



Isolation of Cultivable Periodontal Bacteria from Pre Menopausal and Post Menopausal Women in Chennai, India

Kanakam Elizabeth Thomas

SRM Dental College, Chennai, Tamil Nadu, INDIA

Available online at: www.isca.in, www.isca.me

Received 17th December 2013, revised 20th January 2014, accepted 27th February 2014

Abstract

A woman from the time she attains her puberty till her menopause is undergoing constant hormonal fluctuation. Changes in the hormone, progesterone and estrogen bring changes in general health as well as in the oral health. The gingiva gets inflamed and leads into gingivitis. This condition when untreated becomes periodontitis- inflammation of the gums and tissues surrounding the tooth. This study aims to isolate the cultivable periodontal pathogens, from periodontitis and to correlate with the oral hygiene of women under study. It can be concluded from the datas from the above study that Post menopausal women are prone to periodontitis than premenopausal women. Awareness should be bought among Indian women population, especially in rural areas on the importance of oral health hygiene.

Keywords: Periodontal pathogens, sub gingival plaque, periodontitis, anaerobic oral bacteria.

Introduction

Periodontitis is prevalent at all stages in women. Periodontitis affects the gingival and the supporting tissues of the teeth¹. During the early stages of dentistry periodontitis was considered as a disease of the oral cavity, but advancement in the field of periodontitis shows that periodontal pathogens has a greater role in the different stages of a women's life.

Periodontitis has been associated with changes in the oral cavity when a female reaches puberty, during pregnancy and menopause. Studies show that these changes are due to the hormonal changes during each stage. Periodontitis during pregnancy results in preterm birth. Women on oral contraceptives also suffer from periodontitis. As a woman nears her menopause there is a gradual decline in the estrogen level, which is related to systemic bone loss².

The current study aims at isolating cultivable bacteria from pre-menopause and post menopause women from South Indian population. Both aerobic and anaerobic periodontal cultivable bacteria were isolated.

Material and Methods

The samples were collected aseptically from SRM Dental College, Dept. of Periodontics and Oral Implantology. 30 samples of pre menopausal women and 30 samples of Post menopauseal women were collected. The supra gingival plaque was removed with the help of a Sterile Hi-Media Cotton Swab. With the help of paper point (No: 38) the sub gingival plaque was collected, by inserting the paper point into the periodontal pockets for 20 seconds. From each patient two samples were collected³.

The paper points were transferred into Thioglycollate broth and Brain Heart Infusion Broth for Anaerobic and Aerobic cultivation respectively. The Oral Hygiene Index Simplified (OHIS) value and the Mean Probing depth were calculated. Based on the mean probing depth the subject was categorized as Periodontitic and Non- Periodontitic group. From each subject 2 samples were collected. In the case of Periodontitis sub gingival plaque was collected from the deepest periodontal pocket.

Ethical clearance was obtained from SRM University Ethical committee.

Aerobic Bacteria Isolation: The BHI broth with the paper point was incubated at 37°C for 24 hrs. The broth was subcultured onto Blood Agar plate and Mac. Conkey Agar plate and was kept for overnight incubation at 37°C.

The colonies were identified based on Gram staining, Biochemical tests and Colony morphology⁴.

Anaerobic bacteria Isolation: The paper points were transported to the Microbiology Laboratory in reduced Thioglycollate broth. The bottle containing the paper point was shaken to dislodge the adhered bacteria on the paper point. Using standard loop technique culture was put on blood agar plate containing Hemin and Vitamin K.⁵ The plates were incubated in Anaerobic jar using Hi-Media Gas Pack LE002A for 6 days. Hi Media Indicator tablet LE001B was used as an indicator of anaerobiosis. The bacteria were identified based on Colony morphology, Gram Staining, pigment formation and biochemical tests^{6,7}.

Results and Discussion

The bacteria isolated are divided according to gram staining and their morphology. Post menopausal women have a high microbial flora when compared with the pre menopausal women. The results show the Oral hygiene index is poor in Post menopausal women which are major reason for increase in the oral microflora.

Table-1
Aerobic Bacteria

Bacteria isolated	Pre menopause		Post menopause	
	n	%	n	%
GRAM POSITIVE COCCI				
<i>Staphylococcus sp.</i>	2	6.7	4	13.3
<i>Staphylococcus aureus</i>	1	3.3	2	6.7
<i>Streptococcus mutans</i>	12	40	7	23.3
<i>Streptococcus sp.</i>	-		2	6.7
<i>Enterococcus sp.</i>	1	3.3	1	3.3
GRAM POSITIVE BACILLI				
<i>Lactobacilli sp.</i>	3	10	1	3.3
<i>Actinomycetes</i>	2	6.7	1	3.3
GRAM NEGATIVE COCCI				
<i>Neisseria catrhallis</i>	7	23.3	9	30
GRAM NEGATIVE BACILLI				
<i>Escherichia coli</i>	1	3.3	2	6.7
<i>Haemophilus sp.</i>	1	3.3	1	3.3

