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Anaerobic Microbiological study of periodontitis in Salah Al – Deen City

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Oral microloiology, Periodontis, Anaerobic bacteria

Abstract

Oral flora responsible for periodontal disease is polymorphic. The periodontal infection results either from the penetration of pathogenic microorganisms in the tissues, or even the activation of already existing germs, but not pathogenic under normal conditions. The aims of this study was carried out to evaluate selected bacterial agents causing periodontitis. One hundred eighty samples were examined in the present study. Clinical measurements of periodontal parameters used included dental plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment loss. The results of periodontitis were the most common anaerobic periodontal bacteria isolated from patients were *peptostreptococcus prevotii* which represented 15(8.3%) isolates, while *prevotella intermedia, prevotella melani, prevotella disiens, Bifidobacterium sp., Fusibacterium mortiferum peptostreptococcus tetradius, and Wolinella sp.* represented only 1 (0.5%) isolates.Also another anaerobic subgingival bacteria isolated from inflamed sites in patients were *fusibacterium varium ,vellionella sp., campylobacter gracilis, capnocytophaga sp. ,peptostreptococcus magnus, peptostreptococcus micros, peptostreptococcus niger,peptostreptococcus anaerobius, staphylococcus saccharolyticus, streptococcus consellatus, and gemella morbillorum.* Concerning to the results of this study the researcher concludes *peptostreptococcus prevotii, prevotella intermedia, prevotella melani, prevotella disiens, Bifidobacterium sp., Fusibacterium mortiferum peptostreptococcus tetradius, and Wolinella sp. fusibacterium varium ,vellionella sp., campylobacter gracilis, capnocytophaga sp. ,peptostreptococcus magnus, peptostreptococcus micros, peptostreptococcus niger,peptostreptococcus anaerobius, staphylococcus saccharolyticus, streptococcus consellatus, and gemella morbillorum* were the most common anaerobic periodontal pathogens isolated from patients in the present study.

Introduction

Periodontal disease could be defined as a disorder of supporting structures of teeth, including the gingiva, periodontal ligament and alveolar bone. Periodontal disease develops from a pre-existing gingivitis.

However, not every case of gingivitis develops into a periodontal disease. The inflammation of gingiva alone is termed gingivitis, and the severe inflammation of the periodontal ligament with destruction

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of alveolar bone is called periodontal disease [1] . The current concept concerning the etiology of periodontal disease considers three groups of factors which determine whether active periodontal disease will occur: A susceptible host, the presence of pathogenic species, and the absence of so-called "beneficial bacteria" [2].

It is generally accepted that the oral biofilm in association with anaerobic bacteria is the main etiological factor in periodontal disease [3,4].The oral biofilm consists mainly of microbes and host proteins that adhere to teeth within minutes of a dental oral hygiene procedure. Healthy gingival sulcus has a flora dominated by equal proportions of gram positive cocci, especially Streptococcus spp, and Actinomuces sp. Later, plaque "matures" resulting in a flora consisting from facultative anaerobic microorganisms, spirochaetes and motile rods. The proportions of strict anaerobic, Gram negative organisms increase significantly in accordance with increasing severity of disease. Disease activity in periodontal disease may range from slow, chronic, progressive destruction to brief and acute episodic bursts with varying intensity and duration[5] .The composition of the subgingival microbial flora and the level of pathogenic species differ from subject to subject as well as from site to site. The search for the pathogens of periodontal diseases has been underway for more than 100 years, and continues up today[5] .More than 300 species of bacteria colonize subgingival area and their cell wall components can trigger immune activation[6].

Human is associated with a widely diverse and complex subgingival microbiota encompassing both Gram positive and Gram-negative bacteria, facultative and anaerobic organisms, and possibly yeasts[7].

Whilst numerous studies have investigated the composition of plaque and defined 5 bacterial complexes ranked by the strength of the groups relationship with clinical measures of periodontitis.The normal subgingival flora consists of mainly facultative (both aerobic or anaerobic capabilities) anaerobic Gram positive bacteria, and only 5% spirochaetes and motile rods. Generally ,periodontal disease progresses the proportion of anaerobic, Gram negative rod, and other bacteria increases. In chronic periodontitis approximately 75% of cells are Gram negative of which 90% are strict anaerobes [8]. This shift in microbial composition is represented by a change from mainly purple complex bacteria through to a large proportion of red complex bacteria in sites of active disease [9]. The currently recognized key Gram negative periodontopathogens include:

Porphyromonas gingivalis, Prevotella intermedia, Bacteroides forsythus, Aggregatibacter actinomycetemcomitans , Fusobacterium spesies ,Capnocytophaga species , Campylobacter sp.,wollenilla sp.,and Bifidobacterium [6,10,11,12,13] . Also, the following bacteria could be isolated: Eubacterium spp, Peptostreptococcus species Streptococcus consellatus, and Staphylococcus saccharolyticus [5].

