http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v8i8.37

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Efficacy of Adjunctive Use of Chlorohexidine versus Essential Oil Mouthwashes on *Porphyromonas gingivalis Fim-A genotypes*. A Real Time-polymerase chain reaction study

Authors
Dina H.Younes[1], Mai S. Attia[2*], Nalgaa F. Abd El Haliem[3], Ossama A. Elshall[4]
[1]Lecturer of Periodontology, Oral Medicine and Dental Radiology, Ahram Candian University
[2]Associate Professor, Department of Oral Medicine, Periodontology, Diagnosis and Radiology, Faculty of Dental Medicine, Al-Azhar University (Girls Branch), Cairo, Egypt
[3]Associate Professor, Microbiology & Immunology Department, Faculty of Medicine (for Girls), Al Azhar University, Cairo, Eygpt
[4]Professor, Department of Oral Medicine, Periodontology, Diagnosis and Radiology, Faculty of Dental Medicine, Al-Azhar University (Girls Branch), Cairo, Egypt
*Corresponding Author
Mai Shafik Attia
125 Omr Abn Elkhatab Street, Al-Zahraa Buildings, Nasr City, Egypt

Abstract
Objective: *The objective of this study was to investigate the subgingival infection of P. gingivalis isolates with genetic variation in patients with severe untreated periodontitis and to evaluate the efficacy of antiseptic mouthwashes in periodontal infection.*
Background: *P. gingivalis is a predominant periodontal pathogen that expresses several potential virulence factors with considerable genotypic diversity, and this may be the reason that explains its presence in a healthy patients and in patients with severe periodontitis.*
Subjects and Methods: *30 patients with untreated severe periodontitis randomly divided into: Group I (10): treated with supragingival scaling only (control group), Group II (10): treated with supragingival scaling and essential oil containing antiseptic mouthwash and Group III (10): treated with supragingival scaling and chlorhexidine containing antiseptic mouthwash. Clinical parameters were carried out at baseline and after one week. Samples were collected for anaerobic cultivation and for genotyping by using Real-time polymerase chain reaction (PCR).*
Results: *reduction was observed and significant in all clinical parameters after chlorhexidine and essential oil mouthwashes. However, group III which received Chlorohexidine mouthwash revealing the greatest improvement at the end of the study period. P. gingivalis was detected in 32.5% and 30% of 30 periodontitis patients by conventional culture and Real-time PCR methods, respectively. Fim-A genotypes of P.gingivalis showed that Fim-A genotype IV was the predominant (16.6%) followed by Fim-A II(13.8%) and Fim-AI(11.1%) as identified by PCR.*
Conclusion: *Adjunctive use of chlorhexidine mouthwash was more effective than essential oil mouthwashes as assessed by clinical and microbiological parameters.*
Keywords: *Periodontitis, P.gingivalis, Fim-A genotypes, conventional culture and RT-PCR technique.*

Introduction

Periodontitis is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilm and characterized by progressive destruction of the tooth supporting apparatus .Its main features include loss of periodontal tissue support, manifested by loss of clinical attachment (CAL) and alveolar bone loss assessed radiographically, periodontal pocket and gingival bleeding. [(1)]The majority of periodontal viruelance pathogens typically include gram-negative anaerobic bacteria such as Porphyromonas gingivalis, *Prevotella intermedia, Tannerella forsythensis and Treponemadenticola are strictly anaerobic and Aggregatibacter* actinomycetemcomitans and Campylobacter rectus are facultative. [(2)]

The different microorganisms in the periodontal environment were presented by Socransky and Haffajee[(3)] and united in periodontal complexes . [(4)]Among the red complex, P. gingivalis is present in periodontal pockets that suffer destruction, as well as in healthy gingival margins .[(5,6)] P. gingivalis is a microorganism with considerable genotypic diversity ;Therefore, we can find clones that are more pathogenic than others .This may be the reason why bacteria are present in healthy patients with no signs of periodontitis and in patients with severe periodontitis, where there is evidence of significant destruction of supporting tissue.[(7)]

Among the P. gingivalis virulence factors there are fimbriae which have been considered to be the major virulence factor of this microorganism since it gives it the ability to adhere and invade tissues .In addition, it may also interact with the immune response-dependent inflammatory response in stimulating the secretion of potent inflammatory cytokines, which characterizes its high pathogenicity to periodontal tissue, recurring transient bacteremia leading to high concentrations of systemic cytokines and chemokines.[(6, 8)]

Fimbriae are filamentous components on the cell surface composed of a subunit protein called fimbrillin, which is encoded by a gene denominated *fimA* of which only one copy exists in the *P. gingivalis* chromosome. To date, six fimA genotypes (I, Ib, II, III, IV, V) have been found based on their nucleotide sequence .[(9)] This variant led to the development of a PCR-based fimA genotyping method that identifies possible relationships between the different genotypes, virulence and disease.[(6)]

