Porphyromonas gingivalis fimA genotyping in adult periodontitis population in Kerbala city

Alnasrawy, A.A. 1, Alsalihi, A.A. 2, Abo Almaali, H.M. 3 and Al-Khafaji, Y.A. 4

1,2 Genetic Engineering and Biotechnology Institute for Postgraduate studies-Baghdad University, 3 College of Pharmacy-Kerbala University, 4 Dentistry College – Babylon University, Iraq

Keywords: Periodontitis, *P. gingivalis*, fimA genotyping

Received (November), **Accepted** (December)

ABSTRACT

Periodontitis is a chronic bacterial infection affects the gingiva, periodontium connective tissues and alveolar bone, results in alveolar bone resorption ultimately, tooth loss, indeed, it may causes various serious systemic complications like diabetes mellitus, cardiovascular disorders, rheumatoid arthritis, preeclampsia with low birth weight and orodigestive cancer mortality. Numerous bacteria are associated with periodontitis, *Porphyromonas gingivalis* is considered the main, foremost and strongest periodontal pathogen involved in periodontal diseases. Fimbriae that are encoded by *fimA* gene have been considered the main and the first virulence factor of this bacterium involved in adhesion, colonization, invasion, establishment and persistence within the host. This study was conducted from September, 2013 to July, 2014 to investigate the prevalence of *P. gingivalis* throughout monoplex PCR of *16S rRNA* and multiplex PCR of *fimA* genes amplification in adult periodontitis population in kerbala city. Specific virulent clones of *P. gingivalis* may present in chronic and/or aggressive periodontitis. Some variations are found in the distribution of *P. gingivalis fimA* genotypes among periodontitis patients and the greater prevalence of *fimA* genotypes (II, IV) followed by (III, Ib) in adults with chronic periodontitis.

التنميط الوراثي لجين الاهلاب *fimA* genotyping لبكتريا *Porphyromonas gingivalis* في مجتمع البالغين المصابين بالتهاب اللثة وما حول السن في مدينة كربلاء

عادل عطية عبد علي النصراوي 1 ، علي عبد الامير الصالحي 1 ، حسن محمود موسى ابو المعالي 2 ، يونس عبد الرضا الخفاجي 3

1معهد الهندسة الوراثية و التقنيات الاحيائية للدراسات العليا- جامعة بغداد ، 2 كلية الصيدلة- جامعة كربلاء ، 3 كلية طب الاسنان – جامعة بابل ، العراق.

الكلمات المفتاحية: التهاب اللثة وماحول السن ، بكتريا *Porphyromonas gingivalis* ، الانماط الوراثية لجين *fimA*

الخلاصة

التهاب اللثة وماحول السن هو اصابة بكتيرية مزمنة تستهدف اللثة وماحول السن والانسجة الرابطة والعظم الحويصلي مسببة انهياره وبالتالي فقدان السن وكذلك العديد من المضاعفات الجهازية الخطرة مثل داء السكري، الاختلالات القلبية الوعائية، التهاب المفاصل الروماتزمي، الولادة قبل الاوان مع نقصان وزن المولود والهلاك نتيجة سرطانات الفم والجهاز الهظمي. العديد من البكتريا تشترك في التهاب اللثة وماحول السن، وتعد بكتريا *Porphyromonas gingivali*الرئيسية، الاشهرواقوى الجراثيم المسببة للمرض وتعتبراهلاب fimbriae البكتريا المشفرة بجين *FimA gene*عامل الضراوة الاساس الاول في التصاق، استعمار، غزو، وتموضع البكتريا المزمن داخل المضيف. اجريت هذه الدراسة للفترة من ايلول 2013 لغاية تموز 2014 لمعرفة مدى انتشار جرثومة *P. gingivalis* اعتمادا على تقنية تفاعل سلسلة البلمرة المتعدد لجيني البكتريا *P. gingivalis* وهما (*fimA* gene , *16S rRNA*) في مجتمع البالغين

المصابين بالمرض في مدينة كربلاء. ولوحظ تواجد سلالات ضارية من البكتريا متخصصة في التهاب اللثة وماحول السن المزمن والمدمر، وهناك بعض التباينات في توزيع الانماط الوراثية لجين بكتريا الفم (*P. gingivalis*)
(*fimA* genotypes) بين المرضى وكان اكثرها انتشارا هما النمطان (II , IV) يتلوها النمطين (Ib , III) بين المصابين بالمرض.