The clinical signs of periodontitis are changes in the morphology of gingival tissues, bleeding upon probing as well as periodontal pocket formation. This pocket provides an ideal environment for the growth and proliferation of anaerobic pathogenic bacteria [14]. Most of these microorganisms can produce tissue destruction in two ways: **A.** directly, through invasion of the tissue and the production of harmful substances that induce cell death and tissue necrosis, and. **B.** indirectly, through activation of inflammatory cells which can produce and release mediators that act on effectors, with potent proinflammatory and catabolic activity[15].

Materials And Methods

1- Sampling:

Samples were obtained from periodontal pockets after supragingival plaque was removed from the teeth to be sampled[16]. The supragingival dental plaque was removed with sterile cotton, and the tooth surface was dried with compressed air to prevent contamination with saliva. The exclusion of moisture in the mouth with sterile cotton rolls and subgingival plaque was collected from the most inflamed

sites by inserting a sterile paperpoint into the periodontal pocket for 10 seconds , when the pocket depth was from3-7mm.The sample was mixed with 1 ml thioglycollate broth (transport medium) , sealed tightly to avoid contamination and kept at 4 c^0. Samples were processed within 2 days of collection [17].

2- Cultural Technique:

A plaque sample(loopful from thioglycollate broth containing subgingival paperpoint which inserted into periodontal pocket) was inoculated onto Brucella blood agar supplement with kanamycin 100 mg/l,vancomycin 7.5 mg/l Brucella blood agar, Enriched blood agar ,brain heart infusion agar,and trypticas soy agar with haemin and vitamin K1.The inoculated media were immediately incubated in an anaerobic environment generated with the anaerogens gas pack CO2 system Chemical, for 3 -7 days[17,18].

3- Isolation and Identification of Bacteria:

Isolates were identified using cultural characteristics**,** gram - stain and biochemichal tests (convenentional methods) which includes: indole spot test, catalase test, oxidase test, arginine hydrolysis, lipase test, nitrate reduction , motility test, growth in bile, urease test and growth on kanamycin (1mg), vancomycin (5µg), colistin (10µg) on Brucella blood agar [19,20] **.** Also anaerobic periodontal bacteria were identified by the API RapID ANA II system [remel, USA], API 20A [Biomereiux, France] and VITEK 2 ANC System [Biomereiux, France] [16, 17, 21]**.**

Results

Table 1 showed the types of anaerobic periodontal bacteria isolated. In the present study the most common anaerobic periodontal bacteria isolated were peptostreptococcus prevotii which represented 15 (8.3%) of all isolates, while prevotella intermedia, prevotella melani , prevotella disiens, Bifidobacterium sp., Fusibacterium mortiferum peptostreptococcus tetradius, and Wolinella sp. represented only 1 (0.5%) of all isolates.

Discussion:

