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Bacteriological and Molecular Detection of *Porphyromonas gingivalis* from periodontitis Patients

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Abstract

Objective :The present study was conducted to detect *P gingivalis* by culture and molecular method from clinical oral infection sample. **Method:** hundred fifty (150) paper point were collected from periodontitis patients who attended to specific dental health center and outpatient clinics of dentistry in Al-Hillah city/ Iraq during the duration from (April 2022 to September 2022) . The age of patient (5– 72)years. The samples were collected by paper point the paper point culture on blood agar and selective media(*P. gingivalis* agar (P.GING)) for isolation of *P gingivalis* then diagnosis conform by molecular method. **Results:** The result showed that out of 150 samples 15(10%) *P.gingivalis* isolated from this fifteen isolate only 7(4.66%) confirmed with molecular detection as *P.gingivalis. P.gingivalis* isolated in high rate 6(4%) from age group (25-35) and 4(2.66) in the age group (15-25) while only 2 (1.33%) isolate obtained from the age group (45-55) and 3 (2%) isolate from age group (55-70). **Conclusion:** quantitative evaluation, RT-PCR offers the advantage of eliminating false positives which could otherwise play abysmal role in conventional detection techniques.

Key words: Periodontist, Real Time –PCR , *P.gingivalis* **.**

Introduction

Periodontitis is a chronic inflammatory disease characterized by the destruction of the supporting structures of the teeth. Its high prevalence and negative effects on quality of life make it one of the current problems in dentistry. *Porphyromonas gingivalis (P. gingivalis)* is the predominant periodontal pathogen that expresses a number of virulence factors involved in the pathogenesis of periodontitis : fimbriae, gingipains, haemagglutinin, and lipopolysaccharide[1]. 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 [2]. Invasion of *P. gingivalis* to host cell was relies

on the ability of bacteria to produce gingipains (a trypsin – like cysteine proteinases) which support biofilm formation and regulate host defense response [3].

The expression of several cytokines gingipains was modulate in multi cell kinds involved: gingival fibroblasts, endothelial cells, monocytes and T cells[4-5]. Amongst the virulence factors of *P. gingivalis*, gingipains are the most important virulence factors which are responsible for damage of periodontal tissues inactivate and degrade a number of host defense and structural proteins, also it plays an essential role for *P. gingivalis* nutrient acquisition, colonization, immune subversion and signaling [6]. The major habitat of *P. gingivalis* is the subgingival sulcus of the human oral cavity. It relies on the fermentation of amino acids for energy production, a property required for its survival in deep periodontal pocket, where sugar availability is low[7]. Being an obligate anaerobe, *P. gingivalis* serves as the secondary colonizer of dental plaque, often adhering to primary colonizers such as *Streptococcus gordonii and P. intermedia.*

P. gingivalis can locally invade the periodontal tissues and evade the host defense system by utilizing a panel of virulence factors that cause disruption in the immune and inflammatory reactions. The potential virulence factors of *P. gingivalis* have been extensively described in several reviews [8].

Porphyromonas gingivalis is strongly correlated with chronic periodontitis. Its chronic persistence in the periodontium depends on its ability to evade host immunity without inhibiting the overall inflammatory response, which is actually beneficial for this and other periodontal bacteria. Indeed, the inflammatory exudate (gingival crevicular fluid) is a source of essential nutrients, such as peptides and hemin-derived iron [9].Important features of *P. gingivalis*-mediated chronic periodontitis include the ability of the bacterium to adhere to and invade host cells, disseminate through host cells and tissues, and subvert host immunological surveillance and defense mechanisms[10]. This study aimed to detection of *P .gingivalis* from Periodontitis patient .

Material and methods

Samples Collection

One hundred and fifty (150) samples paper point for isolation *P.gingivalis* collected from each periodontitis patients were admitted to outpatient clinics of dentistry in Babylon city through the duration from (March 2022 to October), These patient were diagnosed by the dentist. Each patient were underwent-detailed history regarding age (the age of patients ranged from 5 to70 years) including both males and females. symptoms of infection .

Samples were obtained from the necrotic pulp or most diseased sites with individual sterile paper points for *P.gingivalis* ,and cotton swab for *C.albicans*,.paper point which were placed in necrotic pulp for 15 sec, In the present study three paper point were used for the collection sample .

