

Porphyromonas gingivalis

PCR

(2011 / 7 / 20 2011 / 6 / 7)

49 *P.gingivalis*

Pocket depth ()

Progressive Chronic

DNA (PCR)

9-3

PCR

- %28.5 %65.3

DNA

30-20 *P.gingivalis*

.PCR *P.gingivalis* :

Detection of *Porphyromonas gingivalis* from Periodontal Pocket Infections by Microbial Cultivation and PCR Techniques

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ABSTRACT

The study aimed to detect *P. gingivalis* from 49 patients with periodontitis at different ages and both sexes, after determination of pocket depth, types of infection whether chronic or progressive by dentists. Routine culture method was done using selective media and anaerobic condition and compared with species specific polymerase chain reaction (PCR) technique.

DNA was extracted from samples and its concentration and purity were determined. The results showed domination of chronic infections and the pocket depths ranged between 3-9mm, as well as the results revealed that isolation percent of *P.gingivalis* by PCR was more higher than culture method, it was 65.3% and 28.5% respectively. The results also showed that phenol-chloroform was the efficient method for DNA extraction comparing with other methods. The study 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.

Keywords: *P.gingivalis*, Culture, PCR.

P.gingivalis

(Shian and Reynolds, 2010 ; Pejic *et al.*, 2011)

– Porphyrin

Loo *et al.*, 2009; Siqueira *et al.*, 2008; Romano *et al.*,)

.(2007

.....*Porphyromonas gingivalis*

(Brown and

Loe, 2000; Anderian *et al.*, 2006; Gaentsch *et al.*, 2009)

(Pejcic *et al.*, 2011;

.Blumenthal *et al.*, 2005)

2006

Anderian

.(Yoshida *et al.*, 2005)

630

15-10

(Gaentsch *et al.*, 2009)

Tannerella (Actinobacillus) *Aggregatibacter actinomycetemcomitans* 1996

American Academy of) *Porphyromonas gingivalis* (Bacteroides forsythus) *forsythia*

.(Periodontology, 1996

Salari and Kodkhoda, 2004; Gaentsch *et al.*,)

.(2009

Microaerophilic

(Pfister, 2002) Viable

.(Boyanova *et al.*, 2009 ; Loo *et al.*, 2009)

PCR

.(Gaentsh *et al.*, 2009)

DNA Probes

ELISA

Pejcic *et al.*, 2011;)

.(Gafan *et al.*, 2004

PCR *P.gingivalis*

PCR

:

49

3≤

Paper point

0.5

3

:

Nacl 9

Normal Saline

:

-

15 °121

Brain Heart Infusion Broth

:

-

/ 1: Cysteine³ /

5:Hemin :

Supplement

(BHI)

3 / 1:Vitamin K³

%2

BHI

:

-

. °20-

%15

:

-

.He *et al.*, 2006

Anaerobic generating system

:

:

.(2011

) %0.0002

.(Oxoid)

.PCR

:

3 0.1

Supplement (BHI)

(Oxoid) Anaerobic Jar

7-5 °37

%2 BHI

P.gingivalis

PCR :PCR

-3 DNA -2 .DNA - 1

DNA -1

DNA 3

: DNA

Bartlet Jones : - DNA -

.DNA (1990)

DNA : -

.(Willis *et al.*, 1999)

: DNA -

(Kasuga *et al.*, 2000; Conrads *et al.*, 1997)

Triton (100x) glass beads

: %0.1

10 °37 250 -1

5 10,000 -2

100 -3

5 10,000

| | | | | | | |
|------|-----|-------------|------|---------------|--|----|
| | 100 | | | | | -4 |
| | | glass beads | %0.1 | Triton (100x) | | |
| | PCR | | 10 | °94 | | -5 |
| .DNA | | 5 | 5 | | | -6 |

DNA

(TE) Tris EDTA 3 1 DNA 10

() UV. Spectrophotometer 100:1

: / DNA . 260

$$\frac{\text{قراءة الامتصاصية عند 260 نانوميتر} \times 50 \times 100}{1000} = \text{DNA} /$$

$$\frac{\text{القراءة عند 260 نانوميتر}}{\text{القراءة عند 280 نانوميتر}} = \text{DNA} : \text{DNA}$$

