2 ml and distilled water – 100 ml.

Reagent B - mordant

Phenol 1 gm, tannic acid 5 gm and distilled water 100 ml.

Reagent C - silver stain

Silver nitrate solution 60 ml and 40 ml, silver nitrate solution 60ml and few drops of ammonia solution till brown precipitate appears, add 0.5-1 ml of ammonia till all precipitate dissolves, from 40ml aliquot, transfer solution to above solution till precipitates reappears and reagent A, reagent B, reagent C must be kept in black stoppered bottle.

Modified Fontana staining procedure

A thin smear of the material was made on a clean glass slide allowed to air dry. The slides were placed vertically in 100ml capacity beaker containing reagent A and allowed to for two minutes. They are removed by forceps, blotted with tissue paper and dipped in a beaker containing absolute alcohol or methanol or rectified spirit for three minutes. The under surface of the slides was cleaned with paper and the smear was air dried. The slides were dipped in a 100 ml beaker containing reagent B pre-heated to 75ºC in hot water bath and allowed to react for a minute. The slides were rinsed in distilled water and air dried. The slides were dipped in a beaker containing reagent B preheated to 75 centigrade and allowed to react for a minute. The slides were rinsed with distilled water, the under surface is cleaned, air dried and examined under bright field microscope.[11] The processing and analysis of data were done by MS word software.

RESULTS

A total no of 55 samples from different individuals were taken for the study. Among 55 cases, 30 were positive for *Treponema denticola* and 25 were negative. The patients are distributed according to age, sex and occupation for the study.

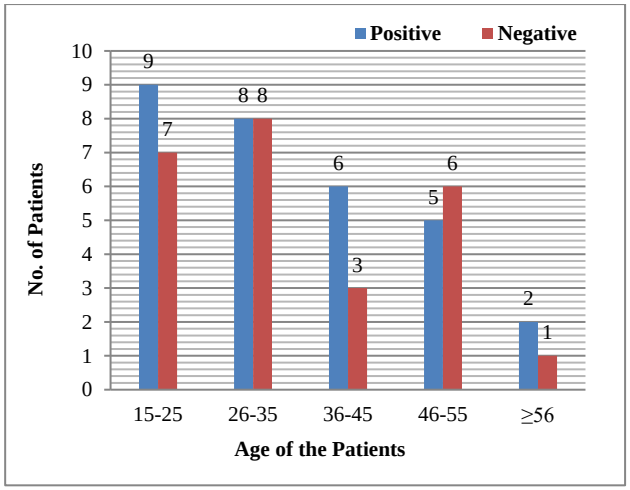

Figure 1: Distribution of patients according to age.

Patients are distributed according to their age group, In the age group 15-25, the positive cases were 56.25% and negative cases were 43.75%. In 26-35 age group, the positive and negative cases were equal i.e. 50-50%. In age group 36-45, the positive cases were 66.66% and negative cases were 33.33%. In 46-55 age groups, the positive cases were 45.45% and negative were 54.55%. In ≥56 age group, the positive cases were 66.66% and negative cases were 33.33% (Figure 1).

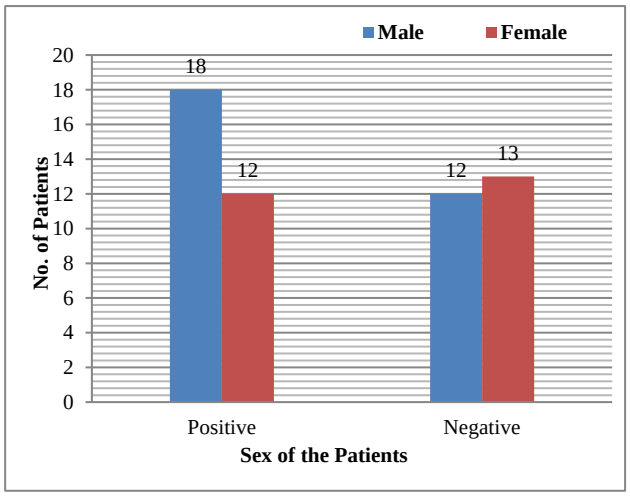

Figure 2: Distribution of patients according to sex.

According to sex distribution of patients, the male patients were 60% positive and 40% were negative, and among the female patients 48% were positive and 52% were negative (Figure 2).

According to the occupation of the individuals, businessman was 28.57% positive and 71.43% negative, farmers were 43.75% positive and 56.25% negative, housewife were 47.05% positive and 52.94% negative, student were 86.66% positive and 13.33% negative (Figure 3).