1. INTRODUCTION

Periodontal disease is the most common chronic inflammatory disorder in the tissues surrounding tooth in adult oral cavity. It is divided into two different disease types, gingivitis and chronic periodontitis [1]. Generally, periodontal diseases are very common, worldwide, they represent a serious oral health problem in adult populations, They have severe forms and they affect about 750 million people or about 10.8% of the population as of 2010 [2].

Numerous bacteria are associated with the initiation and progression of periodontitis [3] among which *Porphyromonas gingivalis* is considered the main, strongest, and foremost periodontal pathogen involved in onset of various forms of periodontal diseases [4]. It harbors an arsenal of virulence factors, including fimbriae, capsule, lipopolysaccharide, collagenases, proteases like gingipains, hemolysin, trypsin, hemagglutinins [5], among which; fimbriae that encoded by *fimA* gene have been considered the main virulence factor of this microorganism involved in colonization, invasion, establishment, and persistence within the host periodontal tissues [6] and may associated with increased risk of various systemic conditions including various cardiovascular diseases like myocardial infarction, atherosclerosis [7], diabetes mellitus[8], rheumatoid arthritis [9] , preeclampsia with low birth weight[10] and orodigestive cancer mortality [11].

Various studies have determined *P. gingivalis 16S rRNA* and *fimA* genotypes prevalence in different world populations, genotypic variability among *P. gingivalis* isolates have been found [12, 13].This study was done to determine the prevalence of *P. gingivalis* by *16SrRNA* and *fimA* genotypes in adult periodontitis patients in kerbala city.

2. MATERIALS AND METHODS

Study populations

One hundred seventy two patients aged between (2o and 70) years attending the Specialist Center of Dentistry, division of periodontology and the clinics of dentistry in Karbala governorate were included in this study during the period from September, 2013 to July, 2014. The patients were divided into three groups depending upon their periodontal status; the first group composed of patient with gingivitis, the second group with chronic periodontitis with a pocket depth with (3-8 mm) and the third group of aggressive periodontitis with attachment loss greater than 1 mm indeed periodontal healthy subjects (n=36), they didn't have any pocket depth or periodontal attachment loss.

Clinical examinations and Gingival Crevicular Fluid (GCF) sampling

The periodontitis patients were diagnosed clinically and a full examination of the entire mouth of each patient was conducted. The periodontal sites to be sampled were air-dried and isolated with cotton rolls, The supragingival plaque was first removed with a sterile Gracey curette, employed with care to avoid bleeding. A sterilized medium size (size 40, T.g., UK) two-four paper points were carefully inserted as deeply as possible into each gingival groove site (periodontal pocket) with a pocket depth (PD $\geq$ 5mm) and kept in site for 30-60 seconds then, the soaked paper points were transferred into 1.5 ml microcentrifuge eppendorf tube contains 1 ml phosphate-buffered saline (PBS, 50 mM potassium phosphate, 150 mM NaCl, pH 7.2) and subjected to molecular bacteriology detection. A pool of periodontal samples from periodontally healthy subjects was also obtained as a control group.

DNA Extraction

The Genomic DNA Mini Kit (Tissue) (Geneaid, Korea) was used for DNA isolation from periodontal samples according to the manufacturer's recommendations. Following extraction, the DNA samples were stored at (-20°C) to be used in molecular detection of *P. gingivalis*.

Detection of *P. gingivalis* by essential genes

Molecular detection of *P. gingivalis* was performed by monoplex polymerase chain reaction (PCR) of *16S rRNA* gene amplification according to[14] and multiplex PCR of species specific *fimA* gene amplification according to[14,15].

Amplification Primers set: this is shown in table 1.