:DNA

(Faik, 2007)

DNA

: -2

: -A

16s rRNA (131 bp)

(Kasuga *et al.*, 2000)

5'-ATA ATG GAG AAC AGC AGG AA-3'
5'-TCT TGC CAA CCA GTT CCA TTG C-3'

:(Forward) -1

/ 100 78.0

:(Reverse) -2

/ 100 138.4

50 DNA : -B

.....*Porphyromonas gingivalis*

| | | | |
|---------------------------|----------------------|--------------|-----------|
| | Master Mix | 25 | -2 |
| | | 1 | -3 |
| | | 1 | -4 |
| | DNA | 5 | -5 |
| | 5 | | |
| | Nuclease free water | 18 | -6 |
| | 50 | | |
| 15 | (Vortex mixer) | | -7 |
| / 4000 | 40 | | -8 |
| Authorized thermal cycler | | | -9 |
| | Labnet International | | |
| | (1) 2 | | °94 |
| | (35) { | 30 | ° 94 |
| | | 1 | ° 60 |
| | | 1 | ° 72 |
| | (1) 5 | | ° 72 |
| | | | -3 |
| | : | | |
| | (1×TAE) | 3 50 | 0.5 -1 |
| | 3 | 0.5 Ethidium | -2 |
| | ° 55-50 | | -3 |
| | | | -4 |
| | | | -5 |
| | | | -6 |
| | | (TAE 1×) | -7 |
| | | | 5 |

DNA 10 -8

(DNA Ladder: 100bp) 5 -9

-10

60 100

-11

260

UV-transilluminator

(%98) 48

49

DP

9-3

(DP)

(%2) 1

3.5

5.03

(Salari and Kadkhoda, 2004 Kumar *et al.*, 2003)

(1999) Armitage

Pfister,)

(DP)

(2003)

Kumar

9-4

(2002

(2005)

Kumar

7.7

≤

3

%30

4

(2008) Chu Lai

27-5

PO₂

.....*Porphyromonas gingivalis*

P.gingivalis

:

:

Supplement

BHI

%2

BHI

-

(2

1

)

.(3

)

(Leon *et al.*, 2007)

-

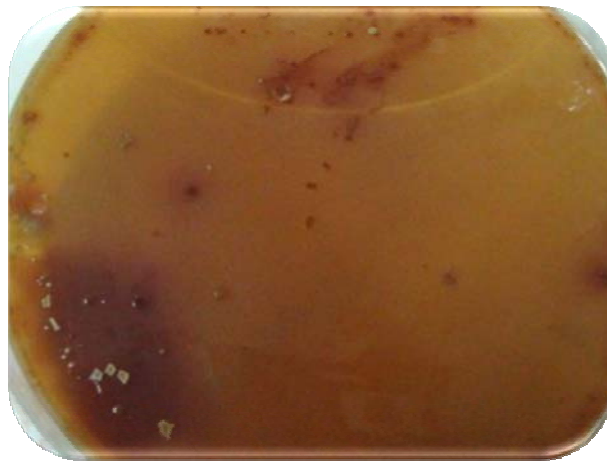
Shelburne *et al.*)