The effect of selected chemicals on oral tissues and their action against microorganisms have been intensively studied both in vitro,ex vivo and in vivo.[(10-13)]These substances should help in avoiding the proliferation of anerobic pathogens in protected oral niches (spaces where mechanical cleaning is more difficult), which may act as reservoir of aggressive bacteria in susceptible individuals .[(14)] As a matter of fact, the reduction in the use of antibiotics should be one of the goals of current medical and dental therapy, considering the increased risk of developing resistant strains.[(15-16)]

Among different mouthwashes chlorhexidine (CHX) remains gold standard among all.[(17)]This substance couples both bactericidal and bacteriostatic effects. [(13)] It has broad antibacterial activity, with very low toxicity and strong affinity for epithelial tissue and mucous membranes.[(17)]On the other hand, essential oil (EO) mouth wash have antimicrobial and anti-inflammatory activities [(18)] and are considered the best alternative to CHX for plaque control. In addition, they are as efficacious as CHX for gingivitis.[(19)]

Due to the strong association between certain microorganisms and periodontal diseases, there is an increasing interest in the use of antimicrobials in their management .Therefore, the aim of this study was to investigate the efficacy of the use of antiseptic mouthwash in subgingival infection of P. gingivalis isolates with its genetic variation in untreated patients with severe periodontitis.

Subjects and Methods

A minimum sample size required for the study was 30 participants according to P. gingivalis count with effect of size was 0.782174, α was 0.05 and β was 0.95 and they were divided into three groups (10 each) who fulfilled the inclusion and exclusion

criteria. patients with untreated severe periodontitis (P) who referred to outpatient clinic, department of Oral Medicine, Periodontology, Diagnosis and Radiology ,Faculty of Dental Medicine for Girls, Al Azhar University. They were 15 males and 15 females with their ages ranged from 25 to 50 years. The study protocol was approved by Research Ethic Committee, Faculty of Dental Medicine for Girls, Al-Azhar University. The individuals were informed about the treatment process, and all of them signed consent forms voluntarily.

The selected patients fulfilled the following inclusion criteria: (1) patients were free from any systemic disease as evidenced by health questionnaire using cornel medical index[20]; (2) Each patient should possess a minimum of 20 teeth and has generalized severe periodontitis with probing depth ≥5mm in each quadrant; Whereas the exclusion criteria were: (1) Patients have congenital valve defects or any other risk situation infectious endocarditis; (2) smokers (3) pregnant females as well as breast feeding mothers;(4) History of periodontal surgery or antimicrobial therapy in the three months preceding the start of our study; and (5) participants allergic to the active ingredients in one of the mouthwashes were excluded from the study.

Clinical Parameters included Plaque Index (PI)[21],Gingival Index (GI)[22] and Probing Depth (PD)[23] were recorded at baseline and after one week for all groups. Probing depth measurements were performed using William's probeat six sites per tooth and recorded to the nearest millimeter .The deepest probing depth was included.

Study Design and Radomization: Each patient was asked to pick an envelope from several opaque sealed envelopes after fulfillment of the inclusion criteria and signing the informed consent to be enrolled in the study. The envelope contained the group to which the selected patient was allocated. Patients were divided into: Group I (10) treated with supragingival scaling and instructed good oral hygiene only (control group); Group II (10) treated with supragingival scaling and received essential oil containing antiseptic mouthwash (*Johnson & Johnson health care products, Division of McNEILPPc, Icc*.) 3 times/day for 1week and Group III (10) treated with supragingival scaling and received 0.12 Chlorhexidine gluconate containing antiseptic mouthwash (*Antiseptal, Kahira Co. for Pharm. And Chem., IND Cairo-ARE*) 3 times/day for 1week.

Participants were given either Group A or Group B mouthwash according to their random allocation. All participants received a similar toothbrush, toothpaste and tooth brushing technique and were instructed to rinse their mouths with the appropriate mouthwashes three times daily at the follow-up . Clinical records were resumed after 7 days.

Collection of Samples: Samples of plaque were collected from the deeper periodontal pocket taken from the selected quadrant at baseline. Sample was taken by means of sterile curette that was inserted to the bottom of the periodontal pocket. The collected sample immediately transferred into screw-caped micro-tubes containing 2 ml of thioglycolate broth as an anaerobic transporting medium. After one week, second plaque sample was taken, followed by full mouth scaling and root planning.

