



## COMPARATIVE EVALUATION OF ORAL MICROBIAL PROFILE IN PERIODONTITIS AND VENTILATOR ASSOCIATED PNEUMONIA PATIENTS

Villas S. Pattar<sup>1</sup>, Renuka Metgud<sup>2</sup>, Sumati Hogade<sup>3</sup> and Prashant A. Karni<sup>4</sup>

<sup>1,2</sup>Department of Periodontics, KLE Academy of Higher Education and Research (KLE University's) KLE VK Institute of Dental Sciences, JNMC Campus, Nehru Nagar, Belagavi-590010. Karnataka, India

<sup>3</sup>Department of Microbiology J N Medical College, KLE University, Nehru Nagar, Belgaum - 590010. Karnataka, India

<sup>4</sup>Department of Prosthodontics, KLE Academy of Higher Education and Research (KLE University's) KLE VK Institute of Dental Sciences, JNMC Campus, Nehru Nagar, Belagavi-590010. Karnataka, India

### ARTICLE INFO

#### Article History:

Received 15<sup>th</sup> May, 2017

Received in revised form 3<sup>rd</sup>

June, 2017

Accepted 16<sup>th</sup> July, 2017

Published online 28<sup>th</sup> August, 2017

#### Key words:

Periomedicine, ventilator associated pneumonia, respiratory pathogens, supragingival plaque.

### ABSTRACT

**Aim:** Dental plaque of patients in the Intensive Care Unit (ICU) can provide an environment for colonization of potential respiratory pathogens (PRP) making it a major risk factor for developing Ventilator associated pneumonia (VAP). This study was aimed to assess and compare the role of dental plaque as a reservoir for respiratory pathogens in causing Ventilator Associated Pneumonia.

**Materials and Methods:** A total of 30 patients belonging to the age group of 40-75 years were enrolled in the study. The subjects were assigned into 3 groups: Group 1 – Healthy, Group 2 – Chronic generalized periodontitis (CGP) and Group 3 – Ventilator associated pneumonia (VAP). Dental examination included recording Simplified Oral Hygiene Index (OHI-S), Gingival Index (GI) and Plaque Index (PI) to assess the oral hygiene status and gingival condition. Supragingival plaque samples were collected from all the 3 groups and subgingival plaque samples were collected only from group 2. The samples were subjected for aerobic and anaerobic microbial analysis. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) software.

**Results:** Aerobic culture of supragingival plaque showed the presence of Klebsiellapneumoniae, Pseudomonas aeruginosa and Escherichia coli in group 3 patients, which was statistically significant ( $P < 0.001$ ) when compared to group-2 and group-1 patients. It also showed a statistically significant difference in the mean scores of OHI-S, GI and PI scores ( $p < 0.001$ ) in group 3 when compared to group 1 suggesting that VAP patients had poor oral hygiene and attributed to lack or abstinence of oral hygiene practices during mechanical ventilation.

**Conclusion:** There was a definitive, qualitative difference of oral microflora seen between above 3 groups and Dental plaque acts as a reservoir for potential respiratory pathogens (Klebsiellapneumoniae, Pseudomonas aeruginosa and Escherichia coli) in causation of ventilator associated pneumonia.

Copyright © 2017 Villas S. Pattar et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### INTRODUCTION

In the era of evidence based dentistry, research in the area of infectious diseases has led to resurgence in the concept of focal infection<sup>1</sup> known as periodontal medicine.<sup>2</sup> Recent evidence suggests that periodontal infection may significantly enhance the risk for certain systemic diseases or alter the natural course of systemic conditions.<sup>3</sup> Nosocomial pneumonia (VAP/Non-VAP) is usually caused by colonization of oropharynx<sup>4</sup> by opportunistic pathogens such as Pseudomonas aeruginosa,

Staphylococcus aureus, Acinetobacter species, and gram-negative enteric bacteria such as Klebsiellapneumoniae, Escherichia coli, and Enterobacter species.<sup>5</sup>

Ventilator associated pneumonia (VAP) is pneumonia in patients receiving mechanical ventilation that was neither present nor developing at the time of intubation.<sup>6</sup> It is a major cause of infection in the hospital, and studies have shown that this infection add to the cost and double the length of stay of the patient in the hospital.<sup>7</sup> Many researches are being carried out to find the causes for development of VAP which has a

high mortality rates ranging from 20% to 41%<sup>8, 9, 10, 11</sup>, so the incidence can be reduced and benefit the mankind.