Hemin

P.gingivalis

(*al.*, 2005

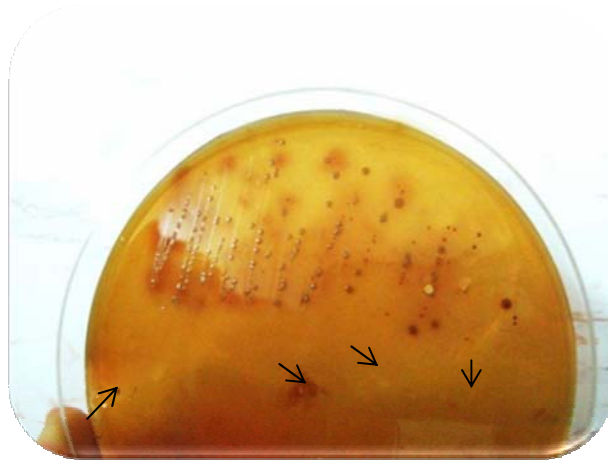
gingivitis



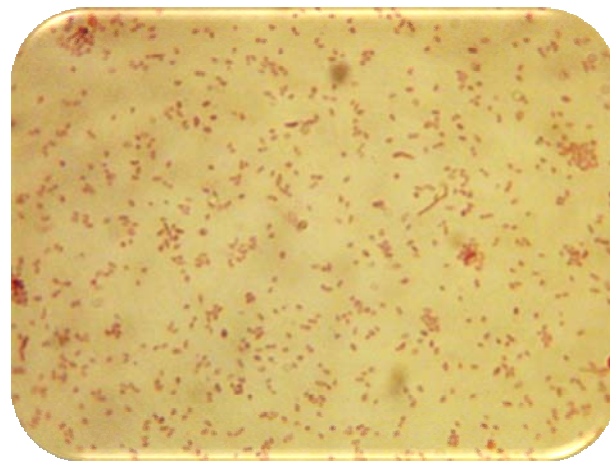
BHI

P.gingivalis

:1



.BHI *P.gingivalis* :2



.100X *P.gingivalis* :3

%28.6 *P.gingivalis* 14 (49)

35

.%71.4

Loo *et al.*, 2009; Salari and Kadkhoda,)

.(2004

.....*Porphyromonas gingivalis*

(Salari and Kadkhoda, 2004)

(2009) Boyanova %21 (2003) Kumar
27 %21.9 %25

Boyanova *et al.*,)

6.03

P.gingivalis

(2009

(2010)

7

T.denticola

3.3

5

P.gingivalis

(He *et al.*, 2006)

PCR :

DNA

.1

DNA

DNA

.(1)

DNA

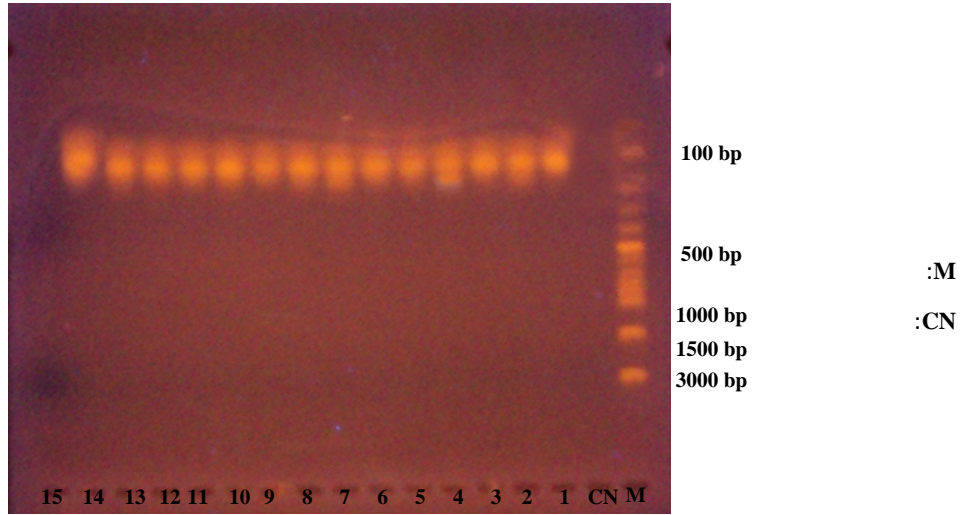
:1

| DNA | DNA / | | |
|-----------|-----------|------------------------|---|
| 0.92-0.82 | 2.50-1.97 | Boiling | 1 |
| 1.09-0.88 | 5.35-3.60 | Freezing and Thawing | 2 |
| 1.75-1.42 | 6.70-5.57 | - Phenol-Chloroform | 3 |

