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RESEARCH ARTICLE

MICROBIOLOGICAL ASSESSMENT IN PLAQUE SAMPLES OF PATIENTS WITH ORAL CANCER WITH OR WITHOUT SMOKING

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ABSTRACT

Aim: To isolate micro-organisms from the GCF, Serum and saliva in patients with oral malignancy with or without Chronic periodontitis. To detect virus from the serum, saliva and GCF in patients with Chronic periodontitis with and without smoking. To evaluate ROM levels from the GCF, saliva and Serum with oral malignancy with and without smoking and Chronic periodontitis.

Objectives

- To Compare the relation between Chronic Periodontitis with Oral carcinoma
- To compare the relation between Chronic periodontitis without Oral carcinoma
- To compare the relation between Chrionic periodontitis with Smoking
- To compare the relation between Chronic Periodontitis without smoking
- To correlate the relation between Oral carcinoma with smoking
- To correlate the relation between Oral carcinoma without smoking

Materials and Methods

25 patients had been selected for each group and samples were obtained respectively. A single dentist was assigned to collect samples to maintain standards. Patients were in the aged between 30 to 55 years and were obtained from the Department of Periodontics, Thai Moogambigai Dental College, Chennai, Tamil Nadu, India and Oral cancer patients were taken from MM Hospital Namakkal, and Cancer Institute, Chennai, TATA Memorial Hospital, Mumbai.

INTRODUCTION

Periodontal diseases are a group of conditions affecting the supporting structures for the dentition. Chronic periodontitis is the result of a response of the host to bacterial aggregations on the tooth surfaces. The outcome of this is an irreversible destruction of the connective tissue attachment, which results in periodontal pocket formation and eventual loss of alveolar bone. While gingivitis is known to be a very prevalent condition among children and adolescents, periodontitis is much less common in this group. The occurrence of severe periodontitis in young adults may have a devastating effect on their dentition and in some cases treatment of these forms of periodontal disease can be unsuccessful. Diagnosis of periodontitis and the identification of affected individuals can sometimes be difficult because there may be no self-reported symptoms. Destructive periodontitis has been described as a consequence of the interaction of genetic, environmental, microbial and host factors Loe *et al* 1994. Hujoel suggests that a hidden periodontitis epidemic related to smoking patterns occurred during the 20th Century and that socio demographic shifts in smoking habits will alter the periodontal needs for the future. The precise mechanism whereby cigarette smoking exerts an effect on the development of periodontal destruction is unknown. A reduction in clinical signs of gingivitis has been reported in smokers and this effect has been shown to be independent of plaque levels. There have been other reports of less bleeding in smokers with periodontitis, suggesting that nicotine could mediate its vasoactive effects on a local basis Graner *et al*, Linden and Mullally found that young smokers had in fact more gingival bleeding than non-smoking regular attenders. The explanation for this finding seemed to be related to the high levels of calculus and plaque reported in this group of young adults. Other studies of older population groups have found little difference in plaque accumulation between smokers and non-smokers.

Nicotine metabolites can concentrate in the periodontium and their effects include the promotion of vasoconstriction, and the impairment of the functional activity of polymorphs and macrophages. The numbers of neutrophils in peripheral blood are also increased by tobacco use and their migration through capillary walls is impaired due also to paralysis of the cell membrane. Cigarette smoking has been demonstrated to activate the release of elastase, which has the capacity to cause tissue damage. The effect of this is particularly well displayed

in lung diseases such as emphysema and in animal studies with elastase-deficient models, which do not develop emphysema when exposed to cigarette smoke. In addition there is an increased production of oxygen species, which can lower tissue levels of alpha- 1 protease inhibitors so enabling elastase and other enzymes with potential for damaging tissue to remain unchecked at active sites Moeker *et al* 2010. The gingival crevicular fluid levels of functional elastase and that complexed with the inhibitor have been demonstrated to be lower in smokers than nonsmoking controls.