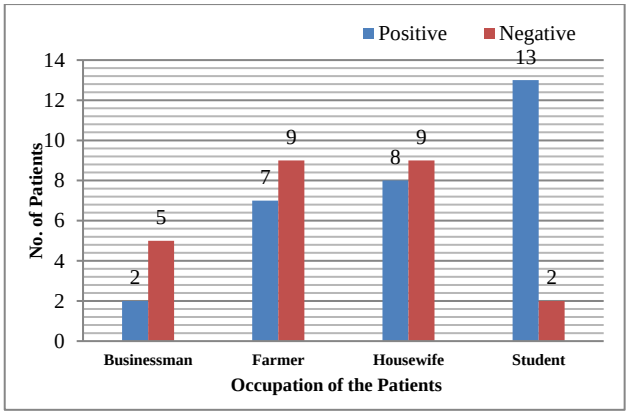

Figure 3: Distribution of patients according to occupation.

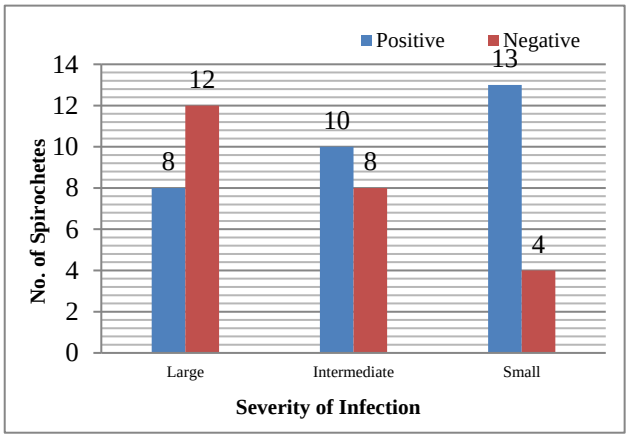

Figure 4: Distribution of spirochetes according to severity of infection.

According to severity of infection, the spirochetes are classified as large, intermediate and small. In patients with diameter 6-8 mm, 76% were positive and 24% were negative. In patients with diameter 7-9 mm, 60% were positive and 40% were negative. In patients with diameter >10 mm, 55% were positive and 45% were negative (Figure 4).

DISCUSSION

Dental carries are a common dental disease occurring in person of poor oral health and hygiene. Despite incredible scientific advances and the fact that caries is preventable, the disease continues to be a major public health problem.[12] The world health organization (WHO) has ranked it as number three among all chronic non-communicable disease that require worldwide at attention for prevention and treatment.[13]

Spirochetes are heterogeneous and complex population of oral cavity. They have very strict nutritional requirements, need strict anaerobic conditions and also show variability on their morphology. Hence, the culture and detection of spirochetes is meticulous process and difficult task.

But one of the methods by which the oral spirochetes can be detected in subgingival plaque samples is by staining with Fontana stain using light microscope. Since data cannot be stored for a longer period of time to study the morphology of oral spirochetes by the wet mount method (dark field, phase contrast), we decided to study oral spirochetes in number of positive and negative cases according to age, sex and occupation of patients in chronic periodontitis patients by using Fontana's method and light microscopy.[14]

Choudhary et al from Maratha Mandal's NGH Institute of Dental Sciences and Research Centre Belgaum Karnataka India, explores the possibility of using staining as a screening procedure for the study of oral spirochetes. The experimental approach of the study included 100 patients with chronic periodontitis and 100 controls. Subgingival plaques were collected and silver nitrate staining was performed. The positive slides were screened for density of organisms and an attempt was also made to determine the relative size of spirochetes and their predominance. Where there was a significant difference in the positivity rate between the patients and controls. Higher number of patients had greater density of spirochetes. The presence of large spirochetes in controls was significantly higher.[15]

Deepa et al from Maratha Mandal's NGH Institute of Dental Sciences and Research Centre Belgaum Karnataka India, revealed statistically significant difference for the positivity of the small and intermediate spirochetes based on their length were higher in CP patients. The thick spirochetes based on diameter were higher in healthy individual.[14]

Cavrini et al from endodontics unit, Department of Oral Sciences, Alma Mater Studiorum, Bologna, Italy; detect of *Treponema denticola* in root canal systems in primary and secondary endodontic infections.[16]

In our study, total no. of 55 samples from different individuals, were taken. Among 55 cases, 30 were positive for *Treponema denticola* and 25 were negative. The patients are distributed according to age, sex and occupation and severity of infection.