Then sterile paper points placed in tube contain 5 ml of BHI broth, after that cultured on Blood agar plates and on selective media anaerobically ;in the anaerobic incubator with using jar and gas back at 37°C for 7-14 days plus (10% Co_2).Then subjected to identification according to the cultural properties, microscopic examination and Biochemical test [11-12] .

Identification of bacteria:

Colonial morphology and microscopic examination:

Each primary positive culture was identified depending on the morphological properties such as (Colony size, shape, color , translucency, edge and elevation of texture) [13]. All gram negative anaerobic bacilli inoculated on blood agar give black-pigmented colonies. The morphology of bacterial cells was investigated by Gram- stain to observe the shape and arrangement of cells under oil immersion (100X magnification) microscopically [14].

Molecular Diagnosis

DNA Extraction

DNA extraction by using G-Spin^{TM} kit (iNtRON/ Korean) according to manufacture company . The extracted genomic DNA was checked by using Nanodrop spectrophotometer

Detection of Specific Gene Markers By Real Time –PCR

DNA was used as a template for RT-PCR to detect *P.gingivalis* . A pair of specific primer were used for the amplification of a fragment gene shown in table(1).

Table (1):The Sequence of Primer and probe that was Used in the Present Study for Detection *P.gingivalis*

Genes	Primer Sequence (5' ----- 3')		Probe (5' ----- 3')	Reference sequence
P. gingivalis ***WaaA***	F	TGGTTTCATGCAGCTTCTTT	CGTACCTCATATCCCGAGG GGCTG	PG1370b
	A	TCGGCACCTTCGTAATTCTT		

PCR Master Mix Preparation and Condition

PCR master mix *P . gingivalis* gene was prepared by using (Trans Script One Step **qRT-PCR Super Mix**) and this master mix done according to company instructions as following table(2):

Table (2): Contents of the qRT -PCR reaction mixture with their volumes

PCR master mix	Volume
DNA tamplate	4µL
forward primer (10µM)	1µL
reverse primers (10µM)	1 µL
One step qPCR SuperMix	9.5 µL

TransScript Greenone step RT/RI Enzyme Mix	0.5 µL
Rnase –free water	4
Total	**20µL**

Thermocycles condition

Rotagene Q (Qia gene , Germany)

95-5 min

95-20 Sec 40 cycle

60-30 Sec 40 cycle

Data collected at green channel.

Statistical Analysis:

Statistical analysis was conducted by using (SPSS 23). Determining the statistical differences among different groups was made using the Pearson Chi-square test and ANOVA tests. The probability of ($P \leq 0.01$) was considered to be statistically significant.

Results

Cultural and Molecular Detection of Isolates

The results of isolation and identification of *P.gingivalis* indicated that 15 (10%) samples were as *P.gingivalis* as shown in table(3) .

Table (3) : Percentage of isolation *P gingivalis* in oral infection samples

Samples	Positive %	Negative%
P.gingivalis	15(10%)	135(90%)
Total	150(100%)	

The Identification of *P gingivalis* isolates depends mainly on the cultural , biochemical characteristics and microscopic examination. The result in Table (4) demonstrates that *P gingivalis* is an aerobic, Gram-negative small coccobacilli. The colonies on blood agar forms black spots, black pigmented colonies, 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 the black pigmentation appear as show in figure (1).

Figure (1): Colonies of *P gingivalis* on Blood agar

P gingivalis showed a positive reaction for Indole and negative reaction for catalase (MacFaddin,2000) .The diagnostic feature summarized on table (4). However, the identification isolates of *P gingivalis* confirmed by PCR.

Table (4):Biochemical Tests and the Microscopic Examination of *P.gingivalis* .

NO	Test	Result
١	Gram stain	G-Ve, Coccobacilli
2	Catalase	-Ve
3	Indole	-Ve
4	Grow on(P.GING)	+Ve
5	Grow on Blood agar	Black pigmented colonies

P.gingivalis isolated in high rate 6(4%) from age group (25-35) and 4(2.66) in the age group (15-25) while only 2 (1.33%) isolate obtained from the age group (45-55) and 3 (2%) isolate from age group (55-70)as show in figure (2).