Much evidence on the effect of cigarette smoking on neutrophil activity suggests that these cells may accumulate at the site of inflamed periodontal tissues. As they may fail to migrate through the gingival crevice they can release their enzymes into the surrounding connective tissue therefore contributing directly to tissue destruction. The role of cytokines has also been extensively examined in the pathogenesis of periodontitis. High levels of prostaglandin influence of Tobacco Smoking on the onset of Periodontitis in young persons PGE2 have been associated with aggressive or early onset periodontitis and these elevated concentrations have been related to an increased responsiveness of circulating monocytes to bacterial challenge and their effect on apoptosis of monocytes by nicotine exposure but these investigators did report that when stimulated by LPS monocytes exposed to nicotine had reduced IL-1 release. This cytokine is proinflammatory and has been associated with osteoclast activity and alveolar bone resorption. Procoagulant activity expression is a function of monocytes related to limiting the spread of infections and its inhibition by nicotine is more evidence of the complexity of effect of tobacco on this part of the host defence mechanism. Our study aimed to detect micro-organisms and evaluate the ROM levels in GCF, saliva and serum of oral malignancy patients with periodontal diseases.

Study Population

The study population consisted of 120 subjects belonging to both sexes and aged between 30-55 years. Chronic Periodontitis subjects were taken from the outpatient clinic of Department of Periodontics, Thai Moogambigai Dental College, Chennai, Tamil Nadu, India. Oral cancer patients were taken from MM Hospital Namakkal, and Cancer Institute, Chennai, TATA Memorial Hospital, Mumbai.

Total of 25 patients had been selected for each group and samples were obtained respectively. Each patients was examined by 1 standard dentist and then assighned to the respective group inorder to maintain levels of standardisation.

Inclusion Criteria

Presence of inflammatory changes in the periodontal tissues
Gingival index ≥ 1
Probing depth and Clinical attachment loss ≥4mm
Radiographic evidence of bone loss
Current smoker (packets >10)
Carcinoma present for more than 8 months

Patients with a history, signs or symptoms of Aggressive Periodontitis, History of periodontal treatment received in the past six months, Under antibiotics and corticosteroidsGross oral pathological findings or history of systemic disease were excluded from the study.

Sampling

- The site with greatest probing depth was selected for GCF collection. After drying the area with a blast of air, supra-gingival plaque was removed without touching the marginal gingiva and the GCF was collected.A standardized volume of 1µl was collected from each site with an extra-crevicular approach, using volumetric capillary pipettes that were caliberated from 1-5µl. The collected GCF was transferred immediately to ependorff tubes and stored at -70 c until the time of assay.
- Whole saliva (2 mL) was collected in disposable, sterile, clean tubes and centrifuged immediately to remove cell debris.
- Venous blood from an anticubital vein was collected in plain tubes without additiveCentrifuged at 3,500rpm for 5 minutes to separate serum.

Real-Time PCR, also known as quantitative polymerase chain reaction (qPCR), is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR).PCR was used to determine the prevalence of periopathic bacteria in subgingival plaque samples of individuals with chronic periodontitis and oral cancer.

Comparison of Saliva, Serum, GCF_ROM values among all four groups using oneway ANOVA and Posthocanalysis. Comparison of mean number PI,PG,TF,SM & HP organisms in all four groups using oneway ANOVA and posthoc Analysis. Mean difference among all the variables among gender using Independent T test.

RESULTS

	N	Mean	Std. Deviation	P value	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CP+CA	15	1.8000	0.67612		1.4256	2.1744	1.00	3.00
CP-CA	15	2.0667	0.59362		1.7379	2.3954	1.00	3.00
CP+SMOKING	15	2.6000	0.50709	0.001	2.3192	2.8808	2.00	3.00
CP-SMOKING	15	2.5333	0.51640		2.2474	2.8193	2.00	3.00
Total	60	2.2500	0.65419		2.0810	2.4190	1.00	3.00