Table 1: The Primer set were used in detection of *P.gingivalis*

Gene	Duplexing primers 5´- 3´	Product size (bp)	Reference
P. gingivalis 16Sribosomal RNA	AGG CAG CTT GCC ATA CTG CG ACT GTT AGC AAC TAC CGA TGT	404	[16]
Type I *fimA*	CTG TGT GTT TAT GGC AAA CTT C AAC CCC GCT CCC TGT ATT CCG A	392	[17]
Type Ib fimA	CAG CAG AGC CAA AAA CAA TCG TGT CAG ATA ATT AGC GTC TGC	271	[15]
Type II *fimA*	ACA ACT ATA CTT ATG ACA ATG G AAC CCC GCT CCC TGT ATT CCG A	257	[17]
Type III *fimA*	ATT ACA CCT ACA CAG GTG AGG C AAC CCC GCT CCC TGT ATT CCG A	247	[17]
Type IV *fimA*	CTA TTC AGG TGC TAT TAC CCA A AAC CCC GCT CCC TGT ATT CCG A	251	[17]
Type V *fimA*	AAC AAC AGT CTC CTT GAC AGT G TAT TGG GGG TCG AAC GTT CTG TC	462	[18]

Amplification Reaction programs: these are shown in tables 2,3.

Table 2: Cycling parameters for monoplex PCR amplification of ribosomal specific *16S rRNA* gene.

No. of Cycles	Stage	Temperature °C	Time
1	Initial denaturation	95	5 min.
35	Denaturation	94	30 Sec.
	Annealing	60	30 Sec.
	Elongation	72	1 min.
1	Final extension	72	10 min.

Table 3: Cycling parameters for Multiplex PCR of Species Specific ***FimA*** gene amplification.

No.ofcycles	Stage	Temperature °C	Time
1	Initial denaturation	95	5 min.
	Denaturation	94	30 Sec.

35	Annealing	58	30 Sec.
	Elongation	72	30 Sec.
1	Final extension	72	7 min.

Agarose Gel Electrophoresis

(1%) Agarose gel electrophoresis of PCR products was accomplished with the use of two types of DNA ladder (Accu Ladder 100 bp Bioneer/Korea) and (50 bp DNA Step Ladder Marker Promega/ USA).

Collected data were analyzed by using the available statistical system package of SPSS-18 (PASW statistical); Statistical analysis was done by using Chi-Square ($\chi 2$) test, Z test when applicable. P-value of ≤ 0.05.

3. RESULTS AND DISSCUSION

The number of positive samples for 16S *rRNA* gene were 140/172 (81.4%) and 9/36 (25%) in patients and control group respectively as demonstrated in figure 1.

Table 4: number and percentage of positive periodontal samples for 16S rRNA gene PCR Amplification.

16S *rRNA* gene	**Patients**		**Control**	
	No.	**%**	**No.**	**%**
Positive	140	81.4	9	25
Negative	32	18.6	27	75
Total	172	100	36	100

The prevalence of *P.gingivalis* in periodontitis patients in the current study was implicated in the frequency of *P. gingivalis* in periodontitis patients which is estimated within the range of 60 to 100%, while it is found in 11 to 25% of healthy subjects [19].

Because of the DNA sequence of *16S rRNA* gene is not unique, they may present in various oral flora or related periodontopathogens of dental plaques or subgingival biofilms. This is agreeing with previous epidemiological studies that revealed molecular detection and quantification of oral bacteria have been mostly achieved by analysis of the ribosomal genes [20, 21].