Poor oral hygiene and periodontal disease may promote oropharyngeal colonization by potential respiratory pathogens (PRPs) including Enterobacteriaceae (Klebsiellapneumoniae, Escherichia coli, Enterobacter species, etc), Pseudomonas aeruginosa, and Staphylococcus aureus.<sup>12</sup> Therefore it has been suggested that dental plaque may serve as reservoir of microorganisms for the causation of Ventilator associated pneumonia in individuals who are on ventilators. Thus the aim of the study was to assess and compare the role of dental plaque as a reservoir for respiratory pathogens in causing Ventilator Associated Pneumonia.

## MATERIALS AND METHODS

Patients of either sex, reported to the Out Patient Department, Department of Periodontics, KLE VK Institute of Dental Sciences, Belgaum, and to medical intensive care unit of KLES Dr. PrabhakarKore Hospital and Medical Research Centre, Belgaum, were selected for the study. A written informed consent was obtained from all the subjects and ethical clearance was obtained by the Ethical Committee, KLE V.K. Institute of Dental Sciences, KLE University, Belgaum. A total of 30 patients belonging to the age group of 40-75 years were enrolled in the study (15 males & 15 females) and divided into 3 groups as follows, Group - 1Healthy (10 patients who were systemically & periodontally healthy), Group – 2 CGP (10 patients with chronic generalized periodontitis), Group – 3 VAP (10 patients with ventilator associated pneumonia).

During oral examination the clinical parameters such Simplified-Oral Hygiene Index (Greene and Vermilion, 1964)<sup>13</sup>, Gingival Index (Loe and Sillness, 1963)<sup>13</sup>, Plaque Index (Sillness and Loe 1964)<sup>13</sup>, were recorded for all the patients to assess the oral hygiene status. Patients within the age group of 40-75 years, who had a gingival index score and OHI-S scores of 1.2 and above for group 2 and 3, who had plaque index score of 1.0 and above for group 2 and 3, and who had been on ventilator for more than 48hrs for any other systemic conditions other than pneumonia in group 3, were included in the study. Patients with systemic conditions like diabetes mellitus, immunologically compromised patients or patients with septicaemia, who have received any scaling and root planning or any other periodontal therapeutic procedures in the last 6 months, and Patients diagnosed as having community acquired pneumonia and non-ventilator associated pneumonia were excluded from the study.

After isolating the teeth with cotton rolls the tooth surfaces were dried with sterile gauze to avoid contamination by saliva. Supragingival plaque samples were collected for all the 3 groups and subgingival plaque samples were collected from group 2 using sterile periodontal curettes and placed in sterile test tube containing 1ml of normal saline solution for aerobic and thioglycolate medium for anaerobic bacteria from the respective groups. The prepared samples were sent to Department of Microbiology, JN Medical College for aerobic and anaerobic culture.

### Statistical analysis

The mean and standard deviation (SD) for OHI-S, GI and PI were calculated from the observations. Intergroup comparison for indices scores was done using Paired 't' test. Intergroup

comparison for the presence of respiratory pathogens in aerobic supragingival plaque culture was done using Chisquare test using SPSS 21.0.

## RESULTS AND OBSERVATIONS

**Clinical parameter evaluation:** The clinical parameters observed for the assessment of oral hygiene status showed that, the mean and standard deviation scores of OHI-S, GI and PI between Healthy group and VAP group showed statistically significant difference (p< 0.001) (Table 1), suggesting that VAP patients had poor oral hygiene when compared to the healthy group. Whereas no difference was found in the mean and standard deviation scores of OHI-S, GI and PI between CGP and VAP (Table 2) suggesting that amount of plaque and gingival inflammation in VAP patients were almost similar to the CGP patients.

