ORIGINAL RESEARCH PAPER Volume - 13 | Issue - 11 | November - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CHRONIC OTITIS MEDIA IN ADULTS - DOES PERIODONTAL PATHOGENS (RED COMPLEX BACTERIA) IN CHRONIC PERIODONTITIS PLAY A ROLE?

Otorhinolaryngology

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ABSTRACT

Periodontitis is an inflammatory is case driven by infection of the tooth-supporting tissue. Otitis media is defined as inflammation in the middle ear cleft, the effusion of fluids in turn may be associated with either absence or presence of tympanic membrane perforation. It has been hypothesized that oral pathogens can translocate to the middle ear cavity through saliva as a transmission vehicle via the eustachian tube. Several bacterial complexes, like the red complex bacteria, consisting of *Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia*, have been found to play a role in the pathogenesis of periodontitis. **Aims:** 1. To detect the red complex bacteria in subgingival plaque sample of periodontally healthy individuals and individuals with chronic periodontitis and in adult chronic otitis media (COM) samples using PCR. 2. To evaluate the correlation between the red complex bacteria in chronic periodontitis and COM in adults using PCR. **Methodology:** 40 individuals with COM in the age group of 20-55 years tested over a period of 1 year and divided into two groups (20 in each group):

1. GROUP 1 - Subjects with chronic periodontitis (TEST)
2. GROUP 2 - Subjects with healthy periodontium (CONTROL)

Pooled subgingival plaque samples and middle ear effusion samples were collected and analyzed using PCR and data was tabulated. **Results:** There was a statistically significantly higher counts of *P.gingivalis and T.denticola* in the test group (Group 1) than in the control group (Group 2) ($p<0.05$). A linear correlation was also seen for *T.denticola* in the test group ($r= 0.297$). **Conclusion:** These results show that there is an increased bacterial load of oral bacterial flora, especially *T. denticolam* in the patients with COM who suffer from periodontitis than in patients who are periodontally healthy. Hence it could be said that Periodontal status of the patient could play a role in the pathogenesis of COM.

KEYWORDS

Chronic Otitis Media, Dental Plaque, Periodontitis, Red complex bacteria, PCR.

INTRODUCTION

Chronic otitis media (COM) is a chronic inflammation of the middle ear and mastoid mucosa, in which the tympanic membrane is not intact and a discharge (otorrhea) is present. COM remains one of the most common chronic childhood infections worldwide, affecting diverse ethnic and cultural groups in both developing and developed countries. It is associated with significant morbidity and can lead to extracranial and intracranial complications. Otitis media was first described by Hippocrates in the 5th century B.C.E. The first recorded surgical incision for treatment of medial ear infection was in the 16th century C.E., performed by French physician Ambroise Paré. German physicians Hermann Schwartze, Anton von Troltsch, and Adam Politzer published the first journal dedicated to ear pathology and treatment in 1865 [1]. Upper respiratory tract infections play an important role in the pathogenesis of middle ear disease. Even though its more common in children, both children and adults experience long-term morbidity with varying degrees of hearing loss. This may be due to the presence of fluid in the middle ear [2]. COM can also affect the adult population and cause significant morbidity.

The periodontium, constitutes a developmental, biologic, and functional unit which undergoes certain changes with age and is, in addition, subjected to morphologic changes related to functional alterations and alterations in the oral environment, Periodontitis is an inflammatory condition driven by infection of the tooth-supporting tissue (i.e., the periodontal tissue). In addition, genetic, environmental and behavioral factors contribute to disease development, exposure of susceptible individuals to the disease, and rate of progression [3]. The structure of the periodontal tissue is diverse. It consists of gingiva, cementum, alveolar bone and periodontal ligament fibers. The junctional epithelium is a unique structure located at the bottom of the gingival sulcus and controls the constant presence of bacteria in this area. The most characteristic feature of periodontitis is the activation of osteoclastogenesis and consequent destruction of alveolar bone, which is irreversible and leads to loss of tooth support. Periodontitis is characterized by microbial associated, host-mediated inflammation leading to loss of periodontal attachment. This is indicated as clinical attachment loss by circumferential assessment of the dentition using a standardized periodontal probe relative to the cement-enamel junction, every part of the human body has typical microflora. Bacterial communities associated with teeth, supragingival and subgingival plaque, form distinctly different clusters than those found at other intraoral or extraoral sites. The main difference between these types of dental biofilms is the lower subgingival redox potential, resulting in the presence of strictly anaerobic genera in gingival biofilms (eg: *Fusobacterium, Prevotella, and Treponema*).Suspected periodontal pathogens can be detected in supragingival plaque at affected sites, whereas subgingival plaque ultimately interacts with periodontal tissue and resides in a special environment, limited space and host immune protection but with abundant nutrition from the gingival crevicular fluid [4].