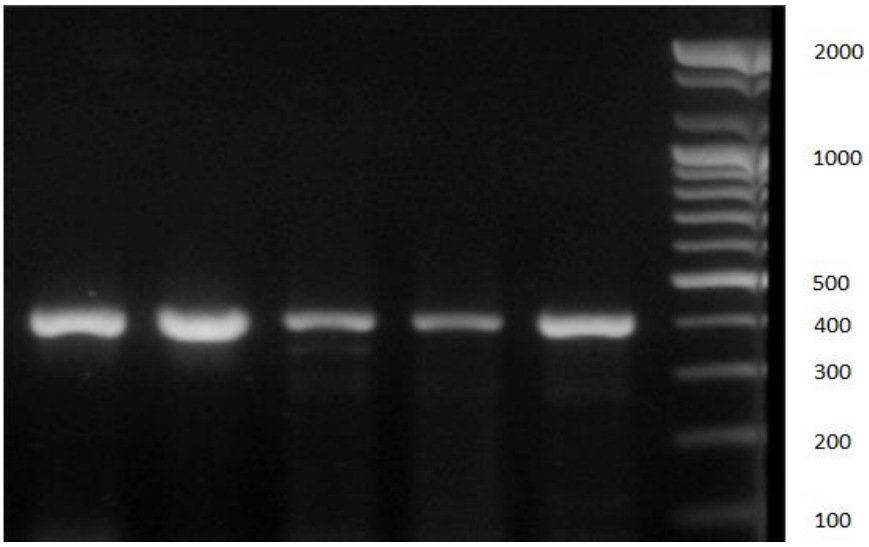

Figure 1: positive results of periodontal samples with *16SrRNA* gene amplification with lanes (404) bp. Lanes 1, 2, 3, 4, 5: PCR products; lane M: DNA ladder (100bp)

However, it has been shown that variations in ribosomal operon copy numbers among species and strains may impair proper detection, determination of cell levels in complex assemblages like periodontal environment in different populations [22]. On the other hand, the total number of periodontal patients that detected with Multiplex PCR Amplification of species specific *fimA* gene was 82(58.6 %) collectively, distributed as 50(61.0%), 19(23.2%) and13(15.8%) in Chronic periodontitis, Gingivitis and Aggressive periodontitis, respectively Table (5).

Table 5: number and percentage of positive periodontal samples with PCR Amplification of *FimA gene.*

Periodontal status	No.	%
Gingivitis	19	23.2
Chronic periodontitis	50	61.0
Aggressive periodontitis	13	15.9
Total	82	100

In the present study, *fimA* genotyping of *P. gingivalis* was conducted because *fimA* genotype/s and virulence FimA protein (fimbirillin) is encoded by the species specific *fimA* gene and occurs as a single copy in the chromosome of *P. gingivalis* [23]. This technique is agree with various clinical, epidemiological, and microbiological investigations depended upon both *16S rRNA* and species specific *fimA* genes for confirmatory diagnosis of *P.gingivalis* [24,25].

In the current study, all *P. gingivalis fimA* genotypes were successfully detected, they have a single band with product size for each allele of *fimA* gene (figures 2 and 3).