Processing of Samples: samples of plaque were sent to the microbiology laboratory and processed within 2 hours. After vortexing for 1 minute a homogenous suspension was divided into two aliquots. One was used for aerobic as well as anaerobic cultivation using gas pack system at 37ºC on Kanamycin Blood Agar; only those organisms which failed to grow aerobically were taken as anaerobes. The other was frozen at -20ºC for Real-time PCR at the molecular biology unit (MIU). Identification of *P. gingivalis* was based on typical colony morphology, pigmentation, haemolysis and gram stain reaction. Number of organism in each sample was expressed in colony forming unit/ml (CFU/ml). If no growth was obtained after 48-72 hrs, reincubation was done for at least 7 days for giving negative reports.(figure 1)

Real-time PCR:

DNA extraction from plaque:

Plaque DNA was extracted using the High Pure PCR template preparation kit (Roche Applied Science, Rotkreuz, Switzerland) with the following modifications: after thawing and vortexing, 50 µl aliquots of each sample were added to 150 µl sterile DNase-free H2O in Eppendorf microtubes (Vaudaux, Switzerland) and heated for 10 minutes at 95ºC. Digestion with proteinase K and isolation of DNA were carried out following the manufacturer's instructions.

Real-time PCR technique:

RT-PCR was carried out on a Light Cycler System (Roche Applied Science) using the Fast Start DNA Master SYBR Green I kit (Roche Applied Science). The sequences of *P. gingivalis* 16S rRNA gene primers used for qualitative detection were:

Forward primer: 5´-TGCAACTTGCCTTACAGAGGG-3´
Reverse primer: 5´- ACTCGTATCGCCCGTTATTC-3´

Primer sets used for *P. gingivalis fimA* genotyping:

Type I fimA:
Forward 5´-CTGTGTGTTTATGGCAAACTTC-3´
Reverse 5´-AACCCCGCTCCCTGTATTCCGA-3´

Type II fimA:
Forward 5´-ACAACTATACTTATGACAATGG-3´
Reverse 5´-AACCCCGCTCCCTGTATTCCGA-3´

Type III fimA:
Forward 5´-AATTACACCTACACAGGTGAGGC-3´
Reverse 5´-AACCCCGCTCCCTGTATTCCGA-3´

Type IV fimA:
Forward 5´-CTATTCAGGTGCTATTACCCAA-3´
Reverse 5´-AACCCCGCTCCCTGTATTCCGA-3´

Type V fimA:
Forward 5´-AAC AAC AGT CTC CTT GACAGTG -3´
Reverse 5´-TAT TGG GGG TCG AACGTT ACT GTC-3´

PCR amplification was performed with a total reaction mixture volume of 20 µl containing; 5 µl Fast Start DNA SYBR Green master mix; 3 µM MgCl2; 5 µl Purified Template DNA and 1 µM of each primer. Samples were submitted to an initial amplification cycle of 95ºC for 10 minutes. This was followed by 40 cycles of denaturation of DNA at 95ºC for 10 seconds, annealing of primers at 58ºC for 5 seconds and extension at 72ºC for 20 seconds.

The fluorescent products detection was monitored once each cycle selectively in the area around the melting point. After amplification, melting curve analysis which is the software analysis used for the detection of the genome of microorganism by measuring the specific melting temperature and express it in the form of a melting curve (it was carried out in the range from 65ºC to 95ºC).*(figure 2)* For the interpretation of results: Qualitative detection analysis is the software analysis used to translate the data resulting from the amplification curves detecting the presence of the *P.gingivalis* strain in each sample.

Statistical Analysis

Data was analyzed Statistical Package for Social Science (SPSS) version 16. Comparing the mean ± SD of two groups was done using paired and unpaired students test. Detecting the sensitivity, the specificity, the positive predictive value (PPV) and the negative predictive value (NPV) of the test were calculated. P value > 0.05 is considered non-significant, < 0.05 is considered significant, and < 0.01 is considered highly significant.