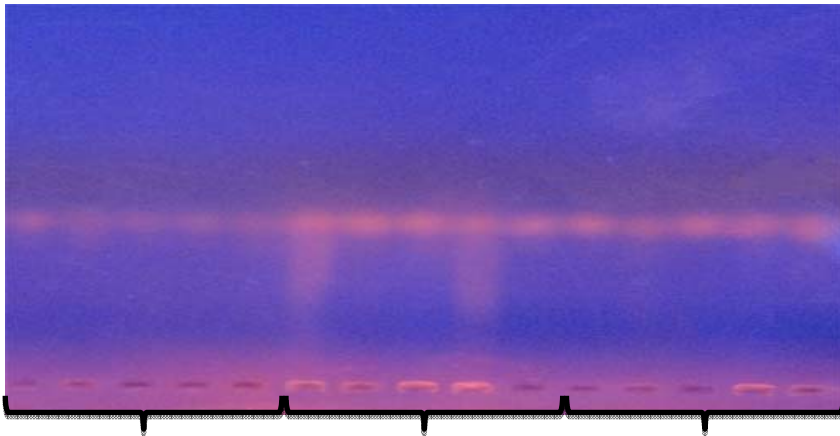
SDS Protinase K -

DNA .DNA DNA (1)

DNA DNA



DNA :1



P.gingivalis 131 bp DNA :2

.100 bp DNA Ladder (M) %1

.....*Porphyromonas gingivalis*

P.gingivalis

DNA

DNA

(131bp)

(2)

16srRNA

(131 bp)

Loo *et al.*, 2009; Castillo *et al.*,)

P.gingivalis

.(2009; Shian and Reynolds, 2010; Pejcie *et al.*, 2011

(32) %65.3

PCR

.(2)

%34.7

17

(49)

.%65

(1999) Rudney Tran

PCR

%61.76

(2002) Pfister

(2009)

Gaentsch

(%75.5)

(2000)

Kasuga

(Loo *et al.*, 2009)

%39.5

%77.6

(%79-20)

³ / 10

PCR

PCR

P.gingivalis

. *P.gingivalis*

A.actinomycetemconitans *T.forsythiu* *P.gingivalis*

.(Boyanova *et al.*, 2009; Guentsch *et al.*, 2009; Kumar *et al.*, 2005) *T.denticola*

PCR

(Pfister, 2002)

PCR

PCR

P.gingivalis

7 (2004) Gafan

PCR

10

Castillo (2009)

Loo (2006)

Vemer

P.gingivalis

PCR

(2009)

PCR

5.5 PCR

.PCR

6.03

%32.7 %67.3

%60.6 21.2

PCR

.(2)

%75 43.8

P.gingivalis

:2

| PCR | | | | | | | | | | | |
|------|----|------|----|------|----|------|---|------|----|--|--|
| | | | | | | | | 49 | | | |
| % | | % | | % | | % | | % | | | |
| 39.4 | 13 | 60.6 | 20 | 78.8 | 26 | 21.2 | 7 | 67.3 | 33 | | |
| 25 | 4 | 75 | 12 | 56.2 | 9 | 43.8 | 7 | 32.7 | 16 | | |

(2003) Bunyaratavei

(2009)

Loo

%47.8

%52.17

%32.2 %67.7

(2010) Shian and Yeynolds

%46 55

(2011) Pejic

%32.7 40-31

%46.7

30-20

PCR

.(3)

(Bunyaratavei, 2003; Matto *et al.*, 1998)*P.gingivalis*

:3

| PCR | | | | | | | | 49 | | |
|------|---|------|----|------|----|------|---|------|----|--------------|
| % | | % | | % | | % | | % | | |
| 66.7 | 2 | 33.3 | 1 | 100 | 3 | 0 | 0 | 6.1 | 3 | 20 |
| 34.8 | 8 | 65.2 | 15 | 82.6 | 19 | 17.4 | 4 | 46.9 | 23 | 30-20 |
| 31.2 | 5 | 68.8 | 11 | 62.5 | 10 | 37.5 | 6 | 32.7 | 16 | 40-31 |
| 0 | 0 | 100 | 3 | 33.3 | 1 | 66.7 | 2 | 6.1 | 3 | 50-41 |
| 50 | 2 | 50 | 2 | 50 | 2 | 50 | 2 | 8.1 | 4 | 60-51 |

Treponema denticola

.(2010)

.(2011)

Treponema

.67-49 (2) **22**

American Academy of periodontology. (1996). Consensus port: section on epidemiology. *Periodontol.* 1216-1218.