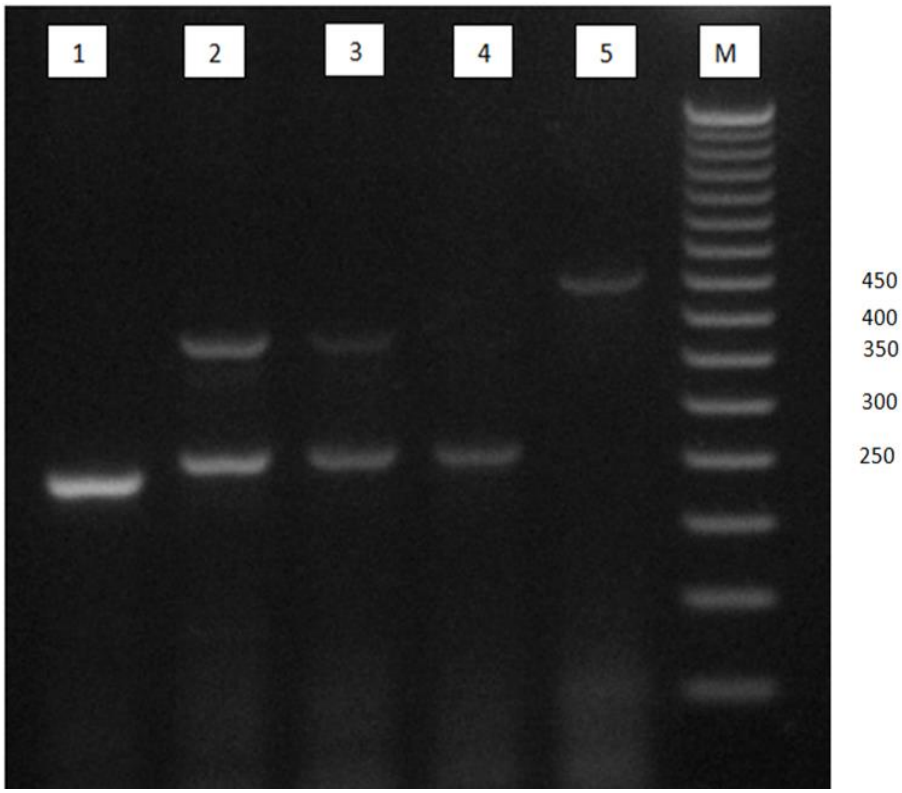

Figure 2: *P. gingivalis* positive periodontal samples for *fimA* genotypes lane (1) *fimA* genotype (III) 247 bp., lanes (2, 3) *fimA* genotypes (1V) 251bp, and *fimA* genotype (I) 392 bp., lane (4) *fimA* genotype (1V) 251bp., lane (5) *fimA* genotype (V) 462 bp.

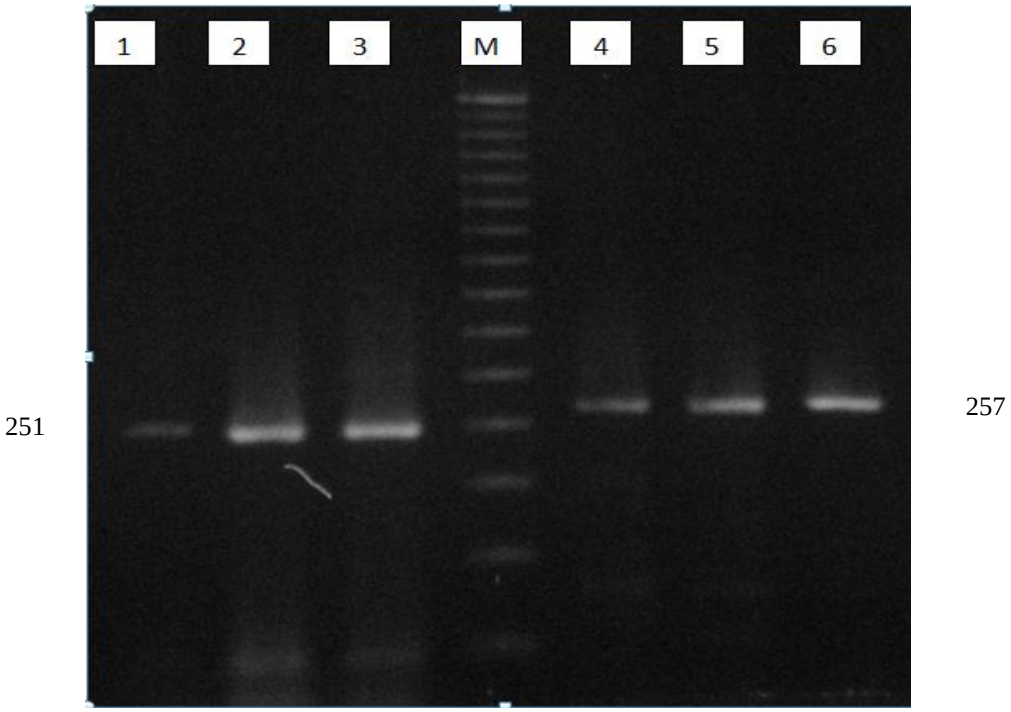

Figure 3: *P. gingivalis* positive periodontal samples for *fimA* genotypes. lanes (1,2,3) *fimA* genotype (1V) 251 bp, lane M= DNA Ladder (50 bp) lanes (4,5,6) *fimA* genotypes (II) 257 bp.

The prevalence of *fimA* genotypes among all of the periodontal patients in the present study were 15 (18.3%), 18 (22%), 70 (85%), 21 (25.6%), 34 (41.4) and 12(15%) in *fimA* genotypes I, Ib, II, III, IV, and V, respectively (Table 6).

