Assessment of Oral Bacterial Microflora in Patients with Stable Chronic Obstructive Pulmonary Disorders

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KEYWORDS
Chronic Obstructive Pulmonary Disorder, Oral Hygiene Index-S, periodontal index, porphyromonas gingivalis, haemophilus influenza

ABSTRACT
Background & Objectives: Chronic obstructive pulmonary disorder is an inflammatory disease characterized by the progressive deterioration of pulmonary function and increasing airway obstruction, includes chronic bronchitis and emphysema. The oral cavity has been considered a potential reservoir for respiratory pathogens. Oral bacteria may play a vital role in the exacerbation of chronic obstructive pulmonary diseases. Hence, we intend to study the bacterial microflora of oral cavity in stable COPD patients.
Methods: In this study 20 patients aged between 20 to 60 years diagnosed with stable COPD and 20 normal healthy controls were selected based on the set inclusion and exclusion criteria. A detailed case history, physical examination and investigations and all the subjects were examined for gingival and periodontal status by recording the Oral hygiene which comprises debris index and calculus index and periodontal index and Quantitative oropharyngeal cultures were obtained by oral washing using isotonic saline as the collecting fluid.
Results: A significant higher score of OHI(S) and PI was observed in COPD patients. There is a direct correlation between periodontal pathogens with the severity of COPD.
Conclusion: The predominant organisms found in the oropharyngeal secretions were porphyromonas gingivalis and haemophilus influenza, in COPD patients.

1. Introduction

The oral microbial ecosystem is remarkably dynamic which consist of multiple bacterial, fungal species and their associated biofilms. Over the past decade, a growing body of scientific evidence suggests an exquisite association between oral infection and systemic diseases [1]. As a consequence, there has been a resurgence of interest in oral microbial ecology, mucosal immunity, and associations with systemic conditions[2]. Chronic obstructive pulmonary disorder is an inflammatory disease characterized by the progressive deterioration of pulmonary function and increasing airway obstruction, includes chronic bronchitis and emphysema. Chronic bronchitis, a clinically defined condition with chronic cough and phlegm whereas emphysema characterized by destruction and enlargement of the lung alveoli. In line with the relationship between the anatomical position of oral cavity and pulmonary infection, oral bacteria can be easily carried into the lung and cause infection[3]. There are four possible ways of contamination of lower airways by microorganisms: aspiration of oropharyngeal secretions, food, or gastric contents, inhalation of infectious aerosols, spread of infections from contiguous sites, and hematogenous spread from extrapulmonary source[2].

It has become apparent in recent years that the oral cavity may be an important reservoir for bacterial pathogens that cause lung diseases and patients with COPD can suffer infectious lung diseases secondary to the aspiration of microorganisms in the presence of deficient immune status. On thorough search of literature, studies on oral bacterial microflora in stable COPD patients are limited. Hence, we intend to study the bacterial microflora of oral cavity in stable COPD patients.

2. Aims and Objectives

1. To study the oral bacterial microflora in stable COPD patients.
2. To study the oral hygiene status in stable COPD patients.
3. To correlate different type of pathogens with severity of COPD.

Source of Data

The present clinical study was conducted in the Department of Oral Medicine and Radiology, Government Dental College and Research Institute in association with department of pulmonology, Victoria hospital, Bangalore, India. In this study 20 patients aged between 20 to 60 years diagnosed with stable COPD, were selected for the study and 20 normal healthy controls were selected based on the set inclusion and exclusion criteria. The study was conducted in full accordance with ethical principles, was independently reviewed, approved by the ethical board of the institution.

Inclusion criteria	Exclusion criteria
1. Diagnosed cases of stable COPD patients with age group between 20-60 years.	1. Patients with Hepatitis, Autoimmune disease or systemic diseases like diabetes, hypertension and HIV, malignancies and other mucosal disorders.
2. Patients under treatment for COPD from the last six months.	2. Patients under systemic steroids, anti-coagulants and immune suppressive drugs.
3. patients with at least six natural teeth present.	3. Pregnant or breast-feeding women.