The present study demonstrated a different microbiota in periodontal pockets. The finding presented here that *vellionella sp* (7.7%) *fusibcterium varium* (5.5%), *campylobacter gracilis* (1.6%) ,*capnocytophaga sp*.(1.1%) ,*prevotella intermedia* (0.5%), *prevotella melani* (0.5%), *prevotella disiens* (0.5%), *bifidobacterium sp.* (0.5%), *fusibacterium nucleatum* (0.5%), *wolinella sp*. (0.5%) were more frequent as isolated from periodontal pocket as showed in Table (1) .It was ,however, interesting to note that gram positive anaerobic bacteria, especially *peptostreptococcus spp*.,were isolated in high rates in periodontal pocket which includes *peptostreptococcus prevotii* (8.3%), *peptostreptococcus magnus*(1.1%), *peptostreptococcus* micros (1.6%), *peptostreptococcus tetadius* (0.5%), *peptostreptococcus niger* (2.2%), and *peptostreptococcus anaerobius* (2.7%) .Also, *staphylococcus saccharolyticus, streptococcus consellatus, and gemella morbillorum* were isolated in the present study in percentages of 2.7%,2.2%,and 1.6% respectively. These results were almost similar to those of Daniluk *et al* who found that the most common periodontal pathogens were *Veillonella* species, *Fusobacterium, staphylococcus saccharolyticus ,streptococcus consellatus, gemella morbillorum , peptostreptococcus spp.* and *prevotella intermedia*[7]. Moreover, these results were almost similar to those of Spartt ,Van Winkelhoff *et al* , whom founds that Veillonella species, Fusobacterium, staphylococcus saccharolyticus ,streptococcus consellatus, gemella morbillorum, peptostreptococcus spp. prevotella intermedia, campylobacter gracilis and bifidobacterium sp. [22,23] . Furthermore , Mohammad et al found that Actinobacillus actinomycetemcomitans (26.8%), Porphyromonas gingivalis (21.9%), Capnocytophaga sp. (16.7%), Eikenella corrodens (13.2%), Prevotella intermedia (10.5%), Prevotella disiens (3.1%), Capnocytophaga gingivalis (2.2%), Peptostreptococcus micros (2.9%), Prevotella corporis (1.8%),

Peptostreptococcus magnus (1.3%) , and Fusobacterium nucleatum (0.4%)were most common isolates[24]. Spratt et al was found that Capnocytophga sp. involvement in some forms of periodontitis [25]. According to Gürsoy et al Prevotella intermedia is associated with periodontal disease [26]. Mayorga-Fayad et al revealed that the frequency increases Prevotella intermedia in patients with periodontitis [27]. The present results were different from those of Yacoubi et al who found that aggregatibacterium actinomycetemcomitans, eikenella corrodens as primary causative agent in periodontitis[28]. Moreover, Cisar et al and Ximenez-Fyvie et al found that actinomyces were the most common component of periodontal disease [29]. The data from the investigation suggest that there was heterogeneity in the subgingival periodontopathogenic bacteria among subjects. However, as many bacteria in the oral cavity cannot be cultured, it was likely that these still uncharacterized bacteria could play a role in the initiation and progression of periodontal disease.

Table (1): Types of anaerobic bacteria isolated from periodontal patients:

Types of isolates	No. (100%) of isolates
prevotella intermedia	1 (0.5%)
prevotella melani	1 (0.5%)
prevotella disiens	1 (0.5%)
Bifidobacterium sp	1 (0.5%)
Fusibacterium nucleatum	1 (0.5%)
Fusibacterium varium	10 (5.5%)
Vellionella sp.	14 (7.7%)
Campylobacter gracilis	3 (1.6%)
Wolinella sp.	1 (0.5%)
Capnocytophaga sp	2 (1.1%)
Peptostreptococcus prevotii	15 (8.3%)
Peptostreptococcus magnus	2 (1.1%)
Peptostreptococcus tetradius	1 (0.5%)
Peptostreptococcus micros	3 (1.6%)
Peptostreptococcus niger	4 (2.2%)
Peptostreptococcus anaerobius	2 (1.6%)
Staphylococcus saccharolyticus	5 (2.7%)
Streptococcus conselatus	4 (2.2%)
Gemella morbillorum	2 (1.6%)
Total	73 (40.7%)

References:

1. Jayamathi G. , Pamela E. ,and Rengarajulu P. Therapeutic effect of proanthocyanidins on the pathogenesis of periodontitis . *Indian J. Experim.Bio*.2011;49:83-93.
2. Wolff L, Dahlen G, Aeppli D. Bacteria as risk markers for periodontitis. J Periodontol 1994; 65: 498-510.
3. Moore WEC. Microbiology of periodontal disease. *J Periodontol .Res* 1987; 22: 335-341.
4. Socransky SS, Haffajee AD, Dzink JL, Hillman JD. Association between microbial species in subgingival plaque samples. *Oral Microbiol Immunol* .1988; 3: 1-7.
   Ljiljana K., Jelena M., Marija I.,and Radmila O. Microbial etiology of periodontal disease. *Medicine and Biology*. 2008;15: 1 – 6.
5. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: Current concepts. *J Periodontol* .1992;63: 322-331.
6. Daniluk T., Tokajuk G., Cylwik-Rokicka D., Rokiewicz D.,Zaremba ML., Stokowska W.Aerobic and anaerobic bacteria in subgingival and supragingival plaques of adult patients. 2006.
7. Samaranayke, L. P. Essential Microbiology for Dentistry. Edinburgh, Churchill Livingstone. 2002.
8. Lisa J. P..Neutrophil extracellular traps in periodontitis.University of Birmingham . Department of Periodontology.College of Medicine .2010.
9. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol*. 2000; 5: 78-111.
10. Zambon JJ. Periodontal diseases: Microbial factors. *Ann Periodontol*.1996; 1: 879-925.
11. Avila-Campos MJ. PCR detection of four periodontopathogens from subgingival clinical samples. *Braz J Microbiol* .2003;34(1): 81-84.
12. Jovanovic Lj. Comparitive study between periodontal bacteria .1997.
13. Schwach-Abdellaouia K. Vivien-Castionib N.and, Gurnya R. .Local delivery of antimicrobial agents for the treatment of periodontal diseases.
14. *Euro. J.Pharma. and Biopharm.* 2000; 50: 83-99.
15. Bascones M A. ,Munoz C M., Noronha S., Mota P., Bascones I C., and Campo Trapero J. Host defense mechanisms against bacterial aggression in periodontal disease :basic mechanisms.*Med Oral Pathol Oral Cir Bucal.*2009;14:680.
16. Francesca C, Guido S, Giuditta C, Bruno R, Giuseppe N, and Anna M. Lo Bue. Isolation of *Porphyromonas gingivalis* and Detection of Immunoglobulin A Specific to Fimbrial Antigen in Gingival Crevicular Fluid. *J Clin Microbiol.* 1998 August; 36(8): 2322–2325.
17. Chetan C,Narayan V, and Vandana KL.The comparitive evaluation of xanthan gel with chlorhexidin (chlosite)in smoker and non smokers:Aclinical and microbiological assessment.2011.
18. Katsuhito F, Naoki K, Haru K, Kunitomo W, and Norichika T. Incidence of *Prevotella intermedia* and *Prevotella nigrescens* Carriage among Family Members with Subclinical Periodontal Disease. *J Clin Microbiol.* 1999 October; 37(10): 3141–3145.
19. Betty A. F. ,Daniel F.S., and Alice S.W. Baily and scotts ,diagnostic microbiology.12th ed.2010.
20. Murray PR., Baron EJ., Pfaller MA., Tenover FC., and Yolker RH. Manual of clinical microbiology.7th ed. 1999.
21. Francine M, Corentine A, Celine M , Philippe R , and Alain L. Evaluation of the New Vitek 2 ANC Card for Identification of Medically Relevant Anaerobic Bacteria . *J Clin Microbiol.* 2009 June; 47(6): 1923–1926.
22. Spratt D. Dental plaque and bacterial colonization. In: Medical biofilms. Jass J, Surman S, Walker J, editors,

23. John Wiley and Sons Ltd, 2003: 175-98.
24. Van Winkelhoff AJ, Rams TE, Slots J. Systemic antibiotic therapy in periodotitics. 1996; 10: 45-78.
25. Mohammad HS., and Zainab K. Rate of cultivable subgingival periodontopathogenic bacteria in chronic periodontitis. J. of Oral Scien.. 2004;46 (3)157-161.
26. Spratt, D.A., Greenman J. , and A.G. Schaffer,. *Capnocytophaga gingivalis*: effects of glucose concentration on growth and hydrolytic enzyme production. Microbiol.1996;142(8): 2161-4.
27. Gürsoy, M., Haraldsson G., Hyvönen M., Sorsa T., Pajukanta R. and Könönen E., . Does the frequency of *Prevotella intermedia* increase during pregnancy? *Oral. Microbiol. Immunol*. 2009;24(4): 299-303.
28. Mayorga-Fayad I., Lafaurie G.I., Contreras A., Castillo D.M., Baron A. , and Aya Mdel R. Subgingival microbiota in chronic and aggressive periodontitis in Bogotá, Colombia: an epidemiologicalapproach Biomedica, .2007;27(1): 21-33.
29. Yacoubi A, Djamila B, Makhrelouf L. and Bensoltane A. Microbiological Study of Periodontitis in the West of Algeria. *World J. Med.Scie.* 2010;5 (1): 07-12.
30. Ximenez-Fyvie L.A., Haffajee A.D. , and Socransky S.S.. Microbial composition of supra and subgingival plaque in subjects with adults periodontitis. *J. Clin. Periodontol.* 2000; 27: 722-732.