Table 6: prevalence of *fimA* genotypes among the positive periodontitis patients for *P. gingivalis.*

***fimA* genotypes**	**Gingivitis**		**Chronic Periodontitis**		**Aggressive Periodontitis**		**Total *fimA* genotypes**		**P-value**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	
fimA genotype I	3	20	7	46.7	5	33.3	15	18.3	0.02
fimA genotype Ib	5	27.8	9	50	4	22.2	18	22	0.3
fimA genotype II	16	22.9	45	64.3	9	12.8	70	85	0.001
fimA genotype III	6	28.6	9	42.8	6	28.6	21	25.6	0.06
fimA genotype IV	6	17.6	22	64.8	6	17.6	34	41.4	0.008
fimA genotype V	2	16.7	8	66.6	2	16.7	12	15	0.3

The prevalence of *P. gingivalis fimA* genotypes in periodontal patients of the present study were relatively less than that of other epidemiological studies reported that *P. gingivalis* is very frequently present in the subgingival plaque of periodontal patients, ranging from 50.3% to 89.4% of cases [14, 17, 26, 27]. However, it has been demonstrated that this bacterium does not appear exclusively in periodontal patients but is also present in the subgingival plaque of periodontally healthy patients, although to a lesser extent, varying between 22.1% and 36.8% [14, 26].

In the present study, The most predominant *fim A* genotypes with statistically significant elevation of II, IV, followed by III and Ib, which were 70 (85%), 34 (41.4), 21(25.6%) and 18 (22%) respectively in table (6)., These results have an agreement with many similar clinical, epidemiological studies about *P. gingivalis fimA* genotyping in chronic marginal periodontitis. They revealed that *P. gingivalis* isolates with *fimA* genotypes II, IV, and Ib have been shown to be significantly more prevalent than isolates with other genotypes [15, 17, 18, 28]. (With one exception of *P. gingivalis fimA* genotyping III which appears relatively elevated 21(25.6%) in periodontal samples in current study) (table 6), this may be implicated in community hypervariable *P. gingivalis fimA* genotypes indicating positive selection as improved by other studies [29, 30].

In addition, *fimA* genotyping of cultured clinical strains of *P. gingivalis* sampled from individuals with periodontitis support the findings that genotypes II, IV, and Ib are related to virulence [31]. In Brazilian periodontal patients, it was found that genotype II was the most prevalent but in this case it was followed by Ib [32]. Similar findings have been reported in studies of the Chinese, Japanese [26] and Spanish populations [33] demonstrated genotype II was the most frequent in chronic periodontitis patients, although differences were found in the prevalence of the other genotypes.

Furthermore, (up to 15) periodontal samples exhibited (2-5) *fimA* genotypes in the same site of periodontal patients and no *fimA* genotypes in the control group enrolled in the current study but the distributions were different in the two groups, these investigations are

similar to a large number of previous experimental, clinical studies suggested various explanations have been proposed, such as the presence of several different *fimA* genotypes colonizing the same periodontal site [12, 17, 32]. Indeed, several investigations from Norway indicated a higher intraindividual heterogeneity of *P. gingivalis* than found earlier. Detection of multiple sequence types (MSTs) from one site in several patients with refractory periodontitis, showed allelic variation in two housekeeping genes indicating recombination between different clones within the periodontal pocket [31, 34].

Another progressive study improved some *fimA* genotypes may be important determinants of virulence for *P. gingivalis*, clonal heterogeneity of subpopulations with both high and low levels of pathogenicity has been suggested to exist among periodontal pathogens harbored by individuals with negligible, slight, or even severe periodontal destruction [35].

CONCLUSION

Specific virulent clones of *P. gingivalis fimA* genotypes may be the cause of chronic and/or aggressive periodontitis. Some variations are found in the distribution of *P. gingivalis fimA* genotypes among periodontitis patients, and the greater prevalence of *fimA* genotypes (II, IV) followed by (III, Ib) in adults with chronic periodontitis.

ACKNOWLEDGEMENTS

Authors are great thankful to assist Prof. Dr. Muhanad Muhsin Ahmed, assist prof. Dr. Hassan A. A. and Dr. Alaamry Ali Mansur for sharing their knowledge and facilities, and to all who participates in this work.

REFERENCES

[1] Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K and Taylor R. (2012). Periodontitis and diabetes: a two-way relationship, **J**. **Periodontol**., 55:21–23.

[2] Flaxman VT, Naghavi AD, et al. (2012). Asystematic analysis for the global burden of disease study, **Lancet**, 380: 21–96.

[3] Holt S, and Ebersole J. **(**2005). *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia*: the "red complex," a prototype polybacterial pathogenic consortium in periodontitis, **Periodontol**., 38:72–122.