**Table 1** Comparison between Healthy group and VAP group

	Group	N	Mean	Std. Deviation	P-Value	t-Value
OHIS	Healthy	10	0.96	0.25	.001	-12.93
	VAP	10	3.66	0.60		
GI	Healthy	10	0.52	0.26	.001	-11.35
	VAP	10	2.21	0.39		
PI	Healthy	10	0.75	0.53	.001	-7.44
	VAP	10	2.27	0.34		

**Table 2** Comparison between CGP group and VAP group

	Group	N	Mean	Std. Deviation	P-Value	t-Value
OHIS	CGP	10	4.07	0.92	.265	1.15
	VAP	10	3.66	0.60		
GI	CGP	10	2.32	0.27	.475	0.73
	VAP	10	2.21	0.39		
PI	CGP	10	2.17	0.36		
	VAP	10	2.27	0.34		

**Microbial analysis:** The microorganisms grown in supragingival plaque Aerobic culture (Table3) were further divided into respiratory organisms (Klebsiellapneumoniae, Streptococcus pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli) and non-respiratory organisms (Oral commensals, Porphyromonas species, Bacteroidesfragilis, Enterococcus species, Micromonas micros, Prevotella species, Fusobacterium species, Peptostreptococcusintermedius, Streptococcus mutans, Peptostreptococcusanaerobius, Citrobacterspp).

**Table 3** Microorganisms isolated in aerobic culture of supragingival plaque (SPA)

Supragingival plaque aerobic (SPA)	Healthy	%	CGP	%	VAP	%	Total
NOGC	1	10%	0	0	0	0	1
Oral commensals	9	90%	1	10%	0	0	10
Klebsiellapneumoniae	0	0	3	30%	5	50%	8
Enterococcus species	0	0	3	30%	0	0	3
Citrobacter spp.	0	0	1	10%	0	0	1
Pseudomonas aeruginosa	0	0	0	0	3	30%	3
Escherichia coli	0	0	0	0	1	10%	1
Klebsiellapneumoniae, Staphylococcus aureus	0	0	1	10%	0	0	1
Enterococcus species, Streptococcus mutans	0	0	1	10%	0	0	1
Klebsiellapneumoniae, Citrobacter spp.	0	0	0	0	1	10%	1
Total	10	100%	10	100%	10	100%	30

Presence of respiratory organisms in VAP was higher compared to CGP group and healthy group which was statistically significant (Table 4).

**Table 4** Association between respiratory and non respiratory pathogen between groups in aerobic culture of supragingival plaque

Organisms	Healthy	CGP	VAP	$\chi^2$ Value	P Value
Respiratory organisms	0	4	10		
Non respiratory organisms	10	6	0	20.36	< 0.001
Total	10	10	10		

Anaerobic culture of supragingival plaque samples did not show presence of any respiratory pathogens in all the three groups. (Table 5)

**Table 5** Microorganisms isolated in anaerobic culture of supragingival plaque (SPAN)

SPAN	Healthy	%	CGP	%	VAP	%	Total
NOGC	9	90%	9	90%	0	0	18
Oral commensals	0	0	1	10%	0	0	1
Porphyromonas spp.	1	10%	0	0	0	0	1
Bacteroidesfragilis	0	0	0	0	5	50%	5
Micromonas micros	0	0	0	0	1	10%	1
Prevotella spp.	0	0	0	0	2	20%	2
Peptostreptococcus anaerobius	0	0	0	0	2	20%	2
Total	10	100%	10	100%	10	100%	30

Subgingival plaque samples were collected only in CGP group. 20% aerobic culture of subgingival plaque samples showed the presence of PRP's such as Klebsiellapneumoniae, Streptococcus pneumonia (Table 6). And none of the subgingival plaque samples showed the presence of PRP's in anaerobic culture of subgingival plaque (Table 7).

**Table 6** Organisms isolated in aerobic culture of subgingival plaque

SGA	CGP	%	Total
NOGC	4	40%	4
Oral commensals	4	40%	4
Klebsiellapneumonia	1	10%	1
Streptococcus pneumonia	1	10%	1
Total	10	100%	10

**Table 7** Organisms isolated in anaerobic culture of subgingival plaque

SGAN	CGP	%	Total
Porphyromonas spp.	2	20%	2
Bacteroidesfragilis	2	20%	2
Micromonas micros	1	10%	1
Peptostreptococcusintermedius	1	10%	1
Peptostreptococcusanaerobius	1	10%	1
Micromonas micros, Prevotella spp.	1	10%	1
Bacteroidesfragilis, Fusobacterium spp.	1	10%	1
Prevotellasp., Fusobacterium spp.	1	10%	1
Total	10	100%	30

## DISCUSSION

Periodontal disease has been reported to be a pre-disposing factor for several diseases and systemic conditions for example cardiovascular diseases, diabetes, kidney diseases, stress related diseases, pre-maturity and/or low birth weight<sup>14</sup> and respiratory diseases such as COPD and pneumonia.