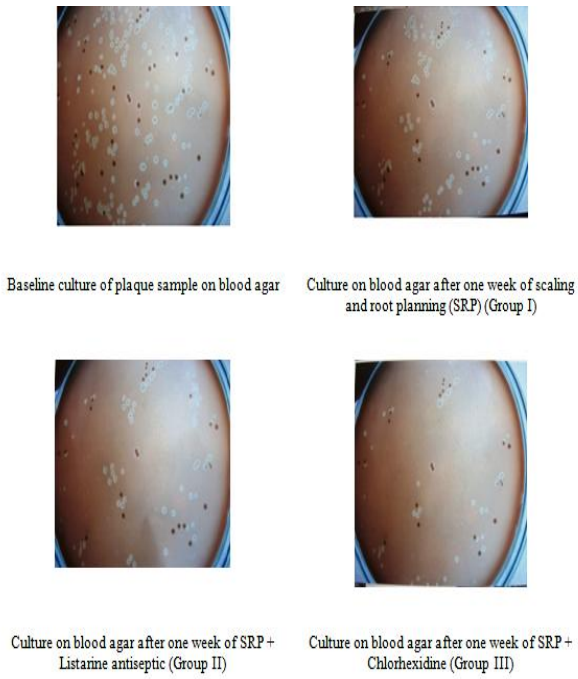

Fig. 1 Culture results

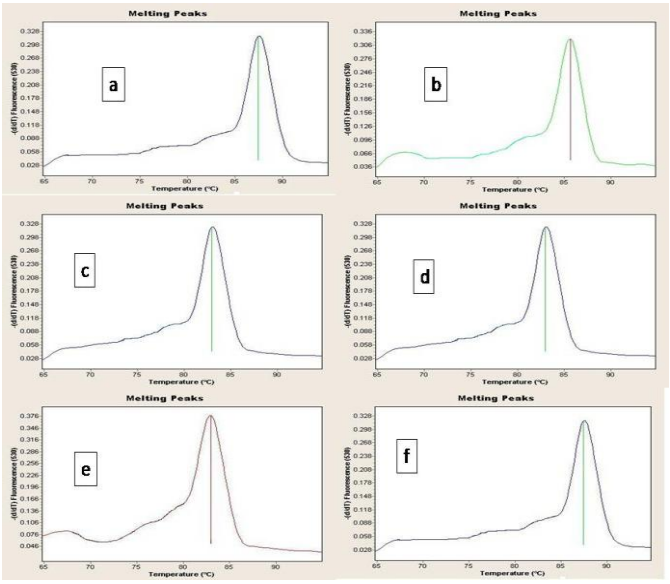

Fig. 2 Melting curve (it was carried out in the range from 65ºC to 95ºC).

Results

I. Clinical parameters

Plaque index

The statistical analysis between the three groups in the percent change of plaque index showed a significant difference between them at one week. It was 23.33 ±21.08 % for the first group, 56.67 ± 19.56 % for second group and 71.67 ± 20.86 % for third one respectively. Moreover, the highest reduction in plaque index was reported in group III 71.67 ± 20.86 % which received chlorohexidine mouth wash (Table 1).

Gingival index

Regarding the mean percent change gingival index the three groups showed a significant difference between them over one week readings with group III revealing greater reduction in GI at the end of the study period which reached 65 ± 31.87%. The mean percent change in gingival index was 41.67±25.15 % and 45 ± 19.33 % respectively in group I and II. While, group III showed a mean percent change in gingival index 65 ± 31.87%at one week (Table 1).

Probing depth

The mean percent change in (PD) in group I showed a decrease in probing pocket depth by 17.39± 5.16 % at one week. While in group II, there was a decrease in probing pocket depth by 22.30 ± 4.29 % at one week and reduced by 23.61 ± 5.2% in group III. The statistical analysis between the groups regarding mean percent change in probing pocket depth showed that there was a statistically significant difference. However, group III showed the greatest reduction (23.61 ± 5.2%) in probing pocket depth measurements at the end of the study (Table 1).

II. Microbiology and Real-time PCR results

A Microbiological study was conducted to detect *P.gingivalis* in adult chronic periodontitis patients in 3 groups. Comparison was done between anaerobic culture & Real-time PCR (considering culture as gold standard). *P. gingivalis* was detected in 39 (32.5%) out of 120subgingivalplaque samples (40 isolates from plaque per group) by conventional anaerobic culture of 30 periodontitis patients (Table2).

P. gingivalis was detected in 36 (30%) out of 120subgingival plaque samples by Real-time PCR of 30 patients with untreated severe periodontitis (Table 3).

Intra-group comparison between anaerobic culture method and Real-time PCR technique for *P. gingivalis*–positive subgingival plaque samples showeda statistical significant difference was detected in Group I, while it was not detected in Group II & III. (Table 2&3).

Real-time PCR results were matching those obtained with conventional anaerobic culture in 92.3% of cases (i.e: 3 samples were culture-positive and Real-time PCR-negative). The sensitivity, specificity, positive and negative predictive values of Real-time PCR to be 92.3%, 100%, 100% and 96.43%, respectively.

Table (1): The mean percent change in PI,GI and PD in group I, II and III.

Parameters	Group I Mean±SD	Group II Mean±SD	Group III Mean±SD	P value
Plaque index (PI)	23.33±21.08	56.67±19.56	71.67±20.86	HS
Gingival index (GI)	41.67±25.15	45±19.33	65±31.87	HS
Probing depth (PD)	17.39±5.16	22.30±4.29	23.61±5.2	HS

P value ≤ 0.001 : Highly significant (HS)

Table (2): Culture results of *P. gingivalis* among the studied groups.