[4] Inaba H, Nakano K, Kato T, Nomura R, Kawai S, Kuboniwa M, et al.(2008). Heterogenic virulence and related factors among clinical isolated of *Porphyromonas gingivalis* with type II fimbriae, **Oral Microbiol. Inmunol**., 23: 29-35.

[5] Mysak J, Podzimek S, Sommerova P, Lyuya M, JirinaBartova Y, Janatova T, Prochazkova T, and Duskova J. (2014). *Porphyromonas gingivalis*: Major periodontopathic pathogen overview, **J. Immunol. Res.,** Review Article ID 476068, 8 pages.

[6] Enersen M. Nakano K. and Amano A. (2013). *Porphyromonas gingivalis* fimbriae, **J. Oral Microbiol.**, 5: 20265.

[7] Boxi Z, Ateia EA, Khalaf H, Basic VT, Kartheyaene J., Robert K., Torbjörn B. and Allan S. (2013). The periodontal pathogen *Porphyromonas gingivalis* changes the gene expression invascular smooth muscle cells involving the TGFbeta/Notch signalling pathway and increased cell proliferation, **BMC Genomics**, 14:770.

[8] Pınar Gümüş and Nurcan Buduneli. (2013). DIABETES MELLITUS AND PERIODONTITIS: SIGNS OF A BIDIRECTIONAL RELATIONSHIP. **EMJ Diabet**., 1:30-36.

[9] Koziel J, Mydel P and Potempa, J, (2014). "The link between periodontal disease and rheumatoid arthritis: an updated review," **Current Rheumatology Report**, vol. 16, article 408.
[10] Perez-Chaparro P, Gracieux P, Lafaurie G, Donnio P, Bonnaure Mallet M. (2008). Genotipic characterization of *Porphyromonas gingivalis* isolated from subgingival plaque and blood sample in positive subjects with periodontitis **J Clin Periodontol.**, 35: 748-53.
[11] Jiyoung Ahn, Stephanie Segers and Richard B. Hayes. (2012). Periodontal disease, *Porphyromonas gingivalis* serum antibody levels and orodigestive cancer mortality, **Carcinogenesis,** 33(5):1055–1058.
[12] Abusleme L, Pozo P, Silva N. (2009). Genotipificacion de *Porphyromonas gingivalis* en pacientes con periodontitis, **Rev Clin Period Implantol Rehabil Oral**, 2: 54-8.
[13] Nagano K, Hasegawa Y, Murakami Y, Nishiyama S, and Yoshimura F. (2010). FimB regulates FimA fimbriation in *Porphyromonas gingivalis,* **J Dental Res.,** 89: 902-8.
[14] Amano A, Kuboniwa M, Nakagawa I, Akiyama S, Morisaki I and Hamada S. (2000). Prevalence of specific genotypes of *Porphyromonas gingivalis fimA* and periodontal health status, **J Dent Res.,** 79:1664-8.
[15] Nakagawa I, Amano A, Ohara-Nemoto Y, Endoh N, Morisaki I, Kimura S, Kawabata S and Hamada S. (2002). Identification of a new variant of *fimA* gene of *Porphyromonas gingivalis* and its distribution in adults and disabled populations with periodontitis, **J Periodontal Res.,** 37, 425-432.
[16] Goncharoff P, Figurski DH, Stevens RH and Fine DH. (1993). Identification of *Actinobacillus actinomycetemcomitans*: polymerase chain reaction amplification of lktA-specific sequences. **Oral Microbiol Immunol.,** 8: 105-110.
[17] Amano A, Nakagawa I, Kataoka K, Morisaki I and Hamada S. (1999). Distribution of *Porphyromonas gingivalis* strains with *fimA* genotypes in periodontitis patients, **J Clin Microbiol.**, 37: 1426-1430.
[18] Nakagawa I, Amano A, Kimura RK, Nakamura T, Kawabata S and Hamada S. (2000). Distribution and molecular characterization of *Porphyromonas gingivalis* carrying a new type of fimA gene, **J Clin Microbiol.**, 38: 1909-1914.
[19] Van Winkelhoff AJ, Loos BG, Van der Reijden WA and Van der Velden U. (2002). *Porphyromonas gingivalis*, *Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction, **J Clin Periodontol.,** 29(11):1023-8.
[20] Lyons SR, Griffen AL and Leys EJ. (2000). Quantitative real-time PCR for *Porphyromonas gingivalis* and total bacteria, **J Clin Microbiol.,** 38:2362–5.
[21] Martin FE, Nadkarni MA, and Jacques NA, et al. (2002). Quantitative microbiological study of human carious dentine by culture and real-time PCR: association of anaerobes with histopathological changes in chronic pulpitis. **J Clin Microbiol**., 40: 1698–704.
[22] Lee ZM, Bussema C 3rd and Schmidt TM. rrnDB. (2009). Documenting the number of rRNA and tRNA genes in bacteria and archaea, **Nucleic Acids Res**., 37:489–93.
[23] Nelson KE, Fleischmann RD, DeBoy RT, Paulsen IT, Fouts DE and Eisen JA, et al. (2003). Complete genome sequence of the oral pathogenic bacterium *Porphyromonas gingivalis* strain W83, **J Bacteriol.**, 185: 5591_601.
[24] Fumiko Hayashi, Mitsugi Okada, Yuki Oda, Taro Kojima and Katsuyuki Kozai1. (2012). Prevalence of *Porphyromonas gingivalis fimA* genotypes in Japanese children, **Journal of Oral Science**, 54(1):77-83.
[25] Moreno, Sandraa; Contreras, and Adolfob. (2013). Functional differences of *Porphyromonas gingivalis* fimbriae in determining periodontal disease pathogenesis: a literature review, **Colombia Médica.**, 44(1): 51-60.