Several bacterial complexes have been found to play a role in the pathogenesis of periodontitis, and the red complex bacteria, consisting of *Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia*, have been known to be the most notorious compared to other bacterial complexes [5]. Saliva appears to be a transmission vehicle for of oral bacteria between the mouth and nasopharynx. It is well known that microorganisms from the nasopharynx can enter the middle ear cavity via the eustachian tube and cause otitis media [6].

Polymerase chain reaction (PCR) developed by Kary Mullis in 1983, is based on harnessing the ability of DNA polymerase to synthesize a new DNA strand complementary to a provided template strand. DNA polymerases can only add nucleotides to an already existing 3'-0H group, so it requires a primer to which it can add the first nucleotide. This requirement allows researchers to delineate specific regions of the template sequence they wish to amplify. At the end of the PCR reaction a specific sequence accumulates in billions of copies (amplicons).

The PCR technique is a more accurate, sensitive, and rapid technique for the detection, identification, and quantification of periodontal bacteria Q-PCR or real-time PCR with species-specific primers provide accurate quantification of individual microbial species and total bacterial count in dental plaque samples. This precise and sensitive method serves as a useful tool for studies on etiology of periodontal diseases. Various putative perio-pathogens such as, *Tannerella forsythia, Prevotella intermedia, Prevotella nigrecens,*

Parvimonas micra, Eubacteria, Campylobacter rectus, Capnocytophaga sputigena, Capnocytophaga ochracea and Capnocytophaga gingivalis have been detected in subgingival plaque samples. Diagnostic tests such as the MicroDentR Test, ParoCheck R kits, MyPerioPathR Test and oral DNAR using multiplex PCR scheme are commercially available to evaluate the microbiota in subgingival plaque samples and they give crucial information for a prevention strategy for healthy patients and treatment plans for 'at risk` patients.

The correlation between the periodontal red complex bacteria and their possible role in pathogenesis of COM in adults is not clearly established. Hence, we aim to-
1. To detect the red complex bacteria in subgingival plaque sample of periodontally healthy individuals and individuals with chronic periodontitis and in adult COM samples using PCR.

2. To evaluate the correlation between the red complex bacteria in chronic periodontitis and COM in adults using PCR.

METHODOLOGY

SOURCE OF DATA:

The study was carried out on patients reporting to the Department of ENT, JJM, Medical College, Davanagere, after obtaining approval from the institutional ethical committee.

Study Of Design: cross sectional study.

Study Duration: February 2021-February 2022

Selection Criteria:
Patients were selected with following inclusion and exclusion criteria from both the sexes.

Inclusion Criteria
Periodontally Healthy With Chronic Suppurative Otitis Media:
- Sulcus depth <3mm
- No sites with clinical attachment loss
- Chronic suppurative otitis media

Subjects With Periodontitis And Chronic Suppurative Otitis Media:
- Probing Pocket Depth 6mm
- Clinical attachment loss > 3mm
- Chronic suppurative otitis media

Exclusion Criteria:
- Individuals with systemic conditions including Diabetes, Rheumatoid Arthritis (RA), Inflammatory Bowel Disease (FD), allergies, etc.
- Individuals who have had antibiotic/anti-inflammatory drugs 3 months prior to the study
- Individuals who have had periodontal therapy within previous 3 months.
- Pregnant and lactating females
- Individuals who consume alcohol and tobacco.
- Individuals with chronic suppurative otitis media who have applied topical antimicrobials either internally or externally in the last 3 months.

40 individuals with COM in the age group of 20-55 years tested over a period of 1 year and divided into two groups (20 in each group):

1. GROUP 1 - Subjects with chronic periodontitis (TEST)
2. GROUP 2 - Subjects with healthy periodontium (CONTROL)

Clinical Parameters:
Following clinical parameters were recorded.:
1. Plaque index (Silness J. &Loe H. 1964)
2. Gingival bleeding index (Ainamo & Bay 1975)
3. Probing pocket depth (PPD) (UNC 15 probe).
4. Clinical Attachment Level (CAL) (UNC 15 probe).