	Plaque (baseline) N=30	Plaque (1 week) N=30	*P value*
Group I	8(26.6%)	6(20%)	< 0.05 (S)
Group II	6(20%)	4(13.3%)	> 0.05 (NS)
Group III	4(13.3%)	2(6.6%)	> 0.05 (NS)

P value ≤ 0.005 : Significant (S)

Table (3): Real-time PCR results of *P. gingivalis* among the studied groups

	Plaque (baseline) N=30	Plaque (1 week) N=30	*P value*
Group I	6(20%)	6(20%)	< 0.05 (S)
Group II	6(20%)	4(13.3%)	> 0.05 (NS)
Group III	3(10%)	2(6.6%)	> 0.05 (NS)

P value ≤ 0.005 : Significant (S)

Distribution and frequencies of *fimA* genotypes of *P. gingivalis*–positive samples were summarized in (table 4). *FimA* genotypes I, II, IV were detected in 4/36 (11.11%), 5/36 (13.8%), 6/36 (16.6%), respectively, among 36 isolates were identified by real-time PCR. Co-infection with more than one genotype was observed in 6/36 (16.6%) of the ninths isolates which caused bacteraemia. *FimA* genotype IV was the predominant one followed by *fimA* II and *fimA*I. *FimA* III and V were not detected throughout all the studied groups of patients (Table 4).

Table (4): Frequencies of *fim A* genotypes of *P. gingivalis*–positive samples among the studied groups

	Plaque(baseline) N=30	Plaque(1 week) N=30	*P value*
Group I			
fimA I	4 (13.3%)	4 (13.3%)	> 0.05 (NS)
fimA II	2 (6.6%)	2 (6.6%)	> 0.05 (NS)
fimA III	ND	ND	-
fimA IV	5 (16.6%)	5 (16.6%)	> 0.05 (NS)
fimA V	ND	ND	-
Group II			
fimA I	0	0	-
fimA II	2 (6.6%)	2 (6.6%)	> 0.05 (NS)
fimA III	ND	ND	-
fimA IV	0	0	-
fimA V	ND	ND	-
Group III			
fimA I	0	0	-
fimA II	1 (3.3%)	1 (3.3%)	> 0.05 (NS)
fimA III	ND	ND	-
fimA IV	1 (3.3%)	1 (3.3%)	> 0.05 (NS)
fimA V	ND	ND	-

P value > 0.005 : Non signifiant (NS)
ND : Not detected = Co-infection with more than one genotype was observed .

Discussion

This work was conducted to investigate subgingival infection of *P.gingivalis* isolates with genetic variation in periodontitis (P) patients and to evaluate the efficacy of using antiseptic mouthwashes on periodontal infection. Although, bacterial culture being the gold standard for the growth and identification of *P. gingivalis*, however, Real-time PCR has proven to be a sensitive and rapid method for detecting and quantifying individual microbial species.[24] The present study therefore combined both microbiological and molecular techniques to provide a more accurate and faster detection method.

The data from this study are in full agreement with the results of several studies and confirm that both chlorhexidine and listerine are highly effective in reducing plaque, gingivitis and periodontitis[25-27]

The finding of previous studies demonstrated the beneficial effects of Chlorehexidine digluconate and Listerine antiseptic**,** though CHX is proven to be significantly better than Listerine**..**

The outcome of the current research showed a significant reduction in plaque index and gingival index in group II and group III compared to the baseline. This could be attributed to the proper scaling and root planing, the improvements in self performed oral hygeine measures as well as antiplaque and anti-inflammatory effects of chlorhexidine and Listerine mouthwashes.

Effectiveness of 0.2% CHX in reducing the clinical parameters was superior in the current study, which is parallel to results of ***Cortelli et al.[28](2009), and Rathand Singh (2013).***[29] CHX is the gold standard among antimicrobial agents whose chemical agent has been continually studied to reduce the formation of plaque and plaque-induced gingivitis .Positively charged CHX molecules are rapidly attracted to the bacterial cell membrane, which is negatively charged, resulting in damage and leakage of intracellular components .This leads to cell death.[30] The mean percent reduction in plaque index in the present study was 23.33%, 56.67%, and 71.76% in Group I, II, and II, respectively. The largest reduction in Group III could be due to the

anti-plaque effect of 0.2% chlorhexidine, which causes proteins precipitation and coagulation in microbe's cytoplasm. This finding was parallel with several studies.[29, 31]

In group III, the gingival index had been reduced about 65% at one week compared to the baseline. This was in accordance with [25] who reported the same reduction in the group which received (CHX) at the first week. This could be attributed to the similarity in the study period time and the age group shared in both studies. In contrast, this was not according to ***Rathand Singh (2013)***[29] who recorded a more reduction in GI about 84%. This may be attributed to the short study period of the present study (one week) and long study period of latter study (90 days).

When comparing anaerobic culture and Real-time PCR technique results for *P. gingivalis*–positive samples, *P. gingivalis* was detected in 39 (32.5%) and 36 (30%) out of 120 plaque of 30 cperiodontitis patients by conventional culture and Real-time PCR methods, respectively.

