International Journal of Dental Research
& Development(IJDRD)
ISSN 2250–2386
Vol. 3, Issue 2, Jun 2013, 1-6

ISOLATION & CHARACTERIZATION OF *PORPHYROMONAS GINGIVALIS* AND DETERMINATION OF SOME IMMUNOLOGICAL ASPECTS IN PATIENTS WITH PERIODONTITIS

NOOR SALMAN AL-KHAFAGEE[1], FATIMA MALIK AL-RUBIAE[2], LUMA JASIM WITWIT[2] & HUSSEIN OLIEWI AL-DAHMOSHI[1]

[1]M.Sc. Medical Microbiology, Biology Department, College of Science, Babylon University, Iraq

[2]M.Sc. Medical Microbiology, College of Derntistry, Babylon University, Iraq

ABSTRACT

Bacteriological and immunological study was done on 150 patients with severe periodontitis at the period from 1- 10-2010 to 1-6-2011in (College of Dentistry). the number of samples gave positive results for growth of *Porphyromonas gingivalis* on blood agar were 35 sample (23.3%) while 115 samples (76.7%) gave appositive growth for others bacteria like *Streptococcus mutans* .The results of immunological study on serum of patients gave positive culture of *P. gingivalis* showed increasing in all immunological testing parameters including concentration of immunoglobulin's such as (IgM, IgG, IgA) , complement (C3&C4) , total protein comparing with healthy individual (20 person) whom didn't have periodontitis and gave a normal results to all tested parameters .

KEYWORDS: *Porphyromonas gingivalis, Streptococcus mutans,* IgM, IgG, IgA

INTRODUCTION

Periodontitis is an infectious, chronic inflammatory disease of the supportive structures of the teeth. Smokers often exhibit periodontal disease that is more severe than in non-smokers, with increased alveolar bone loss, attachment loss, tooth mobility, and tooth loss[1].all apparent and odds ratios of 3 to 7 commonly reported[2].

Moreover, smokers are more likely to be refractory to treatment than non-smokers, Although periodontal disease is localised to the tissues surrounding the tooth, evidence is accumulating that infection with *Porphyromonas gingivalis* may predispose to more serious systemic conditions such as cardiovascular disease and to delivery of preterm infants[3].

Porphyromonas, which are commonly found in the human body and especially in the oral cavity, were originally classified in the Bacteroides genus. *P.gingivalis* are an oral anaerobe associated with periodontal lesions, infections, and adult periodontal disease. *Porphyromonas spp*.are Gram-negative, nonspore forming, anaerobic, rod-shaped bacteria that produce porphyrin pigments (dark brown/black pigments) on blood agar [4].

Cell surface adhesion molecules on the surface of *Porphyromonas* interact with other bacteria, epithelial cells, and extracellular matrix proteins, *P. gingivalis* is thought to spread through tissue, destroy tissue, and evade host defenses by the use of secreted cell-bound proteases, immunoactive cellular compounds, and toxins. *P. gingivalis* cytotoxic metabolic end products, which include butyrate, propionate, have low molecular weights which allows them to easily penetrate periodontal tissue and disrupt the host cell activity [5] .

P. gingivalis is found in the oral cavity, where it is implicated in certain forms of periodontal disease, as well as the upper gastrointestinal tract, respiratory tract, and in the colon. Collagen degradation that is observed in chronic

periodontal disease results in part from the collagenase enzymes of this species[6]. In patients harbouring *P. gingivalis* one finds high levels of specific antibody in the serum [7]. Several observations suggest that the fimbriae or fimbriae- like structures play an important role in the adhesion of the bacteria to the tooth surfaces. [8] have shown that monoclonal antibodies to purified fimbriae and synthetic peptides analogous to the fimbrillin sequence block the adherence of *P.gingivalis* to oral epithelial cells and to oral surfaces.

The humoral immune system might play a role in the mediation of the development of gingivitis and periodontitis, as many investigators have demonstrated the concentration of immunoglobulins specific to whole bacterial cells, to lipopolysaccharide, and to the fimbrial protein of different periodontopathic bacteria are high in patients with adult[9].

This study was aimed to detect some effects of *Porphyromonas gingivalis* on immunity of patients with periodontitis**.**

METHODS

Bacteriological Study

Isolation and all biochemical tests for diagnosis of *Porphyromonas gingivalis* was done according to [10,11].

Immunological Study

Collection of serum: serum samples were collected from patients with periodontitis & from healthy individual as a control samples according to [10]. Radial immunodiffusion for immunoglobulin's and complements C3&C4 (LTA/Italy) concentration was done according to [12].Total serum protein (Biolabo/ France) was done according to [13].

Statistical Analysis

Statistical analysis (mean ± standard deviation) was done depending on [14].

RESULTS

Obtained results of biochemical tests and the microscopic examination of isolated bacteria confirmed that its *Porphyromonas gingivalis* according to morphological , microscopical characteristic and biochemical tests in(table 1).