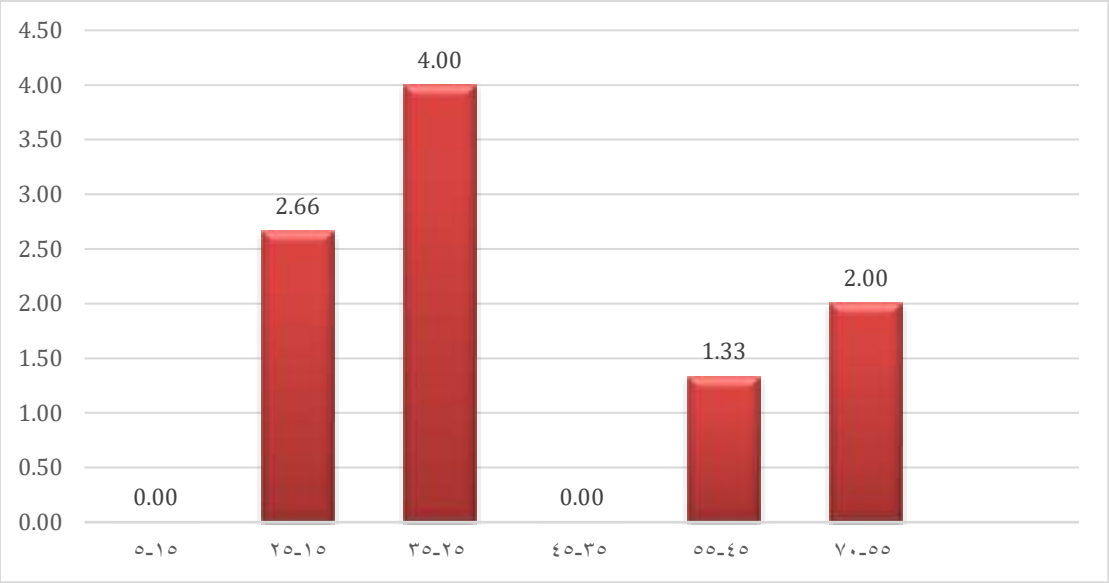

Figure (2): Frequency Distribution Of *P. gingivalis* isolation By Age group.

Molecular diagnosis of *P.gingivalis* by quantitive Real Time PCR

From 15(10%) positive culture to *P.gingivalis* only 10(6.66) isolates give positive result to *P.gingivalis* specific gene (waaA) by q RT-PCR as shown in figure(3).

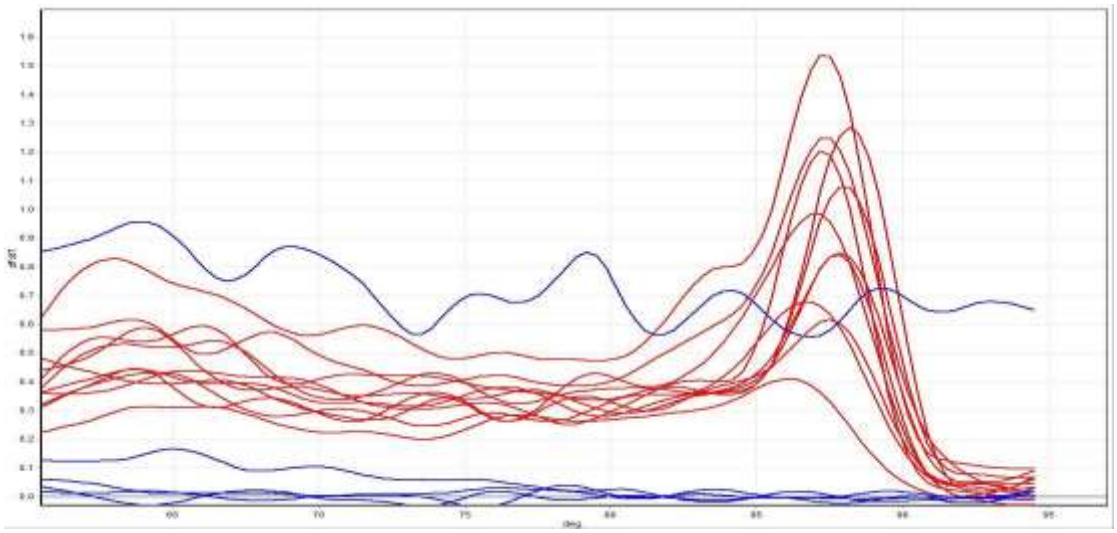

Figure (3) :Detection of *P. gingivalis* by real time PCR , amplification and melting curve , the red curve represent positive detection.

Discussion

P gingivalis is an aerobic, Gram-negative small coccobacilli. The colonies on blood agar forms black spots, black pigmented colonies, 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 the black pigmentation appear[15].