This finding is in accordance with Khalil et al .***(32)*** who found that subgingival plaque samples in (23.8%) were positive cultures for P. gingivalis and the prevalence of P. gingivalis by real-time PCR(33.3%).In addition, P. gingivalis was detected in 111(43%).of 259 subgingival plaque samples based on culture and 138 (53%) samples by real-time PCR .On the other hand, these results are inconsistent with Puig-Silla et al., (2012) [33] who found that P. gingivalis was detected in (66.7%) subgingival plaque samples in periodontitis patients with PCR

In our study, the real-time PCR results in 92.3% of the cases were consistent with those of the anaerobic culture (i.e 3 samples were culture-positive and real-time PCR negative) .The sensitivity, specificity, positive and negative real-time PCR predictive values are 92.3%, 100%, 100% and 96.43%, respectively

Our results were in contrary with a study done by ***Khalil et al.,(2013)***[32] who calculated the sensitivity and specificity values to detect *P. gingivalis* in subgingival plaque samples, with Real-time PCR, were 100%, and 93.8%. For anaerobic culture, were 89.5% and 66.7%, respectively. And a sensitivity of 100% and a specificity of 94% comparing cultivation and Real-time PCR was reported in a previous study[34]. Also, ***Fawzi et al., (2011)*** [35] were reported that Real-time PCR results were matching those obtained with anaerobic culture in 95.7% of cases.

The results of the present study was in accordance to *D'Ercole* ***et al., (2008)***[36] who reported a comparison between culture and PCR procedures in their ability to detect *P. gingivalis* in subgingival plaques, the sensitivity and specificity values were 87.26% and 58.62% by anaerobic culture and 36.17% and 94.48% by PCR.

On studying the distribution and frequencies of *fimA* genotypes of*P. gingivalis*–positive samples.*FimA* genotypes I, II, IV were detected in 4/36 (11.11%), 5/36 (13.8%), 6/36 (16.6%), respectively, among 36 isolates were identified by Real-time PCR. Co-infection with more than one genotype was observed in 6/36 (16.6%) of the ninths isolates which caused bacteraemia. *Fim A* genotype IV was the predominant one followed by *fimA* II and *fimA*I. *FimA* III and V were not detected throughout all the studied groups of patients**.**

Our results was agreed with a study by *Puig-Silla et al* .***(2012)***[33] who reported a strong association between genotypes types II and IV of P. gingivalis fimA and chronic periodontitis. Although genotype IV was more common in the gingivitis group (31.3%), the most common genotype in periodontal patients is II, followed by IV and I (39.4%, 15.2% and 12.1%) .Co-infection with more than one genotype was observed between fimA genotypes II and IV in (3%) and genotypes I, II and Ib in (9.3%) in chronic periodontitis .While fimA genotype III was detected in (9.3%), genotype V was not detected at all studied groups.

Alnasrawy (2014) [37] had found that the greater prevalence of *fimA* genotypes (II, IV) followed by (III, Ib) in adults with chronic periodontitis. 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**.**

On studying the classification of *fimA* genotypes, ***Pérez et al.,(2009)***[38] Identified *P. gingivalis* strains of fifteen Colombian periodontitis patients showed that type II (3/7) is the most common type, collected 15 minutes after the end of the procedure, followed by those collected immediately after the end of the procedure, type IV (2/7) and those collected 30 minutes after the end of the procedure Ib (1/7) and III (1/7) among the blood strains, this finding confirmed the pathogen's capacity to enter the circulatory system. Type V was not detected in the Colombian periodontitis patients. The absence of fimA type V was probably due to the fact that type V was detected mainly in the healthy population .Similar results have been reported in several studies.[39-41]

In a PCR study,[40] in periodontally healthy subjects, *fimA* type I was observed in 76.1%, type II in 9.4%, type III in 7.2%, type IV in 6.5%, and type V in 29.7%; while in subjects with periodontitis, *fimA* type I was observed in 6.7%, type II in 66.1%, type III in 5.8%, type IV in 28.9%, and type V in 17.4%.

Regarding the relationship between fimA genotype and periodontitis aggression, several authors have investigated whether the presence of P. gingivalistype-specific fimA genotypes correlates with periodontitis prevalence and severity, or whether these genotypes are associated with different geographical areas or ethnic patient groups. FimA type II may have an increased pathogenic potential as it was the most common type among patients with generalized aggressive periodontitis . [41] These results are consistent with previous studies showing that strains of type II, IV and Ib have a more virulent potential, while types I, III and V are less virulent or avirulent[42]

Conclusion

The findings of the current study provide useful insight into the effectiveness of various mouthwashes in reducing various clinical signs of periodontal inflammation .However, the chlorohexidine group shows the greatest reduction of all parameters .In addition, this group shows the strongest reduction of P. gingivalis-positive cultures after subgingival scaling .Further studies were recommended to detect the distribution and frequency of Fim A genotypes and their correlation with the periodontal disease pathogenesis.