Two samples were collected from each patient. First sample was pooled subgingival plaque which was collected using 2R/2L or 4R/4L curettes (Hu-Friedy). Second sample was effusion collected-from middle ear from the same patient by swab using a Jobson Horne Probe to which cotton was wrapped at the tip. All the samples were stored in a separate container with transport media at -80°C and was transferred to microbiology lab for identification of periodontal red complex bacteria by using PCR.

Laboratory Procedure
Detection and quantification of *Porphyromonas gingivalis, Treponema denticola and Tannerella forsythia* was done by using PCR method at Maratha Mandal's Central Research Laboratory, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre.

DNA extraction from samples was done by Modified Proteinase-K method.

Detection of amplified products (Agarose gel Electrophoresis): Amplified products were subjected to electrophoresis through Agarose gel containing XI TAE (Tris Acetate EDTA buffer), 20 ml of each amplified product were loaded into each well. Electrophoresis was performed at 25V for 2 hr. The gel was visualized under UV light illuminator after staining with Ethidium bromide(0.5µg/ml). The gel image was captured and analyzed using Gel Documentation System (Major Science, USA). The procedure is as follows:

1. 2gm of Agarose powder was weighed and mixed in 100ml of 1X TAE Buffer (for 2% agarose).
2. The mixture was boiled with gentle stirring till homogenous clear solution was formed
3. The solution was cooled for some time. 2µl of ethidium bromide was added (0.5pg/ml).
4. The solution was then poured into gel mold & the comb was placed. It was then allowed to settle for at least 20min
5. The comb was removed carefully. The gel was kept in electrophoresis unit containing 1X TAE buffer (200ml). The gel was completely submerged in the buffer
6. Coral Load buffer in the amplified sample acted as loading dye
7. 20 pl of this amplified product was taken and added carefully into the wells; molecular weight marker was loaded in the last well.
8. The electrode was fixed; power supply was turned on and the current was adjusted(16A)
9 The gel was run for two hours
10.The photo of gel was taken under UV light transilluminator and the bands were recorded using Gel documentation system (Major Science, USA).

Each organism had specific band size based on the primer sequences selected. *P.gingivalis* had amplified product of 404 base pair, *T.forsythia* gave amplified product of 641 base pair and *T. denticola* gave amplified product of 316 base pair.. The DNA ladder was run simultaneously with each gel to obtain the bands of known sizes which was used in locating the band positions of test samples. Total lab software (UK) was used to obtain the quantification of positive bands based on the intensity of the amplified products. The gel was uploaded into the software and quantification was obtained.

Statistical analysis was done with SPSS software. The data obtained from the 2 groups was analyzed using the following statistical analyses:
1. Independent Sample 't' test
2. Karl Pearson's Coefficient of Correlation.

RESULT:

40 patients within the age group of 20-55 were included in the study based on the inclusion and exclusion criteria and grouped based in cases with periodontitis as Group 1 and those without periodontitis as Group 2.

As the data were non-normally distributed, the mean counts of the three organism types in the two groups were compared using Mann-Whitney U test. The correlation between oral and ear samples was done using Pearson's correlation analysis. A P value of <0.5 was considered significant. As the counts for *T. forsythia* were absent for group 2, they could not be compared statistically.

In the ear samples *P.gingivalis* counts were detected with the mean value of 246.26 and in control the mean value was seen to be 14.5 . Similarly *T.denticola* counts were detected the samples in the test group with the mean value of 219.66 and in the control group it was with a mean value of 15.4 as shown in Table 1.

Table 1. Inter Group Comparison Of Ear Samples Which Were

Significantly Larger In Test Samples Than Control Group. (Bacterial Counts)

Organism compared	Group	Mean	Standard deviation	Mean difference	P Value
P.gingivalis	1	246.26	196.70	231.76	0.006
	2	14.5	17.88		
T.denticola	1	219.66	199.87	204.21	0.011
	2	15.45	16.34		

In the oral samples *P.gingivalis* counts were detected with the mean value of 167.25 and in control the mean value was seen to be 34.65 . Similarly *T.denticola* counts were detected the samples in the test group with the mean value of 191.35 and in the control group it was with a mean value of 17.28 as seen in Table 2.