Anderian, E.; Grenier, D.; Rouabhia, M. (2006). *Prophyromonas gingivalis*-Epithelial cell interactions in periodontitis. *J. Dent. Res.* **85** (5), 392-403.

Armitage, G. (1999). Development of a classification of system for periodontal diseases and conditions-Am. *Perodontol.* **4**,1-6.

Blumenthal, J.; Sherwood, A. Babyakm M.; Watkins, L. Waugh, R.; Georgiads, A. (2005). Effect of exercise of stress management training on makers of car vascular risk in patients with ischemic heart disease *JAMA.* **293**(13),1626-1634.

- Boyanova, L.; Setchanova, L.; Gergova, G.; Rostya, T.; Yordanov, D.; Popova, C.; Kostasilkov, K.; Mitov, L. (2009). Microbiological diagnosis of the severe chronic periodontitis. *J. IMAB*. **2**, 89-94.
- Bunyaratavei, M. (2003). The high frequency of *P.gingivalis*, *P.intermedia*, and *P.nigrescens* in subgingival plaque may be associated with periodontal diseases in subjects aged. 30-49. *H. evidence-based Dental practice*. **3**(2),103-104.
- Castillo, D.; Sanchez. Beltran, M.; Castellanos, J. E.; Leon, R.; Mayorga-Fayad, I.; Sanz, M.; Lafaurif, G. (2009). Detection of *Porphyromonas gingivalis* in bacteremia using different microbiological diagnostics. *J. periodontal Res*. 45-48.
- Conrads, G.; Pelz, K. Hughes, B.; Sefar, L.; Devine, D. (1997). Optimized oligonucleotides for differentiation of *P.intermedia* and *P.nigrescens*-*Oral Microbiol. Immunol.* **12**,117-120.
- Faik, A. (2007). Molecular epidemiology analysis of *Salmonella enteric* serotype Typhi. Ph.D. thesis Al. Mustansirya University.
- Gaentsch, A.; Puklo, M.; Preshow, P.; Glochmann, E.; Pfister, W.; Potempa, J.; Eick, S. (2009). Neutrophils, in chronic and aggressive periodontitis in interaction with *P.gingivalis* and *A. actinomycetemcomitans*. *J. periodontal Res*. **44**(3) 368-377.
- Gafan, G; Lucas, V.; Roberts, G.; Petrie, A.; Spratt, D. (2004). Prevalence of periodontal pathogens in dental plaque of children. *J. Clin. Microbiol.* **42**, 4141- 4146.
- He, J.; Miyazaki, J.; Anaya, C.; Yu, F.; Yeudall. W.; Lewis, J. (2006). Role of *P.gingivalis* feeB2 in metal uptake and oxidative stress protection. *Infection and Immunity.* **74**(7), 4214-4223.
- Jones, J.; Bartlet, A. (1990). Preparation of genomic DNA from bacteria. Experimental techniques in bacterial genetics. In: Wolf, J.B. (ed.) Molecular biology. lab manual.
- Kasuga, Y.; Ishihara, K.; Okuda, K. (2000). Significance of detection of *Porphyromonas gingivalis*, *Bacteroides forsythus* and *Treponema denticola* in Periodontal pockets. *Bull-Tokyo. Dent. Coll.* **41**(3), 109-117.
- Kumar, P.; Griffen, A.; Barton, J.; Paster, B.; Moeschberger, M.; Leys, E. (2003). New bacterial species associated with chronic periodontitis. *J. Dent. Res.* **85** (5), 338-344.
- Kumar, P.; Griffen, A.; Leys, E. (2005). Identification of candidate periodontal pathogens and beneficial species by quantitative 16s clonal analysis. *J. Clin. Microbiol.* **43**, 3944-3955.
- Lai, Y. ; Chu, L. (2008). Novel mechanism for conditional aerobic growth of anaerobic bacterium *Treponema denticola*. *Appl. Environ. Microbiol.* **74**(1), 73-79.
- Leon, R.; Silva, N.; Ovalle, A.; Chaparro, A.; Ahumada, A.; Gajardo, M.; Martinez, M.; Garmonal, J. (2007). Detection of *P.gingivalis* in the amniotic fluid in pregnant women with a diagnosis of threatened premature labor. *International. J. Syst. Evolu. Microbiol.* **78**(7), 1249-1255.
- Loo, T.; Jin, S.; Mary. M.; Cheung, B.; Yi-ding Dou. (2009). Detection of *B.forsythus* and *P.gingivalis* in infected root canals during periapical periodontitis by 16srDNA. *Afr. J. Biotechnol.* **8**(10), 2021-2026.
- Matto, J.; Saarela, M.; Al –Aluusua, S.; Oja, v.; Jousimes-somer, HJ.; Asikainen, S. (1998). Detection of *P.gingivalis* from saliva by PCR by using a simple sample-processing method. *J. Clin. Microbiol.* **36**(1),157-160.