Conflict of Interest

No conflict of interests related to this study

References

1. Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, et al.,: Periodontitis: Consensus report of workgroup 2 of the 2017World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol.*, p.p173–182,2018
2. Vajawat M, Kumar V, Rajeshwari KG, Deepika PC: Clinical, Microbiological and Molecular study of porphyromonas gingivalis in patients with chronic periodontitis – *International Journal of Basic and Applied Medical Sciences* ,3(1), p.p 56-61,2013.
3. Socransky S, Haffajee AD. Periodontal microbial ecology. *Periodontol 2000.* 38, p.p 135-187,2005.
4. Kotsilkov K, Popova C. Real time PCR identification for target adjunctive antibiotic therapy of severe chronic periodontitis. Part II-microbiological effetivenes. *J IMAB*; 20(5), p.p 610_616,204.
5. Yu S, Yuko ON, Kimura M, Nemoto KT, Tanaka M and Kimura S : Dominant prevalence of Porphyromonas gingivalis fimA types I and IV in healthy Japanese children. *Journal of Dental Sciences*; 12, p.p 213-219,2017.
6. Enersen M, Nakano K and Amano A: Porphyromonas gingivalis fimbriae. *Journal of Oral Microbiology*, 5, p.p 20265-2027, 2013.

7. Simone S, Jamal MS, Sareh SY, Michael K, Dilara OK, Adrian K, Stefan R and Susanne S: Failure to detect an association between aggressive periodontitis and the prevalence of herpesviruses. *Journal of Clinical Periodontol.* , 40(1), p.p 1–7,2013.
8. Singhrao SK, Harding A, Poole S, Kesavalu L, and Crean ST: Porphyromonas gingivalis Periodontal Infection and Its Putative Links with Alzheimer's disease. *Mediators of Inflammation* , p.p 1- 10 , 2015.
9. Fabrizi S, León R, Blanc V, Herrera D, Sanz M: Variability of the fimA gene in *Porphyromonas gingivalis* isolated from periodontitis and non periodontitis patients. *Med Oral Patol Oral Cir Bucal* ,18 ,p.p100-5,2013.
10. Pan PC, Harper S, Ricci-Nittela D, Luxb Rand Shi W. In-vitro evidence for efficacy of antimicrobial mouthrinses. *J Dent.*, 38, p.pS16–S20,2010.
11. Sreenivasan PK, Haraszthy VI, Zambon JJ. Antimicrobial efficacy of 0.05% cetylpyridinium chloride mouthrinses. *Lett Appl Microbiol.*,56, p.p14–20,2013.
12. Garcia-Godoy F, Klukowska MA, Zhang YH, Anastasia MK,Cheng R,Gabbard M et al., Comparative bioavailability and antimicrobial activity of cetylpyridinium chloride mouthrinses in vitro and in vivo. *Am J Dent*. ,27, p.p185–190,2014.
13. Wilder RS, Bray KS. Improving periodontal outcomes: merging clinical and behavioral science. *Periodontol 2000.* ,71: p.p65–81,2016.
14. Kumar PS. Oral microbiota and systemic disease. *Anaerobe.*,24, p.p90–93,2013.
15. Eick S, Renatus A, Heinicke M, Pfister W,Statufl SI and Jentsh H. Hyaluronic acid as an adjunct after scaling and root planing: a prospective randomized clinical trial. *J Periodontol*.,84:p.p 941–949,2013.
16. Kulik EM, Waltimo T, Weiger R, et al. Development of resistance of mutans streptococci and Porphyromonas gingivalis to chlorhexidine digluconate and amine fluoride/stannous fluoride-containing mouthrinses, in vitro. *Clin Oral Investig*. ,19, p.p1547–1553,2015.
17. Balagopal S, Arjunkumar R. Chlorhexidine: The gold standard antiplaque agent. *J Pharm Sci Res.*,5(12),,p.p 270-274,2015.
18. Martin BJ, Campbell PM, Rees TD, et al. A randomized controlled trial evaluating antioxidant-essential oil gel as a treatment for gingivitis in orthodontic patients. *Angle Orthod.* ,86, p.p 407–412,2016.
19. Van Der Weijden FA, Van Der Sluijs E, Ciancio SG, et al. Can chemical mouthwash agents achieve plaque/gingivitis control? *Dent Clin North Am*. ,59, p.p799–829,2015.
20. Abramson J. The corneal medical index as an epidemiological tool. American Journal of Public Health , 56, p.p 287-298,1996.
21. Silness J, Loe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition. Acta Odontologia Scandinavica , 22,121,1964.
22. Löe H. and Silness J. Periodontal Disease in Pregnancy I. Prevalence and Severity, 21(6),p.p533-551,1963.
23. Polson AM, Caton JG, Yeaple RN and Zander HA. Histological determination of probe tip penetration into gingival sulcus of humans using an electronic pressure-sensitive probe. *Journal of Clinical Periodontology* ,7 , p.p479-488,1980.
24. López NJ, Mellado JC, Leighton GX. Occurrence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Prevotella intermedia* in juvenile periodontitis. *J Clin Periodontol* , 23,p.p101- 105,1996.
25. Goutham BS, Manchanda K, Sarkar AD, Prakash R, Jha K, Mohammed S. Efficacy of two commercially available Oral Rinses - Chlorohexidine and Listrine on Plaque and Gingivitis - A Comparative Study. *J Int Oral Health* , 5(4),p.p56-61,2013.