Table 2. Inter Group Comparison Of Oral Samples Which Were Significantly Larger In Test Samples Than Control Group. (Bacterial Counts)

Organism compared	Group	Mean	Standard deviation	Mean difference	P Value
P.gingivalis	1	167.25	174.53	132.6	0.040
	2	34.65	69.38		
T.denticola	1	191.35	193.18	174.07	0.044
	2	17.28	27.69		

Overall *P.gingivalis* was detected in 67.5% of the samples in test group with the mean value of 206.75 and in control group it was detected in 55% of the samples and the mean value was seen to be 24.56 . Similarly *T.denticola* was detected in 60% of the samples in the test group with the mean value of 205.51 and in the control group it was detected in 50% of the samples with a mean value of 16.36 as seen in Table 3.

Table 3 Inter Group Comparison Of Overall Counts In Samples Which Were Significantly Larger In Test Samples Than Control Group. (Bacterial Counts)

Organism compared	Group	Mean	Standard deviation	Mean difference	P Value
P.gingivalis	1	206.75	187.86	182.19	0.001
	2	24.56	51.03		
T.denticola	1	205.51	194.55	189.15	0.001
	2	16.36	22.46		

After PCR, the data was obtained and statistically analyzed. The results showed that there was statistically significant higher count of *P. gingivalis and T.denticola* in Group 1 samples. A positive correlation for *T. denticola* was seen between the oral and ear samples of the test group.

Pearsons correlation between oral and ear counts for *P.gingivalis and T. denticola* is depicted in table 4. It shows a positive correlation was seen between oral and ear samples of *T.denticola* in the test group.

Table 4: Pearson's Correlation

Organism compared	Group	Variable 1	Variable 2	r value	P Value
P.gingivalis	1	ORAL	EAR	-.143	0.379
T.denticola	1	ORAL	EAR	.297	.204
P.gingivalis	2	ORAL	EAR	-.020	0.935
T.denticola	2	ORAL	EAR	-0.20	0.935

T. forsythia was detected only in 7.5% of the samples in the test group and was not detected in any samples of the control group.

DISCUSSION

Periodontal pathogens, in particular the red complex bacteria comprising of *P.gingivalis, T.denticola and T. forsythia* are known be the cause of severe and destructive periodontal disease and the periodontal pocket serves as a niche for these pathogens to survive and multiply. Hence in the present study, pooled subgingival plaque samples were collected to enhance the chances of detection of these organisms. Middle ear effusion samples from COM patients were collected from within the ear canal and not from near the external auditory meatus to prevent contamination of the samples.

Both the collected samples were placed in a transport media (TE buffer solution) and then sent to the microbiological lab for detection and quantification of the red complex bacteria in each sample. 40 patients enrolled in the present study and hence 80 samples (40 MEE-+ 40 subgingival plaque) were analyzed.

After PCR, the data was obtained and statistically analyzed. The results showed that there was satistically significant higher count of *P. gingivalis and T.denticola* in the Group 1 samples. A positive correlation for *T. denticola* was seen between the oral and ear samples of the test group.

This is the first study comparing subgingival plaque samples and middle ear effusion samples in adults. Based on the above findings, we could hypothesize that periodontal health of an individual could directly influence the pathogenic load in the middle ear effusion of the same patient. We could also infer that patients undergoing routine periodontal therapy could have a lesser risk of developing recurrent chronic otitis media.

Within the limits of this study, it can be concluded that periodontal pathogens could translocate from oral cavity to the middle ear and play a significant role in middle ear infections. Furthermore, oral hygiene maintenance could help in reducing the bacterial load of the pathogens in the middle ear and could also limit the further spread and recurrence rate of the disease. We could also infer from the study that in cases that reoccur and not resolving with conventional therapy, oral pathologies should also be looked at as possible source of pathogens.

CONCLUSION

Following the statistical analysis of the data, following conclusions were drawn.

1. The number of oral and middle ear red pathogens were significantly higher in the periodontitis group than the periodontally healthy group.

2. A positive correlation was seen for *T. denticola* in the periodontitis group.

This study shows that red pathogens were seen significantly higher in middle ear amongst those with periodontitis. Hence it could be said that chronic periodontitis in patients could be a factor in pathogenesis of chronic otitis media, however further research is necessary to confirm the same.