P gingivalis is cultured on selective media (P.GING) it is enriched selective media for the isolation and presumptive identification of *P gingivalis* [16].On these media the bacteria appears to be required Nalidixic acid ,Colistin and Bacitracin requirement ,these reqirment considered as a good enrichment agents , to provide the bacteria with the needed nutritional factors [17].

A previous study done in Iraq by AL-Bdery and Al-Yasseen, [18] found that out of 150 subgingival dental plaque samples only 78 isolates were belonged to *P. gingivalis*, which appeared as a small to large colonies convex, semi mucoid, translucent after 48 hr of incubation anaerobically and formation of black pigmented colonies after 7 days of incubation anaerobically on blood agar supplemented with 5% sheep blood, hemen and vitamin K and all isolates were negative to oxidase, catalase, methyl red and simmon citrate while it's gave positive results to indole test and Alk/Alk without gas and H_2S production on TSI agar.

According to the culturing identification 15(10%) *P.gingivalis* were detected only this result is agrees with Gomes *et al*. [19] they found that *P. gingivalis* was rarely isolated by culture methods (1%).

However , other studies have indicated that the prevalence rate of *P. gingivalis* in healthy subjects was 36.8% and in the periodontitis patients was 87.1% [20].

Another study done by Hajishengallis[21]who found that *P.gingivalis* isolation rate from subgingival sample was(4%).

In the diagnosis of *P.gingivalis* infection ,currently rely on the traditional culture method requires the isolation ,culture and identification of the microorganism ,and has the disadvantages of being time –consuming insensitive , and cumbersome with the development of molecular biology techniques , rapid diagnostic studies of *P.gingivalis* have progressed rapidly [22].

The present study agree with study done by Al-Rawi[23]who revealed that there are effects of age and sex on isolation rate and the results indicated that percentage of *P.gingivalis* was detected in 20-30 years old and males were more infected than females.

The current result similar to study conducted by Kugaji, *et al*. [24] who found a higher rate of detection of *P. gingivalis* In the older age groups. Also Oka *et al*., [25] found Patient in their 80 years old showed a higher rate of co-infection of *C. albicans and P. gingivalis* (23.5%) compared with other participants.

The older age groups were detected with more number of positive cases compared to younger age groups, which could mean the older age group is at higher risk to get infected with *P. gingivalis*. The pathogenicity of *P. gingivalis* is attributed to different virulent factors which directly or indirectly destabilize the immunogenic responses from host and help the bacterium to invade the host tissue.

Study by Kugaji, *et al*[26]detected *P. gingivalis* by qRT-PCR Chronic Periodentitis group in a rate 79.16%, whereas 29.17% samples were positive in the Healthy group.

Also these result correlated with other result ALwarid, [27]that indicate the percentage of *p.gingivalis* by PCR (17.7%);but this result is not agreement with Gomes *et al*, [28] that indicate the isolate of *p. gingivalis* by PCR is (38%) .

Joshi *et al*. [29] found the percentage of prevalence of *P. gingivalis* was 66% in the chronic periodontitis (CP)group in a study which was carried out in Indian subjects.

In few other studies prevalence of *P. gingivalis* in the chronic periodontitis (CP) group which was ranging from 45% to 53%[30]

The difference in the prevalence of bacteria in the CP group could be due to different geographical locations, inclusion criteria, technique used for detection, and variation in sample size between other previous studies.

The PCR examine gave more positive results than culture techniques when traditional culture methods are used, laboratories may need 7-14 days to identify anaerobic strict bacteria, followed by biochemical and other tests to identify the microorganism; the time required for identification can be even longer for slow-growing microorganisms or samples with low microbial counts, while PCR can offer information in only a few days and faster [31]Previous studies found higher prevalence rate (49.1%) of *P. gingivalis* was reported by using Real-Time PCR [32].

However, all the mentioned references agreed on that the rate of detection is higher in disease than health. Presence of this bacterium in low number of healthy individuals (9/35) and in a significantly higher number of subjects in Chronic Periodontitis group indicates that it is an opportunistic pathogen. Healthy periodontium is maintained through a good oral hygiene of the individual. Opportunity for higher growth rate of *P. gingivalis* is usually generated through plaque accumulation in the sub-gingival area in which the growth of early plaque colonizers (gram positive cocci and rods) provide necessary growth factors such as attachment sites, substrate, reduced oxygen tension and an area away from host's oral immunity [33].