[26] Zhao L, Wu YF, Meng S, Yang H, OuYang YL and Zhou XD. (2007). Prevalence of *fimA* genotypes of *Porphyromonas gingivalis* and periodontal health status in Chinese adults, **J Periodontal Res.**, 42:511-7.
[27] Herrera D, Contreras A, Gamonal J, Oteo A, Jaramillo A and Silva N, et al., (2008). Subgingival microbial profiles in chronic periodontitis patients from Chile, Colombia and Spain **J Clin Periodontol.,** 35:106-13.
[28] Perez P, Lafaurie G, Gracieux P1, Meuric V1 Tamanai Z, Castellanos J and Bonnaure-Mallet M. (2009). Distribution of *Porphyromonas gingivalis fimA* genotypes in isolates from subgingival plaque and blood sample during bacteremia, **Biomedica**, 29: 298-306.
[29] Bryson K, McGuffin LJ, Marsden RL, Ward JJ, Sodhi JS and Jones DT. (2005). Protein structure prediction servers at University College London, **Nucleic Acids Res**., 33: 36_38.
[30] Laerdal J, and Enersen M. (2010). Preliminary study of *Porphyromonas gingivalis* major fimbriae (FimA). Unpublished data, **University of Oslo**.
[31] Enersen M, Olsen I, Kvalheim O and Caugant D. (2008). *fimA* Genotypes and Multilocus Sequence types of *Porphyromonas gingivalis* from patients with periodontitis, **J Clin Microbiol**., 46: 31-42.
[32] Missailidis CG, Umeda JE, Ota-Tsuzuki C, Anzai D and Mayer M. (2004). Distribution of *fimA* genotypes of *Porphyromonas gingivalis* in subjects with various periodontal conditions, **Oral Microbiol Inmunol**., 19:224-9.
[33] Miriam Puig-Silla, Francisco Dasí-Fernández, José-María Montiel-Company, and José-Manuel Almerich-Silla. (2012). Prevalence of *fimA* genotypes of *Porphyromonas gingivalis* and other periodontal bacteria in a Spanish population with chronic periodontitis, **Med Oral Patol Oral Cir Bucal**., Nov 1;17 (6):e1047-53.
[34] Morten Enersen. (2011). *Porphyromonas gingivalis*: a clonal pathogen (Diversities in housekeeping genes and the major fimbriae gene). **Journal of Oral Microbiology,** 3: 8487 - 8489.
[35] Morten Enersen, Kazuhiko Nakano and Atsuo Amano. (2013). *Porphyromonas gingivalis* fimbriae. **Journal of Oral Microbiology**, 5: 20265.