Twenty patients with stable COPD, who satisfied the above inclusion and exclusion criteria and 20 normal patients with no past or present history of COPD were included in the control group. All the patients were explained the aims of the study, methodology, risks and benefits of participation in the study and a formal written informed consent was obtained. The pulmonologist made the diagnosis of respiratory diseases and non-respiratory diseases. Lung function was estimated by spirometry in both the groups by calculating the ratio of forced expiratory volume in 1 sec and forced vital capacity FEV1/FVC < 70% was diagnosed as COPD. All 40 patients of case and control group were examined for gingival and periodontal status by recording the following indices:, Oral hygiene index and periodontal index.

Oral hygiene status was assessed by Simplified Oral Hygiene Index (OHI-S)[27] which has two components, the Debris Index-Simplified (DI-S) and the CalculusIndex-Simplified(CI-S),which are calculated separately and are summed up to get OHI-S for an individual. The interpretation of index is as follows:good—0to1.2 , fair—1.3 to3.0, poor—3.1 to 6.0.

The periodontal disease index (PDI)[28] was developed by Sigurd P Ramfjord in 1959. The PDI is a clinician's modification of Russell's periodontal index (PI) for epidemiological surveys of periodontal disease. The PDI is primarily concerned with an accurate assessment of the periodontal status of the individual person. Emphasis is placed on recording of the attachment level of the periodontal tissues relative to the cementoenamel junction. Such accurate measurable assessments are essential for longitudinal studies of periodontal disease and as a scientific basis for clinical trials. Scoring methods Only six selected teeth are scored for assessment of the periodontal status of the mouth; however, for short-term clinical trials and where a limited number of patients are available, one may consider all of the teeth in the mouth. The six selected index teeth are: 16—Maxillary right first molar, 21—Maxillary left central incisor,24—Maxillary left first premolar,36—Mandibular left first molar, 41—Mandibular right central incisor, 44—Mandibular right first premolar.

Table 1: Periodontal disease index: scoring criteria

0	Absence of inflammation
1	Mild to moderate inflammatory gingival changes not extending all around the tooth.
2	Mild to moderately severe gingivitis extending all around the tooth.
3	Severe gingivitis, characterized by marked redness, tendency to bleed, and ulceration.
4	Gingival crevice in any of the four measured areas (mesial, distal, buccal, lingual), extending apically to the cementoenamel junction but not more than 3 mm.
5	Gingival crevice in any of the four measured areas extending apically to the cementoenamel junction 3–6 mm.
6	Gingival crevice in any of the four measured areas extending apically more than 6 mm from the cementoenamel junction.

Calculation of PDI scores: The PDI score for the individual is obtained by totalling the scores for each tooth examined and then, dividing by the number of teeth examined. The PDI score ranges from 0 to 6. The PDI score for a group is obtained by totalling the individual PDI scores and then, dividing by the number of people

examined. The average PDI score for a group range from 0 to 6.

3. Sample for Microbial Culture

Quantitative oropharyngeal cultures were obtained by oral washing using isotonic saline as the collecting fluid. The subject rinsed and gargled with 10ml of saline for 30s which was collected in a sterile vial. From this sample 1 ml was added to 9 ml of brain heart infusion (BHI) broth, to make serial tenfold dilutions. Dilution series were made in trays of 64 (8x8) cups of 1.5 ml. Each cup was filled with 0.45 ml of BHI. A 0.05 ml sample from the 1: 10 diluted oropharyngeal suspension was mixed with 0.45 ml BHT in the first cup, resulting into a 1: 100 oropharyngeal suspensions. All dilution steps were prepared in BHI with 0.05 ml micro diluters. The oropharyngeal samples were incubated at 37°C for 18 h. The number of cups showing turbidity due to growth of bacteria indicates the logarithm of the concentration of microorganisms per ml of oropharyngeal washing (quantitative determination). Thereafter, all the dilutions with growth were subculture on MacConkey agar, yeast morphology agar and blood agar for qualitative determination. Enterobacteriaceae, Pseudomonadaceae and Acinetobacter spp. were evaluated on MacConkey agar, yeasts on yeast morphology agar and streptococci and staphylococci on blood agar. Morphologically distinct colonies were isolated in pure culture. Predominantly grown organism were taken into consideration.