Conclusion

Although microbiological culture technique is still considered as a gold standard for the detection of *P. gingivalis*, RT-PCR provided several advantages over the conventional methods. In addition to quantitative evaluation, RT-PCR offers the advantage of eliminating false positives which could otherwise play abysmal role in conventional detection techniques.

References

[1]**Oleinik, E. A., & Goncharenko, A. V. (2022).** The Relationship between Mutations in Gene-Specific Domains of Salivary Fibronectin (cFn) and Dynamin-2 (Dynm-2) and the Development of Porphyromonas gingivalis-Initiated Periodontitis. *Journal of Molecular Pathology*, *3*(3), 182-189.

[2]**Almaali, A. (2014).** *Porphyromonas gingivalis* fimA genotyping in adult periodontitis population in Kerbala city. *karbala journal of pharmaceutical sciences*, *5*(8), 11-20.

[3]**Ren, J., Sang, Y., Lu, J., & Yao, Y. F. (2017).** Protein acetylation and its role in bacterial virulence. *Trends in microbiology*, *25*(9), 768-779.

[4]**Palm, E., H. Khalaf and T. Bengtsson (2015).** Suppression of inflammatory responses of human gingival fibroblasts by gingipains from *Porphyromonas gingivalis*. *Mol Oral Microbiol*., **30** : 74–85.

[5]**Kariu. T., R. I. Nakao, T. Keda, K. Nakashima, J. Potempa and T. Imamura (2017).** Inhibition of gingipains and *Porphyromonas gingivalis* growth and biofilm formation by prenyl flavonoids. *J Periodont Res.*, **52** : 89–96

[6]**Mahato, N., X. Wu and L. Wang (2016).** Management of periimplantitis: a systematic review, 2010-2015. *Springer Plus*, **5** : 105.

[7]**Bostanci, N., & Belibasakis, G. N. (2012).** *Porphyromonas gingivalis:* an invasive and evasive opportunistic oral pathogen. *FEMS microbiology letters*, *333*(1), 1-9.

[8]**Mysak, J., Podzimek, S., Sommerova, P., Lyuya-Mi, Y., Bartova, J., Janatova, T., ... & Duskova, J. (2014).** Porphyromonas gingivalis: major periodontopathic pathogen overview. *Journal of immunology research*, *2014*.

[9]**Carvalho-Filho, P. C., Gomes-Filho, I. S., Meyer, R., Olczak, T., Xavier, M. T., & Trindade, S. C. (2016).** Role of *Porphyromonas gingivalis* HmuY in immunopathogenesis of chronic periodontitis. Mediators of inflammation, 2016.

[10]**Maccfadin, J. K.(2000).** Biochemical Test for Identification of Medical Bacteria. 3rd ed. Lippincott Williams and Winkins . Awolter Klumer Company . Philadelphia Baltimor .New York.

[11]**Forbes, B.A., Sahm, D.F. and Weissfeld , A.S .(2007).** B Marín-*Ramos* ailey and Scott's Diagnostic Microbiology. 12th ed. Mosby, USA.

[12] **Wanger, A., Chavez, V., Huang, R., Wahed, A., Dasgupta, A., & Actor, J. K. (2017).** Microbiology and Molecular Diagnosis in Pathology: A Comprehensive Review for Board Preparation, Certification and Clinical Practice. Elsevier.

[13]**Brenner, D. J., Krieg, N. R. and Staley, J. S.(2005).** Bergey's Manual of Systematic Bacteriology. Vol, 2, 2nd edition. Part B, pp. 556–578. New York: Springer.

[14] **Ogrendik, M., Kokino, S., Ozdemir, F., Bird, P. S., & Hamlet, S. (2005).** Serum antibodies to oral anaerobic bacteria in patients with rheumatoid arthritis. *Medscape General Medicine*, *7*(2), 2.

[15]**AL-Khafagee,N.S;AL-Rubiae,F,M;Witwit,L.J. and AL Dahmoshi,H.O.(2013).** Isolation and Characterization of *Porphyromonas gingivalis* and Determination Some Immunological Aspect In Patient With Periodontitis.International Journal of Dental Research and Development (IJDRD);3:1-6.

[16]**Jousimies-Somer, H. R; Summanen, P; Citron, D. M; Baron, E; Wexler, H. M. and Finegold, S. M. (2002).**Wadsworth – KTL Anaerobic Bacteriology Manual.Sixth Edition. Star Publishing Co.Belmont, CA 94002.