According to the distribution of age group, in the age group 15-25, the positive cases were 56.25% and negative cases were 43.75%, in 26-35 age group the positive and negative cases were equal i.e. 50-50%, in age group 36-45, the positive cases were 66.66% and negative cases were 33.33%, in 46-55 age group, the positive cases were 45.45% and negative were 54.55%, in ≥56 age group, the positive cases were 66.66% and negative cases were 33%. Epidemiologic studies and long-term clinical trials have documented that the severity of periodontal disease increases with age and that once initiated, periodontitis will continue to progress in the absence of treatment, with eventual loss of the teeth.[17-24] Over a 10 years period, the progression of periodontal disease in all age groups was slow among patients who initially had mild gingivitis but not periodontitis.[6]

According to the distribution of sex of the patients, the male patients were 60% positive and 40% were negative, and among the female patients 48% were positive and 52% were negative. A survey of employed adults and elderly people conducted by the National Institute of Dental Research from 1985 through 1986 provided new information about the prevalence of periodontal disease.[25] This study indicated that the overall prevalence of some loss of attachment was high, with 80 percent of employed men and 73 percent of working women having a loss of 2 mm or more involving one or more teeth. However, the prevalence of more severe levels of periodontal destruction appeared to be lower than previously believed. Advanced periodontitis, defined as a loss of attachment of 6 mm or more at one or more sites, affected roughly 15 percent of those between the ages of 60 and 64.[6,25]

According to the occupation of the individuals, businessman was 28.57% positive and 71.43% negative, farmers were 43.75% positive and 56.25% negative, housewife were 47.05% positive and 52.94% negative, student were 86.66% positive and 13.33% negative.

According to the severity of infection of individuals, in infection of 6-9 mm, among the population, 76% were positive and 24% were negative and >10 mm i.e. severe infection among the population, 55% were positive and 45% were negative. Thirty-five percent of the teeth with severe gingivitis at the beginning of the study had periodontitis at the end of the study.[22] These studies demonstrate that untreated periodontal disease eventually leads to tooth loss, and they imply that disease progression is continuous. This, however, is probably not the case. The progression of periodontal disease is believed to be episodic, with exacerbations and remissions.[6] Socransky and co-workers studied the progression of periodontal disease by measuring the changes in the level of periodontal attachment over time.[26-28] Periodontal disease was characterized by recurrent acute episodes followed by remission and was not necessarily continuous. Our current understanding of the disease is that various sites in the mouth may have episodes of active progression followed by periods of no further loss, then later may have other episodes of loss of attachment.[6]

CONCLUSION

The present method using Fontana stain is a simple, rapid and inexpensive method, for demonstration of oral spirochetes. This method can be effectively used to semi-quantify the spirochetes in patients with periodontitis. It also aids in establishing treatment, follow up and prognosis of patients with chronic periodontitis.