Also table (2 and 3) showed the isolation and purity percentage of this bacteria from all sample were collected in this study.

Immunological study to serum of (35) patients with severe periodontitis in *vitro* showed that *p.gingivalis* induced humeral immunity by increased immunoglobulin's (IgM, IgG, IgA) and complement (C3&C4) concentration also increased in total protein (table 4,5).

Table 1: Results of Biochemical Tests and the Microscopic Examination of *Porphyromonas gingivalis*

Type of Test	Result of Test
Growth on blood agar	Black colonies with β hemolytic
Gram stain reaction and shape on microscope examination	G-ve, coccobacillli
Catalase	-
Lipase	-
Indole	+
Urease	-
Motility	-
Glucose	Fermentative

Table 2: Number of Bacterial Isolates from Patients with g*ingivalis*

Bacteria	Number of Isolates	%
Porphyromonas gingivalis	35	23.3%
Other bacteria	115	76.7%
Total number	150	100%

Table 3: Number of Pure and Mix Growth of *Porphyromonas gingivalis*

Type	Number of Isolates	%
Pure isolates	27	77.14 %
Mixed isolates	8	22.86 %
Total number	35	100 %

Table 4: Mean of Immunoglobulin's & Complement Concentration (Mg/Dl)

Parameters	Test Serum M ± S.D	Control Serum M ± S.D
IgM	312.3 ± 6.1	215.4 ± 3.1
IgG	2006.5 ± 30.5	1669.2 ± 20.7
IgA	606.0 ± 15.2	310.3± 19.04
C3	177.2 ± 3.4	143.8± 0.9
C4	60.2 ± 0.9	41.9± 3.2

- M= Mean; - S.D = Standard deviation

Table 5: Mean of Total Protein Concentration (G/Dl)

Serum Samples	M ± S.D
Total proteins for test sample	13.2± 0.4
Total protein for control sample	6.68 ±0.09

DISCUSSIONS

The result in table (1) demonstrate that *Porphyromonas gingivalis* is an anaerobic, Gram-negative. It is observed to be non-motile and rod-shaped. When colonized on blood agar it forms black spots due to it takes part in Iron Transport, the way it does this is by using a hemin as a device to help it transport iron. When this builds up it results in the black pigmentation that is detected[9,4] .from table (2,3) the high isolation percentage results showed that *P. gingivalis* was very important causative agent of periodontitis disease and this agreed with [16].Also the result in table (4,5) showed the effect of infection with this bacteria on the increasing concentration of immunoglobulin's, complement (C3&C4) and total proteins concentration in patients serum compared with healthy control group , this results agreed with [17, 9,18,19] Who explained the role of this bacteria in patient immunity when B cells interact with macrophages in gingival tissue and become plasma cells which produce antibodies. There are also B cells series that carry the memory of a particular antigen and can quickly produce antibodies, this memory is dependent on interactions with T cells. Plasma cells (B cells) produce immunoglobulin's within gingival tissue which bind to and inactivate bacterial antigens including Exotoxins. Antibody-Antigen complexes also activate Complement . Plasma cells produce a variety of immunoglobulin's with each one being specific for a particular bacterial antigen and have memory to become quickly activated. Patients with periodontal disease have high serum levels of IgG specific for plaque bacteria. Activation of complement is another part of the immune response seen in Gingivitis Activation of complement produces molecules such as C2a C5b that are cytotoxic, increase vascular permeability and are chemo tactic for PMNs and Macrophages. T Lymphocytes are very common in gingivitis.

They interact with bacterial Antigens processed by Macrophages. Activated T Lymphocytes produce cytokines. These are bioactive molecules that enhance inflammation and also can cause damage to gingival and periodontal cells. Two important cytokines are Interleukin-1 (IL-1) and Tumor Necrosis Factor (TNF) all these increasing in immunoglobulin's and complement component concentration combined with increasing in total protein concentration [17].