- Pejcic, A.; Kesic, L.; Milasin, J.; Pesic, Z.; Mirkovic, D. (2011). The effect of periodontal therapy on C. reactive protein and periodontal pathogens in periodontitis patients. *Acta. Stomatol. Croat.* **45**(1), 14-23.
- Pfister, S. (2002). Comparison of microbial cultivation and a commercial PCR based methods for detection of periodonto pathogenic species in subgingival plaque samples. *J. Clin. Periodontal.* **29**, 638-644.
- Romano, F.; Barbui, A.; Aimetti, M. (2007). Periodontal pathogens in periodontal pockets and in carotid atheromatous plaques. *Minerva Stomatol.* **56**(4), 169-179.
- Salari, M. and Kadkhoda, Z. (2004). Rate of cultivable subgingival periodonto pathogenic bacteria in chronic peridoutitis. *J. Oral Science.* **46**(3), 157-161.
- Shelburne, C.; Gleason, R.; Coulter, W.; Lantz, M.; Lopatin, D.(2005). Differential display analysis of *P. gingivalis* gene action response to heat and oxidative stress. *Oral Microbiology and Immunology.* **20**(4), 233-238.
- Shian, J. ; Reynolds, M. (2010). Sex differences in destructive periodontal diseases: Exploring the biologic basis. *J. Periodontology.* **80**(11), 1505-1517.
- Siqueria, J.; Rogas, I.; Deblan, G.; Garmo, F.; Paiva, S.; Alves, F.; Rosado, A.S. (2008). Profiling of root canal bacterial communities associated with chronic apical periodontitis from Brazilian and Norwegian subjects. *J. Endod.* **34**: 1457-1461.
- Tran, S. ; Rudney, J.D. (1999). Improved multiplex PCR using conserved and specific-specific 16srRNA gene primers for simultaneous detection of *A. actinomycetemcomitans*, *B.forsythus* and *P.gingivalis*. *J. Clin. Microbiol.* **37**, 3504-3508.
- Vemer. C.; Lemaitre, P.; Daniel, A.; Glumeli, B.; Lakhssassi, N.; Sixou, M. (2006). Real time polymerase chain reaction Vs. anaerobic culture for periodontal pathogen identification. *Oral Microbiology and Immunology.* **21**(6), 341-346.
- Willis, S.; Smith, K.; Dunn V.; Gapter, L.; Rivere, K.; Riviere, G. (1999). Identification of seven *Treponema* species in health and disease associated dental plaque by nested PCR. *J. Clin. Microbiol.* **37** (3), 867-869.
- Yilma, O.; Watanabe, K.; Lamont, R. (2002). Involvement of integrins in fimbria-mediated binding and invasion by *P.gingivalis*. *Cell. Microbiol.* **4**, 305-314.
- Yoshida, A. Nagashima, S.; Ansai, T.; Kato, H.; Takehara, T. (2005). Loop-Mediated isothermal amplification method for rapid detection of the peridontopathic bacteria *P.gignivalis*, *T.forsythia* and *T.denticola*. *J. Clin. Microbiol.* **43**, 2418-2424.