Funding: This research was supported by JNMC, KLE University, Belgaum, India
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Grossi SG, Genco RJ. Periodontal disease and diabetes mellitus: A two ways relationship. Ann Periodontol. 1998;3:51-61.
2. Loesche WJ. The role of spirochetes in Periodontal disease. Adv Dent Res. 1988;2(2):275-83.
3. Novak MJ. Classification of diseases and conditions affecting the periodontium. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA, editors. Carranza's Clinical Periodontology. 10th ed. St. Louis Missouri: Elsevier; 2007:103-104.
4. Rylev M, Kilian M. Prevalence and distribution of principal periodontal pathogens worldwide. J Clin Periodontol. 2008;35:346-61.
5. Saini S, Aparna, Gupta N, Mahajan A, Arora DR. Microbial flora in oro-dental infections. Indian J Med Microbiol. 2003;21:111-4.
6. Williams RC. Periodontal disease. New England J Med 1990;322(6):373-82.
7. Keyes PH, Likins RC. Plaque formation, periodontal disease, and dental caries in Syrian hamsters. J Dental Res. 1946;25:166.
8. Choi BK, Paster BJ, Dewhirst FE, Gobel UB. The Diversity Cultivable and Uncultivable Oral spirochetes from patients with severe destructive periodontitis. Infection Immunity. 1994;62(5):1889-95.
9. Listgarten MA, Levine S. Positive correlation between the proportions of subgingival spirochetes and motile bacteria and susceptibility of human subjects to periodontal deterioration. J Clin Periodontol. 1981;8:122-38.
10. Benachinmardi KK, Nagamoti J, Kothiwale S, Metgud SC. Microbial Flora in Chronic Periodontitis: Study at a Tertiary Health Care Centre from North Karnataka. J Lab Physicians. 2015;7(1):49-54.
11. Gangadhar NL, Rajashekar M. A modified Silver Impregnation staining for leptospires. Indian Vet J. 1998;75:349-51.
12. Bansode P, Deshpande S, Ahire C, Dekate K. Dental Caries Occurrence, Types and Occurrence of Trauma to Anterior Teeth, Their Severity in Relation to Pulpal Involvement in Patients Visiting O. P. D. of Government Dental College and Hospital, Aurangabad. J Evolution Med Dent Sci. 2014;3(44):10924-9.
13. Marrs JA, Trumbley S, Malik G. Early childhood caries: determining the risk factors and assessing the prevention strategies for nursing intervention. Pediatr Nurs. 2011;37(1):9-15.
14. Deepa B, Kishore B, Nayak R, Ingalgi P. Detection, semi-quantification and morphology of oral spirochetes in chronic periodontitis: a light microscopic study. J Pharm Biomed Sci. 2013;29(29):735-40.
15. Varsha Choudhary from Maratha Mandal's NGH Institute of Dental Sciences and Research Centre Belgaum Karnataka India. Utility of silver nitrate staining in detection of spirochete from oral cavity of periodontally healthy and diseased individuals.
16. Cavrini F, Pirani C, Foschi F, Montebugnoli L, Sambri V, Pratic C. Detection of Treponema denticola in root canal systems in primary and secondary endodontic infections. A correlation with clinical symptoms. New Microbiol. 2008;31(1):67-73.
17. Scherp H. Current concepts in periodontal disease research: epidemiological contributions. J Am Dent Assoc. 1964;68:667-75.
18. Williams RC, Howell TH. Periodontology: section A: definition, epidemiology and etiology of periodontal disease. In: Shaw JH, Sweeney EA, Cappuccino CC, Meiler SM, eds. Textbook of oral biology. Philadelphia: W.B. Saunders; 1978: 1019-1031.
19. Loe H, Anerud A, Boysen H, Smith M. The natural history of periodontal disease in man: the rate of periodontal destruction before 40 years of age. J Periodontol. 1978;49:607-20.
20. Becker W, Berg L, Becker BE. Untreated periodontal disease: a longitudinal study. J Periodontol. 1979; 50:234-44.
21. Lindhe J, Haffajee AD, Socransky SS. Progression of periodontal disease in adult subjects in the absence of periodontal therapy. J Clin Periodontol. 1983;10:433-42.
22. Buckley LA, Crowley MJ. A longitudinal study of untreated periodontal disease. J Clin Periodontol. 1984;11:523-30.
23. Hausmann E, Dunford R, Wikesjo U, Christersson L, Henry MK. Progression of untreated periodontitis as assessed by subtraction radiography. J Periodontal Res. 1986;21:716-21.
24. Loe H, Anerud A, Boysen H, Morrison E. Natural history of periodontal disease in man: rapid, moderate and no loss of attachment in Sri Lankan laborers 14 to 46 years of age. J Clin Periodontol. 1986;13:431-45.
25. Epidemiology and Oral Disease Prevention Program, National Institute of Dental Research. Oral health of United States adults: The National Survey of Oral Health in U.S. employed adults and seniors, 1985-86: national findings. Bethesda, Md.: Department of Health and Human Services, Public Health Service, National Institutes of Health; 1987.
26. Socransky SS, Haffajee AD, Goodson JM, Lindhe J. New concepts of destructive periodontal disease. J Clin Periodontol. 1984;11:21-32.
27. Goodson JM, Acheal T, Haffajee AD, Somberger GC, Socransky SS. Patterns of progression and

regression of advanced destructive periodontal disease. J Clin Periodontol. 1982;9:472-81.
28. Goodson JM, Haffajee AD, Socransky SS. The relationship between attachment level loss and alveolar bone loss. J Clin Periodontol. 1984;11:348-59.

Cite this article as: Paudel S, Nagamoti JM, Mishra VP, Twanabasu S, Kusma S. Detection of oral spirochete (*Treponema denticola*) by modified Fontana staining technique from sub-gingival plaques of patients suffering from periodontal disease and to analyse the morphology of spirochetes with respect to the disease's status. Int J Sci Rep 2020;6(8):286-91.