Mean Gingiva Index among all four groups was statistically significant

	N	Mean Probing Depth	Std. Deviation	P value	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CP+CA	15	6.6000	1.05560		6.0154	7.1846	5.00	8.00
CP-CA	15	6.5333	1.12546		5.9101	7.1566	5.00	9.00
CP+Smoking	15	7.3333	0.89974	0.001	6.8351	7.8316	6.00	9.00
CP-Smoking	15	7.9333	0.96115		7.4011	8.4656	6.00	9.00
Total	60	7.1000	1.14537		6.8041	7.3959	5.00	9.00

Groups	N	Mean Clinical Attachment loss	Std. Deviation	P value	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CP+CA	15	5.6000	1.05560		5.0154	6.1846	4.00	7.00
CP-CA	15	5.5333	1.12546		4.9101	6.1566	4.00	8.00
CP+SMOKING	15	6.3333	.89974	0.001	5.8351	6.8316	5.00	8.00
CP-SMOKING	15	6.8667	.91548		6.3597	7.3736	5.00	8.00
Total	60	6.0833	1.12433		5.7929	6.3738	4.00	8.00

Mean probing depth & CAL among all four groups was statistically significant, themean values were higher in CP than in CA

	N	Mean	Std. Deviation	P value	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CP+CA	15	289.73	21.07967		278.0598	301.4069	268.00	356.00
CP-CA	15	289.47	13.94820		281.7424	297.1909	267.00	312.00
CP+Smoking	15	342.13	56.94216	<0.001	310.5998	373.6669	259.00	468.00
CP-Smoking	15	348.73	67.29197		311.4683	385.9984	271.00	492.00
Total	60	317.52	52.85429		303.8630	331.1704	259.00	492.00

GCF_ROM values among all four groups was statistically significant. The mean values was slightly higher in smokers than in and was similar in cancer and CP

(I) groups	(J) groups	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
CP+CA	CP-CA	1.06667	1.000	-79.8936	82.0269
	CP+Smoking	-121.06667*	0.001	-202.0269	-40.1064
	CP-Smoking	-116.13333*	0.002	-197.0936	-35.1731
CP-CA	CP+CA	-1.06667	1.000	-82.0269	79.8936
	CP+Smoking	-122.13333*	0.001	-203.0936	-41.1731
	CP-Smoking	-117.20000*	0.002	-198.1603	-36.2397
CP+Smoking	CP+CA	121.06667*	0.001	40.1064	202.0269
	CP-CA	122.13333*	0.001	41.1731	203.0936
	CP-Smoking	4.93333	0.998	-76.0269	85.8936
CP-Smoking	CP+CA	116.13333*	0.002	35.1731	197.0936
	CP-CA	117.20000*	0.002	36.2397	198.1603
	CP+Smoking	-4.93333	0.998	-85.8936	76.0269

Serum ROM levels was statiscally significant in smokers with periodontitis and oral cancer On comparison of mean salivary ROM levels were significant I all 4 groups but was greater in smokers than non smokers

Groups	N	Mean	Std. Deviation	P value	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
CP+CA	15	3.5333	1.55226		2.6737	4.3929	1.00	6.00
CP-CA	15	3.7333	1.53375	0.489	2.8840	4.5827	1.00	6.00
CP+SMOKING	15	4.0667	1.57963		3.1919	4.9414	2.00	7.00
CP-SMOKING	15	4.3333	1.39728		3.5595	5.1071	2.00	6.00
Total	60	3.9167	1.51032		3.5265	4.3068	1.00	7.00

When PI,PG,TF,SM&HP levels were compared among all 4 groups, SM and HP were statistically insignificant