Table 2
Anaerobic Bacteria

Bacteria isolated	Pre menopause		Post menopause	
	n	%	n	%
GRAM POSITIVE COCCI				
<i>Stomatococcus sp.</i>	2	6.7	6	20.0
<i>Gemella sp.</i>	1	3.3	-	
<i>Peptostreptococcus sp.</i>	7	23.3	1	3.3
<i>Peptococcus sp.</i>	-		2	6.7
GRAM POSITIVE BACILLI				
<i>Bifidobacterium</i>	-		2	6.7
<i>Eubacterium</i>	1	3.3	1	3.3
<i>Propionibacterium</i>	1	3.3	1	3.3
<i>Aggregatibacter sp.</i>	-		-	
<i>Actinomycetes</i>	1	3.3	-	
GRAM NEGATIVE COCCI				
<i>Vellionella sp.</i>	6	20.0	7	23.3
GRAM NEGATIVE BACILLI				
<i>Bacteroides</i>	4	13.3	5	16.7
<i>Prevotella sp.</i>	1	3.3	-	
<i>Porphyromonas sp.</i>	2	6.7	1	3.3
<i>Fusobacterium</i>	3	10	4	13.3
<i>Leptotricha</i>	-		-	

Table 3
Isolation frequency of aerobic bacteria according to OHIS

Bacteria Isolated	Pre Good n=13	meno Fair n=10	pause Poor n=7	Post Good n=6	meno Fair n=8	pause Poor n=16
GRAM POSITIVE COCCI						
<i>Staphylococcus sp.</i>	-	1	1	-	-	4
<i>Staphylococcus aureus</i>	-	-	1	-	-	2
<i>Streptococcus mutans</i>	8	4	-	3	4	-
<i>Streptococcus sp.</i>	-	-	-	1	-	1
<i>Enterococcus sp.</i>	-	-	1	-	-	1
GRAM POSITIVE BACILLI						
<i>Lactobacilli sp.</i>	2	1	-	1	-	-
<i>Actinomycetes</i>	-	-	2	-	1	-
GRAM NEGATIVE COCCI						
<i>Neisseria catrhallis</i>	3	3	1	1	3	5
GRAM NEGATIVE BACILLI						
<i>Escherichia coli</i>	-	-	1	-	-	2
<i>Haemophilus sp.</i>	-	1	-	-	-	1

Table 4
Isolation frequency of anaerobic bacteria according to OHIS

Bacteria Isolated	Pre Good n=12	meno Fair n=10	pause Poor n=8	Post Good n=5	meno Fair n=9	pause Poor n=16
GRAM POSITIVE COCCI						
<i>Stomatococcus sp.</i>	-	2	-	1	1	4
<i>Gemella sp.</i>	-	1	-	-	-	-
<i>Peptostreptococcus sp.</i>	1	2	4	1	-	-
<i>Peptococcus sp.</i>	-	-	-	-	2	-
GRAM POSITIVE BACILLI						
<i>Bifidobacterium</i>	-	-	-	-	-	2
<i>Eubacterium</i>	-	-	1	-	-	1
<i>Propionibacterium</i>	-	-	1	-	1	-
<i>Aggregatibacter sp.</i>	-	-	1	-	-	-
<i>Actinomyces</i>	-	-	1	-	-	-
GRAM NEGATIVE COCCI						
<i>Vellionella sp.</i>	-	2	4	1	1	5
GRAM NEGATIVE BACILLI						
<i>Bacteroides</i>	1	1	2	2	2	1
<i>Prevotella sp.</i>	-	-	1	-	-	-
<i>Porphyromonas sp.</i>	-	-	2	-	1	-
<i>Fusobacterium</i>	-	1	2	-	1	3
<i>Leptotricha</i>						

OHIS: 1: Good, 2: Fair, 3: Poor

Discussion: In the current study it was found that pre menopausal women have lesser gingival related problems compared to those who are post menopausal. The Oral Hygiene Index is also found to be good in premenopausal women. The presence of periodontal pathogens does not signify chronic periodontitis, they should be present above a significant level. This in association with environmental and genetic factors will lead to periodontitis⁸.

Among the samples collected, only one Pre menopausal patient was found to harbor *Candida albicans*. Reports have shown that even though the yeast colonize tongue, palate and buccal mucosa they are also found to occur in the subgingival plaque of patients with chronic periodontitis^{9,10}. From periodontal pockets *C.albicans* have been isolated in large numbers (>15%)^{11,12}.

India being a highly populated country with approximately 1000 million population, several studies reveals that periodontal health status in very poor. The main reasons for the high rate of poor oral health status is due to, over 72% of the population are from rural area. In the villages of India there are no parodontal facilities for the people¹³. Compared to rural areas the Periodontal health is better in urban area. Hospital and dental colleges are found to be more in urban area, which help in giving awareness to nearby villages and towns. Apart from this continuous monitoring of bone loss in post menopausal women

and restoration of calcium will prevent periodontitis¹⁴. According to Vipin Agarwal oral disease affects 50% of the Indian community¹⁵. Dental health education and awareness have been shown to be correlated to periodontal health.