4. Statistical Analysis

The data were compiled and analysed using Statistical Package for Social Sciences (SPSS) for Windows, Version 22.0. IBM. Corp. The collected data was summarized by calculating frequency and percentage for discrete variables and mean, standard deviation for continuous variables. Comparison of socio-demographic variables including the smoking habit prevalence between 02 study groups were done using Fischer Exact Test. The association b/w the presence of various Bacterial Microflora among the 02 study groups was done using chi square test. The clinical parameters like FVC (%), OHI & PI was compared between the 02 study groups using student unpaired t test. Statistical significance was set at $P < 0.05$.

Age distribution:

In the study population out of twenty cases 60% of patients were in the age range of 35-50years whereas the rest of the 40% patients were in the age range of 51-70 years. 60% of Controls were in the range of 51-70 years and the rest of 40% were in the range of 35-50 years.

Gender distribution: In both the study population percentage of males were 70%, compared to the female population which was around 30%.

Comparison of clinical parameters among the study population Out of 20 cases the mean respiratory function (FEV1 /FVC) was 62.15% (severe respiratory infection), whereas out of 20 controls the mean respiratory function (FEV1 /FVC) was 81.60% (normal respiratory function). Which is statistically significant ($p<0.001$)

The mean OHI(S) score of the cases was 2.99 (fair oral hygiene status), whereas the controls had the mean OHI(S) score of 2.03(fair oral hygiene status), which is statistically significant with the p value of 0.009.

The mean PI score of the cases was 5.5(i.e. Gingival crevice in any of the four measured areas extending apically to the cementoenamel junction 3–6 mm), whereas the mean PI score of the controls was 2.3(ie Mild to moderately severe gingivitis extending all around the tooth), which statistically significant with the p value of 0.001. (table 2)

Table 2: comparison of clinical parameters among the 02 study groups using student unpaired t test

Variables	Groups	N	Mean	SD	S.E.M	Mean Diff	95% CI for the Diff		t	P-Value
							Lower	Upper		
FEV1 / FVC(%)	Cases	20	62.15	4.83	1.08	-19.45	-22.54	-16.36	-12.740	<0.001*
	Controls	20	81.60	4.83	1.08					
OHI(S)	Cases	20	2.99	1.27	0.28	0.97	0.25	1.68	2.730	0.009*
	Controls	20	2.03	0.94	0.21					
PI	Cases	20	5.55	1.73	0.39	1.85	0.85	2.85	3.740	0.001*
	Controls	20	2.30	1.38	0.31					

- - Statistically Significant

Comparison of the presence of various bacterial microflora among the study groups Out of 20 cases 40% of patients had haemophilus influenza, 40% of patients had porphyromonas gingivalis and 20% of patients had noramal microbial flora in their oropharyngeal secretions. Out of 20 controls 85% of patients had normal microflora, 15% of patients had porphyromonas gingivalis in their oropharyngeal secretions. On comparison of the presence of bacterial microflora between the two groups was statistically significant with the p value of <0.001.(table 3)

Table 3: Comparison of the association b/w the presence of various Bacterial Microflora among the study groups using chi square test

Microflora	Cases		Controls		χ^2 value	P-Value
	n	%	N	%		
Normal Flora	4	19.0%	17	81.0%	18.320	<0.001*
H. Influenza	8	100.0%	0	0.0%		
P. Gingivalis	8	72.7%	3	27.3%		

- - Statistically Significant

5. Discussion

The oral cavity hosts a highly diverse microbiota. Because of its humidity and temperature, the mouth provides an appropriate environment for the development of organized bacterial communities. These occur as biofilms on both hard surfaces (teeth) as well as the soft tissue of the stomatognathic system. The anatomical continuity between the lungs and the oral cavity makes the latter a potential reservoir of respiratory pathogens.In the study population out of twenty cases 60% of patients were in the age range of 35-50years whereas the rest of the 40% patients were in the age range of 51-70 years. 60% of Controls were in the range of 51-70 years and the rest of 40% were in the range of 35-50 years. In patients with COPD, 70% were males compared to the female population which was around 30%. The predominance of COPD among males is explained because of the habit of smoking in males. Assessment of periodontal health was done using OHI(S) and PI. The oral hygiene index-Simplified (OHI-S) is depicted as a sensitive simple method for assessing group, individual oral hygiene quantitatively and the PI is primarily concerned with an accurate assessment of the periodontal status of the individual. In our study a significant higher score of OHI(S) and PI scores was noted in individuals with decreased respiratory functions (FEV1 /FVC) i.e. in COPD patients compared to the normal controls.The possible factor causing increased scores of OHI(S) and PI could be related with maintenance of oral hygiene. These results are in accordance with the studies conducted by Scannapieco et al, Gracia and Hayes et al and Benazir ghani et al.