The anatomical continuity between the lungs and the oral cavity makes the latter a potential reservoir of respiratory pathogens. Within 48 hours of admission to the intensive care unit (ICU), patients have changes in oral flora towards a predominantly gram-negative spectrum, which is more virulent. Dental plaque can provide an environment for colonization of potential respiratory pathogens making it as a

major risk factor for developing VAP in patients who are on ventilators.<sup>15</sup>

In the present study, Statistically significant difference (p <0.001) in OHI-S, Plaque index and Gingival index scores was seen in CGP and VAP patient group when compared to healthy group (Table 1,2). This increased amount of plaque in VAP patients may be attributed to lack or abstinence of oral hygiene practices during mechanical ventilation. Increase in the plaque scores in ventilator patients in the present study is in agreement with investigations carried out by Scannapieco *et al.*,<sup>15</sup> and by Fourrier *et al.*<sup>16</sup>

The microorganisms grown in aerobic culture of supragingival plaque were further divided into respiratory pathogens and non-respiratory pathogens. Results showed that VAP patients harboured greater number of potential respiratory pathogens compared to chronic periodontitis patients followed absence of PRPs in healthy subjects and the difference were statistically significant, (Table 4) (p< 0.001).

Growth of respiratory pathogens in aerobic supragingival plaque culture was 100% in VAP patients, and 40% in CGP patients (Table 4). The lack of oral hygiene, the decline in activity of daily living, and the presence of polypharmacy-related xerostomia in institutionalized elders disturb the delicate equilibrium between tooth structure and oral fluids, thus providing favourable conditions for the proliferation of potential respiratory pathogens in dental plaque. Further weakened swallowing and cough reflexes<sup>17</sup>, decreased mucociliary clearance of airways result in the aspiration of the respiratory pathogens present in dental plaque into the lungs.<sup>18, 19, 20</sup>. This explains the biological plausibility for dental plaque to act as potential reservoir for respiratory pathogens. The present study is in agreement with the investigations done by Russell & Colleagues<sup>21</sup> and Ali A El- Solh & Colleagues<sup>22</sup> who isolated similar respiratory pathogens in hospital acquired pneumonia patients (Klebsiellapneumoniae, Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa).

Subgingival plaque was collected only in CGP group, as in healthy group subgingival plaque cannot be procured.<sup>23</sup>In VAP patients subgingival plaque sample collection was avoided as it can induce bleeding, increases the risk of aspiration pneumonia which may complicate the disease severity and increases the mortality of the patient.

Aerobic culture of subgingival plaque showed presence of respiratory pathogens in 2 samples (Table 6). The supragingival and subgingival zones is not watertight compartment which substantiate the presence of respiratory pathogens (20%) in aerobic culture of subgingival plaque and thereby endorses the possibility that dental plaque could be a potential reservoir for respiratory pathogens.

Anaerobic culturing of supragingival and subgingival plaque samples did not show presence of PRPs in any of the group. (Table 5, 7) This can be attributed to high mortality of respiratory pathogens in anaerobic conditions.

The study showed that respiratory pathogens (Klebsiellapneumoniae, Pseudomonas aeruginosa and Escherichia coli) colonizing dental plaque could be implicated in development of Ventilator associated pneumonia in hospitalized elderly patients and it is suggested that active programs be instituted by all health-care practitioners to enhance the access of institutionalized patients to dental care

services and to improve daily oral hygiene. So that the incidence of ventilator associated pneumonia can be decreased and reduce the mortality rate of VAP

## CONCLUSION

The present study suggested that there was a definitive, qualitative difference present between the microflora of Healthy, Chronic generalized periodontitis and Ventilator associated pneumonia patients and Dental plaque acts as a reservoir of respiratory pathogens (Klebsiellapneumoniae, Pseudomonas aeruginosa and Escherichia coli) in causation of ventilator associated pneumonia. Further multicentric studies with large sample size are required to substantiate the causal relationship of dental plaque and Ventilator associated pneumonia.