Natural therapy an alternative: A better alternative for prevention and treatment for periodontitis is natural therapy. The use of tender neem twigs is found to be used as toothbrush in many parts of India. This will help in maintaining good oral health and reduces bad breath¹⁶. The predominant type of oral disease is Periodontal disease, which is mainly induced by plaque formation. The main causative agent of dental caries is *Streptococcus mutans*. Resin forming tree Pistachio extract is found to be having antibacterial activity against *Streptococcus mutans*¹⁷. In another study mango extracts were found to be having antimicrobial effect on *Staphylococcus aureus*¹⁸. It is also reported white cedar crude extract is also effective against *Enterococcus faecalis*, *Micrococcus* and *E.coli*¹⁹.

Conclusion

It can be concluded from the data from the above study that Post menopausal women are prone to periodontitis than Premenopausal women. Awareness should be brought among Indian women population, especially in rural areas on the importance of Oral Health hygiene.

References

1. Spratt D., Dental Plaque and bacterial colonization In medical biofilms. Jass J. Surman S. Walker editors Wiley and Sons Ltd. 175-98 (2003)
2. Friedlander A.H., The physiology, medical management and oral implications of menopause, *J. of American Den. Association* , **133**, 73-81 (2002)
3. Mane A.K., Karmarkar A.R., Bharadwaj RS., Anaerobic bacteria in subjects with chronic periodontitis and in Periodontal health, *J. of Oral health Community Dentistry*, **3(3)** 49-51 (2009)
4. John Lindquist, An Introduction to Bacteriology- 2001 www.jlindquist.net (2001)
5. Slots J., Rosling B.G., Genco R.J., Suppression of penicillin resistant oral *Actinobacillus actinomycetemcomitans* with tetracycline, considerations on endocarditis prophylaxis, *Journal of Periodontology*, **54** 193-196 (1983)
6. Baron E.J., Citron D.M., Anaerobic infection flowchart using minimal laboratory resources, *Clinical infectious Diseases*, **25(2)**, 143-146 (1997)
7. Koneman E.W., Allen S.D., Janda M.V. and Schredenber P.C., The anaerobic bacteria *Colour atlas and text books of diagnostic Microbiology 5th edition* Philadelphia Lippincott JB Ch **14** 725-727 (1997)
8. Colombo Ana Paula V., Subgingival Microbiota of Brazilian subjects with untreated chronic periodontitis, *Journal of Periodontology*, **73**, 360-369. (2002)
9. Slots J., Rams T.E. and Listgarten M.A., Yeasts enteric rods Psuedomonads in sub gingival flora of adult periodontitis, *Oral Microbiology and Immunology*, **3**, 47-52 (1988)
10. Jarvensivu A., Hietanen J., Rautemaa R., Sorsa T., Richardson M., Candida yeasts in chronic periodontitis tissues and subgingival microbial films, *Oral Diseases*, **10**, 106-112 (2004)
11. Dahlen G., Wikstrom M., Occurrence of enteric rods staphylococcus and candidia in subgingival samples, *Oral Microbiology Immunology*, **10**, 42-46 (1995)
12. Reynaud A.H., Nygaard-Otsby B., Boygard GK., Eribe E.R., Olsen I. and Gjermo P., Yeasts in periodontal pockets, *J. of Clinical Periodontology*, **28**, 860-864 (2001)
13. Vimal Sikri, Poonam Sikri, National Community Dentistry **1st edition**, CBS Publication, 19-38 (1999)
14. ShukhlanJharna, Shukhla Nupur, Dey Sarkar Purnima, Bafna Agoorbala, Assessment of Bone loss in post menopausal women by evaluation of Urinary hydroxyproline and serum status of osteocalcin, *Int. Res. J. of Biologic. Sci.*, **2(9)**, 11-14 (2013)
15. Vipin Agarwal, Manish Khatri, Guljot Singh, Geeti Gupta Marya Vimal Kumar. Prevalence of Periodontal diseases in India, *Journal of Oral health and Community Dentistry*, **4**, (Spl) 7-16 (2010)
16. Inaam Hashmat, Hussain azad, Ajj Ahmed. Neem (*Azadirachta indica A.Juss*) A nature's drugstore: An overview, *Int. Res. J. of Biologic. Sci.*, **1(16)**, 76-79 (2012)
17. Farzenh Hosseini, Afsoon Adlgostar, Fariba Sharifma. Antibacterial activity of *Pistachio atlantica* extracts on *Streptococcus mutans* biofilm, *Int. Res. J. of Biologic. Sci.* **2(2)**, 1-7 (2013)
18. Prakash Alok, V.keerthana, Jha Chandana Kumar, Kumar Ratan Agarwal Dinesh. Antibacterial property of two different varieties of Indian mango Kernel extracts at various concentrations against some Human Pathogenic bacterial strains, *Int. Res. J. of Biologic. Sci.* **2(4)**: 28-32 (2013)
19. Sharma Abhis, Patil Ujwala, Kakkar Sharma, Bhot Meeta. Evaluation of *Tecomella undlata* leaves crude extracts, *Int. Res. J. of Biologic. Sci.*, **2(6)**, 60-62 (2013)