As the periodontal infection increases the mean FEV1 /FVC (%) is decreased, hence there is an inverse relation between periodontal infection and lung function in patients with COPD. In untreated periodontal disease cytokines produced by epithelial and connective tissue in response to these bacteria include IL-1a.IL-1ß, IL-6, IL-8 and TNF-α. They recruit neutrophils to infiltrate airway parenchyma and release proteolytic enzymes and toxic oxygen radicals damage respiratory epithelium making it more susceptible to COPD (Travis et al 1994). The oral washing method, described in this study is a modification of the technique used by Johnston and Bodey. Oral washing using normal saline has three advantages in assessing the microbial characterization, (I) Gargling permits sampling of surfaces of the entire oropharyngeal cavity including tonsillar crypts and possibly otherwise inaccessible areas. (2) Using a defined volume of saline and letting the subject gargle for a specified period of time, there is a minimal variation in sampling. (3) The mean concentrations of micro- organisms received from gargle samples were higher than the mean concentrations of microorganisms obtained by the swab technique. Hence in the present study we have used oropharyngeal washing with saline for the assessment of oral bacterial microflora in COPD patients. we observed that the predominant organisms grown was porphyromonas gingivalis and haemophilus influenzae in COPD patients compared to the normal control group. Porphyromonas (P) gingivalis, is the gram-negative, anaerobic, nonmotile asaccharolytic rods that usually exhibit coccal to short rod morphologies.It adheres and invades epithelial cells by targeting specific host receptors, modulating host signaling events and deregulating the host cytokine network. Effectors of the innate immune system, proinflammatory cytokines, chemokines, MMPs, and antimicrobial peptides are up regulated and may have a

direct impact on disease progression and the inflammation processes, which may contribute to bacterial persistence and the progression of chronic manifestations of periodontal diseases (Andrian et al. 2006; Yilmaz 2008). Haemophilus influenza is a gram negative cocobacillus with a variable shape.The most common cause of chronic bacterial airway colonization in patients with COPD is haemophilus influenzae and it also contributes to airway inflammation in stable chronic COPD.

P. gingivalis, Haemophilus influenza, several other periodontal pathogens are proteolytic organisms capable of degrading fibronectin. It has been hypothesized that the proteolytic activity of a periodontopathic bacterium could alter the fibronectin coated oral mucosal epithelial cell surfaces. In turn, this results in fewer non-specific host defense mechanisms in high-risk individuals. These micro-organisms produce enzymes that degrade these fibronectin and other enzymes that degrade the salivary film on the mucosal surface, thereby exposing adhesion receptors to respiratory pathogens thereby compromising a protective barrier and enabling colonization of oral mucosal surfaces with potential respiratory pathogens. A study by Erb-Downward et al. in patients with Moderate or Severe COPD revealed a core of lung microbiome that included Pseudomonas, Streptococcus, Prevotella, Fusobacterium, Haemophilus, Veillonella, and Porphyromonas. Hence observations of our study showed that COPD patients with poor oral hygiene and elevated levels of proteolytic bacterias such as P.gingivalis and H.influenza may alter the oral bacterial microflora and its interaction with the host immune mechanism, which can accelerate the progression of COPD.

6. Conclusion

This is a preliminary study which aimed at assessing the oral bacterial microflora in stable COPD patients. The following observations were made in this study, The predominant organisms found in the oropharyngeal secretions were porphyromonas gingivalis and haemophilus influenza, in COPD patients. A significant higher score of OHI(S) and PI was observed in COPD patients. This is a baseline study with small sample size, the result of the study encourages further studies on a larger sample size and follow up for a longer duration.

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