DISCUSSION

Periodontitis history is associated with poorly differentiated tumours in the oral cavity. These results support additional, confirmatory basic science, and prospective clinical studies. Although both are called periodontal disease, gingivitis and periodontitis are distinct diseases. Gingivitis is a non-destructive reversible inflammation of the gums strongly associated with poor oral hygiene. On the other hand, only a small subset of the population with poor oral hygiene develops destructive periodontitis, leading to epithelial migration and bone loss. Factors that initiate periodontitis are poorly understood. Smoking reduces gingivitis but it is a strong risk factor for periodontitis. Gingivitis is mostly associated with Gram-positive facultative bacteria, whereas periodontitis with Gram-negative anaerobic. Accumulating evidence supports a role of viruses in the initiation and progression of periodontitis. Recent study also suggests a synergy between chronic periodontitis and oral HPV infection in base of tongue cancers. A link between poor oral hygiene has been suggested. It is possible that, subjects with poor oral hygiene, those who develop periodontitis are at higher risk for developing cancer. Prospective clinical studies including both periodontitis patients and those with gingivitis without a periodontitis history will allow testing this hypothesis. Alveolar bone loss associated with periodontal inflammation is a slow chronic process that is usually irreversible. The rate of bone loss ranges between 0.04 and 0.28 mm annually. In recent studies, panoramic radiographs were taken at admission before the initial cancer diagnosis. It is not likely that cancer preceded periodontitis. The biological mechanism of the association between chronic infection/inflammation and cancer has been described extensively. However, well-designed longitudinal studies are required to prove.

Over a number of years, epidemiological studies established several well-defined risk factors for cancer such as tobacco, diet, age, hereditary. H. pylori became the first bacterial species to be officially recognized by the World Health Organization as a definite cause of cancer in humans. Since then, there has been a growing body of evidence supporting an association between specific microorganisms, including those in the oral cavity, and various types of cancers. In this study herpes simplex viruses were significantly increased in patients with oral cancer and chronic periodontitis. Subsequently, other community constituents, such as Fusobacterium nucleatum, can become opportunistically pathogenic, and the combined effect of a dysbiotic microbial community along with a dysregulated immune response ultimately causes periodontal disease.

Although smoking is a well-recognized risk factor for periodontal attachment loss, smokers often exhibit less gingival bleeding than would be predicted. Previous studies have shown that the clinical signs of inflammation are less pronounced in smokers when compared with nonsmokers. These observations may be due to alterations in the inflammatory response in smokers, or due to alterations in the vascular response of the gingival tissues. Although no significant differences in the vascular density of healthy gingiva have been observed between smokers and nonsmokers, the response of the microcirculation to plaque accumulation appears to be altered in smokers when compared with nonsmokers. With developing inflammation, increases in gingival crevicular fluid flow,' bleeding on probing,' and gingival blood vessels' were less in smokers when compared with nonsmokers. In addition, the oxygen concentration in healthy gingival tissues appears to be less in smokers than nonsmokers, although this condition is reversed in the presence of moderate inflammation. Subgingival temperatures are lower in smokers than nonsmokers, and recovery from the vasoconstriction caused by local anaesthetic administration takes longer in smokers.

The involvement of ROS and the antioxidant defense mechanisms in human saliva has been demonstrated in various processes of the oral cavity: healing periodontal disease, preventing oral carcinogenesis, reducing oral mucosa inflammatory reactions, and ameliorating metal-based restoration reactions. Oxidative stress is caused by an imbalance between the production of ROS and the body's ability to produce sufficient antioxidants and repair the resulting damage. The action and protective mechanism of a single antioxidant depends on the concentration, specific reactivity of the ROS, and condition of the antioxidant interaction.

CONCLUSION

Microbiological & biochemical parameters are assisted in smoking & non-smoking patients with or without carcinoma were assessed. On assessing the microbiological parameters, Micoplasmic bacteria was significant in chronic periodontitis with carcinoma patients. There was also a significant increase in herpes virus in plaque samples from chronic periodontitis with carcinoma patients when compared with chronic periodontitis. With respect to biochemical parameters, Reactive Oxidase metabolite (ROM) level was highly significant in carcinoma with chronic periodontitis patients. Various etiology with oxidative stress are few of the contributing factors for the malignancy. In the present study there was significant increase with respect to herpes virus and ROM in chronic periodontitis with malignancy patients. There was a relationship with malignancy and chronic periodontitis patients. Further longitudinal studies are needed before we could confirm the relationship with malignancy and chronic periodontitis patients.

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