REFERENCES

1. McGuire M K, Nunn M E.(1996). Prognosis versus actual outcome III. The effectiveness of clinical parameters in accurately predicting tooth survival. J Periodontal; 67:666–674.
2. Bagaitkar J, Demuth D R, Scott D A.(2008). Increased susceptibility to bacterial infections in tobacco smokers. Tobacco Induced Diseases; 4.
3. Palmer R M, Wilson R F, Hasan A S, Scott D A .(2005). Mechanisms of action of environmental factors–tobacco smoking. J. Clin Periodontol ; 32(Suppl 6):180–195.
4. Shelburne C E, Gleason R M, Coulter W A, Lantz M S, Lopatin D E.(2005). Differential display analysis of *Porphyromonas gingivalis* gene activation response to heat and oxidative stress. Oral microbiol and immunol; 20(4):233-8.
5. Grenier D, Dang L. (2011) . Proteases of *Porphyromonas gingivalis* as Important Virulence Factors in Periodontal Disease and Potential Targets for Plant-Derived Compounds : A Review Article .Current Drug Targets .12(3):322-331.
6. Naito M, Hirakawa H, Yamashita A. (2008). Determination of the Genome Sequence of *Porphyromonas gingivalis* Strain ATCC 33277 and Genomic Comparison with Strain W83 Revealed Extensive Genome Rearrangements in P. gingivalis. DNA Res ; 15 (4): 215–25.
7. Wegner N, Wait R., Sroka A., Eick S, Nguyen K A., Lundberg K, Kinloch A., Culshaw S, Potempa J, Venables P J.(2010) .Peptidylarginine deiminase from *Porphyromonas gingivalis* citrullinates human fibrinogen and α-enolase: Implications for autoimmunity in rheumatoid arthritis. Arthr. & Rheum. ;62 (9): 2662–2672.
8. Lee J Y T, Sojar G S, Bedi R J.(1992). Synthetic peptides analogous to the fimbrillin sequences inhibit adherence of *Poryphomonas gingivalis*. Infect. Immun. ; 60: 1662- 1670.
9. Ogrendik M, Kokino S, Ozdemir F, Bird P S, Hamlet S.(2005)."Serum Antibodies to Oral Anaerobic Bacteria in Patients With Rheumatoid Arthritis". MedGenMed ;7 (2): 2.
10. Forbes B A, Daniel F S, Alice S W. Bailey and Scott's.(2007). Diagnostic Microbiology . 12th ed. ,Mosby Elsevier company . USA , ,pp 62-465
11. MacFaddin J F.(2000). Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams and Wilkins, USA; pp 57-800.
12. Mancini G, Carbonaro A O, Heremans J F.(1965). Immunochemical quantitation of antigens by single radial immunodifution. Immunochemistry; 2:235-54.
13. Tietz N W, Burtis C A., Ashwood E R, Saunders W B .(1999). Text Book Of Clinical Chemistry .3rd Ed ; pp 477-530.
14. Dawed K, Al-Yas Z A .(1991). Statastical Methods In Agricultural Researches: Mosul university . (Arabic)

15. Haffajee A D, Socransky S S.(2000). Microbial etiological agents of destructive periodontal diseases. Periodontol ; 5:78–111.

16. Moore W E, Moore L V. (2000). The bacteria of periodontal diseases. Periodontol; 5:66–77.

17. Michae G N, Fermin C, Henry T. Carranza's.(2002). Clinical Periodontology. 9th Ed., W.B. Saunders Co.

18. Honma1 Y, Sugita1 N, Kobayashi T, Abiko3 Y, Yoshie1 H.(2008).Lower antibody response to *Porphyromonas gingivalis* associated with immunoglobulin G Fcγ receptor IIB polymorphism. J.of Period. Research; 43: 706–711.

19. Kenichi I, Hiroshi H, Katsutoshi I, Tatsuo A, Mikio S, Koji N, Kazuhisa S. (2010). *Porphyromonas gingivalis* Peptidoglycans Induce Excessive Activation of the Innate Immune System in Silkworm Larvae. J. of Biolog. Chemis; 285: 33338-33347.

عزل وتوصيف لبكتريا *Porphyromonas gingivalis* وتحديد بعض المعايير المناعية لمرضى التهاب ماحول السن

نور سلمان الخفاجي[1] , فاطمة مالك الربيعي[2] , لمى جاسم وتوت[2]

و حسين عليوي الدهموشي[1] *

***ماجستير احياء مجهرية ,جامعة بابل - كلية العلوم - قسم علوم الحياة[1],ماجستير احياء مجهرية ,جامعة بابل – كلية طب الاسنان [2]. العراق**

الخلاصة

اجريت دراسة مناعية وبكتيرية على 150 مريض بالتهاب ما حول السن الحاد للفترة من 1-10-2010 الى 1-6-2011 في كلية طب الاسنان –جامعة بابل . كان عدد العينات التي اعطت نتيجة موجبة لنمو بكتريا *Porphyromonas gingivalis* على وسط اكار الدم هو 35 عينة وبنسبة (23.3%) في حين 115 عينة وبنسبة (76.7%) أعطت نتيجة ايجابية لانواع بكتيرية اخرى مثل *Streptococcus mutans* . بينت نتائج الفحوصات المناعية التي اجريت على امصال المرضى الذين اعطوا نتيجة ايجابية لزرع بكتريا *P. gingivalis* حدوث زيادة في كل المعايير المناعية المختبرة او المدروسة والتي شملت تركيز الاجسام المضادة (IgM, IgG, IgA) و مكوني المتمم (C3&C4)وكذلك تركيز البروتين الكلي , مقارنة مع الاشخاص الاصحاء (20 شخص) والذين لايعانون من التهاب ما حول السن الحاد فقد اعطوا نتائج طبيعية لكل المعايير المدروسة .