Limitations

Long term clinical studies are needed to assess the clonal similarity of the pathogens could help us confirm the translocation of these pathogens from the oral cavity to the middle ear

REFERENCES

1. Bento RF, Fonseca AC. A brief history of mastoidectomy. Int Arch Otorhinolaryngol. 2013 Apr;17(2):168-78. doi: 10.7162/S1809-97772013000200009. PMID: 25992009; PMCID: PMC4423283.
2. Danishyar A, Ashurst JV. Acute Otitis Media. [Updated 2023 Apr 15]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK470332
3. Könönen E, Gursoy M, Gursoy UK. Periodontitis: A Multifaceted Disease of Tooth-Supporting Tissues. J Clin Med. 2019 Jul 31;8(8):1135. doi: 10.3390/jcm8081135. PMID: 31370168; PMCID: PMC6723779.
4. Sedghi LM, Bacino M, Kapila YL. Periodontal Disease: The Good, The Bad, and The Unknown. Front Cell Infect Microbiol. 2021 Dec 7;11:766944. doi: 10.3389/fcimb.2021.766944. PMID: 34950607; PMCID: PMC8688827.
5. Mohanty R, Asopa SJ, Joseph MD, Singh B, Rajguru JP, Saidath K, Sharma U. Red complex: Polymicrobial conglomerate in oral flora: A review. J Family Med Prim Care. 2019 Nov 15;8(11):3480-3486. doi: 10.4103/jfmpc.jfmpc_759_19. PMID: 31803640; PMCID: PMC6881954.
6. Topcuoglu N, Keskin F, Ciftci S, Paltura C, Kulekci M, Ustek D, Kulekci G. Relationship between oral anaerobic bacteria and otitis media with effusion. Int J Med Sci. 2012;9(3):256-61. doi: 10.7150/ijms.4382. Epub 2012 May 7. PMID: 22606045; PMCID: PMC3354330.
7. Segata N, Haake SK, Mannon P, Lemon KP, Waldron L, Gevers D,Huttenhower C, Izard J. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. Genome biology. 2012 Jun; 13:1-8. https://doi.org/10.1186/gb-2012-13-6-r42
8. Jervee-Storm PM, Koltzscher M, Falk W, Dörfler A, Jepsen S. Comparison of culture and real-time PCR for detection and quantification of five putative periodontopathogenic bacteria in subgingival plaque samples. Journal of clinical periodontology. 2005 Jul;32(7):778-83. PMID: 15966886 DOI: 10.1111/j.1600-051X.2005.00740.x
9. Yücetürk AV, Ünlü HH, Okumuş M, Yildiz T, Filiz Ü. The evaluation of eustachian tube function in patients with chronic otitis media. Clinical Otolaryngology & Allied Sciences. 1997 Oct;22(5):449-52. DOI: 10.1046/j.1365-2273.1997.00029.x
10. Roland PS. Chronic suppurative otitis media: a clinical overview. Ear, nose & throat journal. 2002 Aug 2;81(8):8
11. Gmür R, Guggenheim B. Monoclonal antibodies for the detection of periodontopathic bacteria. Archives of Oral Biology. 1990 Jan 1;35:145-51.
12. Jervee-Storm PM, Koltzscher M, Falk W, Dörfler A, Jepsen S. Comparison of culture and real-time PCR for detection and quantification of five putative periodontopathogenic bacteria in subgingival plaque samples. Journal of clinical periodontology. 2005 Jul;32(7):778-83 DOI: 10.1111/j.1600-051X.2005.00740.x
13. Turgeon ML. Linne & Ringsrud's Clinical Laboratory Science-E-Book: The Basics and Routine Techniques. Elsevier Health Sciences; 2015 Feb 10
14. Relationship between oral anaerobic bacteria and otitis media with effusion. -Topcuoglu

Keskin F , Ciftci S , Paltura C , Kulekci M , Ustek D , Kulekci G. 2001 Oct 1;7(5):241-6 https://doi.org/10.7150/ijms.4382

15. Kamen Kotsilkov,Chrisitina Popova,Lyudmila Boyanova,Lena Setchanova &Ivan Mitov -Comparison of culture method and real-time PCR for detection of putative periodontopathogenic bacteria in deep periodontal pockets Biotechnology & Biotechnological Equipment Volume 29, 2015 - Issue 5 https://doi.org/10.1080/13102818.2015.1058188
16. Feigin RD. Otitis media: closing the information gap. N Engl J Med. 1982 Jun 10;306(23):1417-8. doi: 10.1056/NEJM198206103062309. PMID: 6978993.