## References

1. Paquette DW. The concept of risk and the emerging discipline of periodontal medicine. *The journal of contemporary dental practice*. 1999 Jun 25; 1(1):1-8.
2. Offenbacher S. Periodontal diseases: pathogenesis. *Annals of periodontology*. 1996 Nov; 1(1):821-78.
3. Mealey BL. Influence of periodontal infections on systemic health. *Periodontology* 2000. 1999 Oct 1; 21(1):197-209.
4. Scannapieco FA. Role of oral bacteria in respiratory infection. *Journal of periodontology*. 1999 Jul 1; 70(7):793-802.
5. American Thoracic Society. Hospital-acquired pneumonia in adults: diagnosis, assessment of severity, initial antimicrobial therapy, and preventive strategies. A consensus statement. *Am j respircrit care med*. 1996 May 1; 153(5):1711-25.
6. Munro CL, Grap MJ, Elswick RK, McKinney J, Sessler CN, Hummel RS. Oral health status and development of ventilator-associated pneumonia: a descriptive study. *American Journal of Critical Care*. 2006 Sep 1;15(5):453-60.
7. Bacteria from patients dental plaque causes Ventilator-Associated Pneumonia. *Science Daily*. Mar.27, 2007.
8. Kollef MH. Ventilator-associated pneumonia: a multivariate analysis. *Jama*. 1993 Oct 27; 270(16):1965-70.
9. Bercault N, Boulain T. Mortality rate attributable to ventilator-associated nosocomial pneumonia in an adult intensive care unit: A prospective case control study. *Crit Care Med*. 2001; 29: 2303-09.
10. Heyland D, Cook D, Griffith L, et al. The attributable morbidity and mortality of ventilator-associated pneumonia in the critically ill patient. *Am J RespirCrit Care Med*. 1999; 159: 1249-56.
11. Safdar N, Dezfulian C, Collard HR, Saint S. Clinical and economic consequences of ventilator-associated pneumonia: A systematic review. *Crit Care Med*. 2005; 33:2184-93.
12. FA Scannapieco, JM Mylotte. Relationships between periodontal disease and bacterial pneumonia. *Journal of periodontology* 1996; 67: 1114-22.
13. Soben Peter. Essentials of Preventive and Community dentistry. Second edition. Page 133-54.
14. Gomes-Filho IS, Santos CML, Cruz SS et al. Periodontitis and nosocomial lower respiratory tract infection: preliminary findings. *J Clin Periodontol* 2009; 36: 380-87.
15. Scannapieco FA. And Mylotte JM. Colonization of dental plaque by respiratory pathogens in medical Intensive Care patients. *Crit Care Med* 1992; 20: 740-45.
16. Francois Fourrier, Benoit Duvivier, HerveBoutigny, MichelineRD, Claude Chopin.Colonization of dental plaque: A source of nosocomial infections in Intensive care Unit (ICU) patients. *Crit Care Med* 1998; 26: 301-08.
17. Limeback H. Implications of oral infections on systemic diseases in the institutionalized elderly with a special focus on pneumonia. *Ann Periodontol* 1998; 3: 262-75.
18. Kikuchi R, Watabe N, Konno T, Mishima N, Sekizawa K, Sasaki H. High incidence of silent aspiration in elderly patients with community acquired pneumonia. *Am J Respir* 1994; 150: 251-53
19. Sekizawa K et al. Lack of cough reflex in aspiration pneumonia. *Lancet* 1990; 335: 1228-29.
20. Nakagawa T, Sekizawa K, Arai H et al. High incidence of pneumonia in elderly patients with basal ganglia infarction. *Arch Intern Med* 1997; 157: 321-24.
21. Russell SL, Boylan RJ, Kaslick RS, Scannapieco FA, Katz RV. Respiratory pathogen colonization of the dental plaque of institutionalized elders. *Spec Care Dentist*. 1999 May-Jun; 19(3): 128-34.
22. Ali A. El-Solhet al.Colonization of dental plaques: A Reservoir of Respiratory Pathogens for Hospital - Acquired Pneumonia in Institutionalized Elder. *Chest* November 2004; 126: 5; 1575-82.
23. Loe H, Theilade E, Jensen SB: Experimental gingivitis in man. *J Periodontol* 36; 177: 1965.

\*\*\*\*\*