26. Kirti S, Khuller N, Bansal P, Singh P. Clinical and microbiological effects of subgingival irrigation with propolis extract (Propolis PlatinumTM) and chlorhexidine (Periogard®) as an adjunct to scaling and root planing in patients affected with chronic periodontitis – a comparative study. *J Pharm Biomed Sci* ,07(11), p.p387–399,2017.
27. Azad MF, Schwiertz A and. Jentsch HF.Adjunctive use of essential oils following scaling and root planing –a randomized clinical trial .*BMC Complementary and Alternative Medicine* , 16,p.p171-179,2016.
28. Cortelli SC and Cavallini F & Alves M F & Bezerra AA & Queiroz CS and Cortelli JR. Clinical and microbiological effects of an essential-oil-containing mouth rinse applied in the "one-stage full-mouth disinfection" protocol—a randomized doubled-blinded preliminary study. *J Clin Oral Invest*, 13,p.p189–194,2009.
29. Rath SK and Singh M: Comparison of efficacy of 0.2% and 0.12% chlorhexidine mouthwashes. *Dental Research Journal* 2013; 10 (3):364-369.
30. Kulkarni P, Singh DK , Jalaluddin M and Mandal A : Comparative evaluation of antiplaque efficacy between essential oils with alcohol-based and chlorhexidine with non alcohol based mouthrinses. *J Int Soc Prevent Communit Dent* ,7, p.p 36-41,2017.
31. Lim KS and Kam PC: Chlorhexidine--pharmacology and clinical applications. *Anaesth Intensive Care* 2008 ;36(4):502-512.
32. Khalil B, Winkelhoff AJ, Christina MJ, and Savelkoul HM. Comparison of Real-Time PCR and Culture for Detection of *Porphyromonas gingivalis* in Subgingival Plaque Samples. *Journal of clinical microbiology* , 41(11), p.p4950–4954,2013.
33. Puig-Silla M, Dasí-Fernández F, Montiel-Company JM, Almerich-Silla JM.Prevalence of fimA genotypes of Porphyromonas gingivalis and other periodontal bacteria in a Spanish population with chronic periodontitis .*Med Oral Patol Oral Cir Bucal.* , 17, p.p1047-53,2012.
34. Storm PM, Koltzscher M, Falk W, Do¨rfler A, Jepsen S. Comparison of culture and real-time PCR for dedection and quantification of five putative periodontopathogens in subgingival plaque samples. *J Clin Periodontol* , 32, p.p 778–783,2005.
35. Fawzi MM, Fawzi M M, El-Amin HM, Elafandy MH : Detection and Quantification of *Porphyromonas gingivalis* from Saliva of Schizophrenia Patients by Culture and Taqman Real-Time PCR: A Pilot Study. *Life Science Journal* ,8(2), p.p65-74,2011.
36. D'Ercole S, Catamo G, Piccolomini R. : Diagnosis in periodontology: a further aid through microbiological tests. Crit Rev Microbiol. , 34(1), p.p 33-41,2011.
37. Alnasrawy AA, Alsalihi AA, Abo Almaali HM and Al-Khafaji YA: *Porphyromonas gingivalis fimA* genotyping in adult periodontitis population in Kerbala city .*Kerbala journal of pharmaceutical science* ,8, p.p11-20,2014.
38. Pérez P, Lafaurie G, Gracieux P1, Meuric V1 Tamanai Z, Castellanos J, Bonnaure-Mallet M. Distribution of *Porphyromonas gingivalis* fimA genotypes in isolates from subgingival plaque and blood sample during bacteremia. *Biomédica.*, 29, p.p298-306,2009.
39. Sandra M and Adolfob C. Functional differences of *Porphyromonas gingivalis* fimbriae in determining periodontal disease pathogenesis: a literature review. Colombia Médica -, 44, p.p48-56,2013.
40. Sandra M, Adriana J, Beatriz P, Botero JE and Adolfo C. Porphyromonas gingivalis Fim-A genotype distribution among Colombians. Colombia Médica, 46, p.p122-129,2015.
41. Soares RR, Tahim CM, Silveira VR, Nogueira NAand Rego RO . Prevalence of Porphyromonas gingivalis fimA II genotype

in generalized aggressive periodontitis patients. Braz J Oral Sci., 15(3), p.p176-180,2016.

42. Mahalakshmi K, Padma K, Chandrasekaran SC. Detection of *Porphyromonas gingivalis fimA* Type I genotype in gingivitis by Real-Time PCR–A Pilot Study:" *Journal of Clinical and Diagnostic Research*,10, p.p32-35,2016.