[17]**NCCLS. Quality Control for Commercially Prepared Microbiological CultureMedia; Approved Standard- Third Edition. (2004).** NCCLS document M22-A3. NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898.

[18]**Grenier, D., & Dang La, V. (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.

[19]**AL-Bdery, A. S. J., & Al-Yasseen, A. K. (2018).** Genotypic and Phenotypic Detection of some Virulence Factors among Porphyromonas gingivalis Related with Periodontitis in Al-Najaf Al-Ashraf City/Iraq. *Plant Archives*, *18*(2), 2345-2353.

[20]**Gomes, B. P. F. A., Jacinto, R. C., Pinheiro, E. T., Sousa, E. L. R., Zaia, A. A., Ferraz, C. C. R., & Souza-Filho, F. J. (2005).** *Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia and Prevotella nigrescens* in endodontic lesions detected by culture and by PCR. *Oral microbiology and immunology*, *20*(4), 211-215.

[21]**Bostanci, N., & Belibasakis, G. N. (2012).** *Porphyromonas gingivalis:* an invasive and evasive opportunistic oral pathogen. *FEMS microbiology letters*, *333*(1), 1-9.

[22]**Clais, S., Boulet, G., Kerstens, M., Horemans, T., Teughels, W., Quirynen, M., ... & Cos, P. (2014).** Importance of biofilm formation and dipeptidyl peptidase IV for the pathogenicity of clinical *Porphyromonas gingivalis* isolates. *Pathogens and disease*, *70*(3), 408-413.

[23]**Atanasova, K. R., & Yilmaz, Ö. (2014).** Looking in the Porphyromonas gingivalis cabinet of curiosities: the microbium, the host and cancer association. *Molecular oral microbiology*, *29*(2), 55-66.

[24]**Al-Rawi, A. M. (2012).** Detection of Porphyromonas gingivalis from periodontal pocket infections by microbial cultivation and PCR techniques. *Rafidain journal of science*, *23*(1A).

[25]**Kugaji, M. S., Muddapur, U. M., Bhat, K. G., Joshi, V. M., Kumbar, V. M., & Peram, M. R. (2019).** Quantitative evaluation of Porphyromonas gingivalis in Indian subjects with chronic periodontitis by Real-Time Polymerase Chain Reaction. *Journal of Advanced Oral Research*, *10*(2), 137-144.

[26]**Oka, I., Shigeishi, H., & Ohta, K. (2022).** Co-infection of oral Candida albicans and Porphyromonas gingivalis is associated with active periodontitis in middle-aged and older Japanese people. *Medicina*, *58*(6), 723.

[27]**Al-Warid**, **R. Jasim**. **(2017).**Molecular And Bacteriological Detection For Bacteria Isolated From Endodontic Treatment: Pak. J. Biotechnol. Vol. 14 (4) 711-715 .

[28]**Joshi, V. M., Bhat, K. G., Kugaji, M. S., & Ingalagi, P. S. (2016).** Prevalence of Porphyromonas gingivalis and its relationship with herpesvirus in Indian subjects with chronic periodontitis: A cross-sectional study. *Journal of the International Clinical Dental Research Organization*, *8*(2), 106.

[29]**Ercan, E., Dalli, M., Yavuz, İ., & Özekinci, T. (2006).** Investigation of microorganisms in infected dental root canals. *Biotechnology & Biotechnological Equipment*, *20*(2), 166-172.

[30]**Chen, L. L., Wu, Y. M., Yan, J., Sun, W. L., Sun, Y. Z., & David, O. (2005).** Association between coinfection of *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans and Treponema denticola* and periodontal tissue destruction in chronic periodontitis. *Chinese medical journal*, *118*(11), 915-921.

[31]**Chaudhuri, S., Pratap, S., Paromov, V., Li, Z., Mantri, C. K., & Xie, H. (2014).** Identification of a diguanylate cyclase and its role in Porphyromonas gingivalis virulence. *Infection and Immunity*, *82*(7), 2728-2735.

[32]**Boutaga, K., Van Winkelhoff, A. J., Vandenbroucke-Grauls, C. M., & Savelkoul, P. H. (2006).** The additional value of real-time PCR in the quantitative detection of periodontal pathogens. *Journal of clinical periodontology*, *33*(6), 427-433.

[33]**Siqueira Jr, J. F., & Rôças, I. N. (2003).** PCR methodology as a valuable tool for identification of endodontic pathogens. Journal of dentistry, 31(5), 333-339.