Discussion

Periodontitis is a complex disease in which disease expression involves intricate interactions of the biofilm with the host immunoinflammatory response and subsequent alterations in bone and connective tissue homeostasis ***(Tatakis and Kumar., 2005).***

As such, conceptual models of the pathogenesis of periodontitis may benefit from a systems approach, in which biologic mechanisms are studied and interpreted in a hierarchical set of functional modules, such as the microbial ecosystem or the immunoinflammatory response, which may be modified by factors (e.g., smoking) that operate at the patient level ***(Taubman et al., 2007).***

In the past 20 years there has been an increasing awareness of the role of tobacco use on the prevalence and severity of periodontal diseases ***(Haber et al., 1993).*** As proposed by ***Gelesky SC (1999),*** smoking meets the majority of criteria given for causation of a disease. Experimental evidence accumulated over the last two decades has indicated cigarette smoking is a true risk factor for periodontitis ***(Tonetti MS., 1998, Vandana and Sridhar., 2008).***

Saliva in humans is a mouth fluid possessing several functions involved in oral health and homeostasis with an active protective role in maintaining oral healthiness. It is a complex secretion whose components exert a well documented role in health and disease ***(Johan Aps and Luc Martens, 2005).***

Similar to other biological systems, the salivary antioxidant system includes various molecules and enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), etc., which can be used as biomarkers for diagnosis of various periodontal diseases ***(Nurdan Ozmeric, 2004).***

Sathishkumar et al (2008), has proved that saliva possess a significant antioxidant activity in the non smokers than the smokers, through various in vitro antioxidant assays.

Antioxidants are present in all body fluids and tissues and protecting against endogenously formed free radicals. Antioxidant (AOX) enzymes such as superoxide dimutase and glutathione peroxidase provide protection within cells whilst low molecular weight scavenging antioxidants and antioxidant vitamins are present in extra cellular fluid. In addition, dietary derived components such as uric acid non protein thiols and glutathione also act as antioxidants ***(Halliwell B., 1991).***

Total antioxidant activity has been reported to be reduced in saliva of patients with periodontitis relative to that in non-periodontitis subjects ***(Rai et al., 2006).***

Glutathione and uric acid are low molecular weight antioxidants. In particular, glutathione can directly scavenge free radicals or act as a substrate for glutathione peroxidases and glutathione S-transferases during the detoxification of hydrogen peroxide, lipid hydroperoxides, and electrophilic compounds ***(Pompella et al., 2003, Sies H., 1999, Pastore et al., 2003).***

In the present study, we are testing the hypothesis that total glutathione of saliva is higher in subject of periodontitis in smoker as compared to non-smoker and control, and salivary total glutathione concentrations before and after scaling and root planning in smoker as well as in non smoker.

The present study was performed on a total of 40 medically healthy individuals divided into three groups. The first group (Group 1) was composed of 15 non-smoking patients with generalized moderate to severe chronic periodontitis. The diagnostic criteria for chronic periodontitis will be in accordance with the criteria of the International Classification Workshop proposed by ***Armitage (1999).*** This group was subjected to clinical examination, radiological examination and laboratory investigations before supragingival scaling and subgingival clinical debridement in 3-4 sessions and 2 & 4 months after the non-surgical periodontal treatment.

The second group (Group 2) was composed of 15 smoking patients with generalized moderate to severe chronic periodontitis. According to ***Buduneli et al. (2006)*** smokers are defined as those who smoked $\geq$10 cigarettes per day for more than 5 years. This group was also subjected to clinical examination, radiological examination and laboratory investigations before supragingival scaling and subgingival clinical debridement in 3-4 sessions and 2 & 4 months after the non-surgical periodontal treatment.

The third group (Group 3) was a control group which was composed of 10 non-smoker subjects (controls) with clinically healthy periodontium. This group was also subjected to clinical examination, radiological examination and laboratory investigations.

Levels of total salivary glutathione level were determined using kinetic enzymatic recycling assay according to manufacturers' instructions.

Saliva is a complex secretion whose components exert a well documented role in health and disease and its diagnostic use is spreading ***(Bald and Glowacki., 2005).*** Moreover, saliva contains various antioxidants, including uric acid, which contributes more than half of the total radical trapping capacity ***(Moore et al., 1994),*** and the remaining half by the glutathione and its precursors ***(Zappacosta et al., 2002, Sathishkumar et al., 2010).***

Genetic and biochemical evidence has demonstrated that glutathione and glutathione-dependent enzymes play a central role in the cellular defense against toxic environmental agents ***(Ludmila Gavriliuc et al., 2007).***

In the present study there was a statistically significant difference in the mean probing depth value before treatment where p-value was (0.03) and after treatment of chronic periodontitis with p-value (0.01). There was slight significant difference in Plaque Index in pre treatment values where p-value was (0.01), while there was no significant difference after treatment with p values (0.08).

The Gingival Index shows significant difference in pre treatment values where p-value was (0.01). And there was a significant difference after treatment with p values (0.02).

Balwant Rai. (2008) showed that the mean probing depth and bleeding on probing were 4.82 + 0.12 mm & 64.2 + 0.5 % & 3.94 + 0.14 mm & 54.3 + 0.6% & 1.92 + 0.14 mm & 25.2 + 0.4 % in periodontitis in smokers & non-smokers and healthy periodontium. Significant reductions in

clinical recordings were obtained in smoker and non-smoker patients following periodontal treatment.

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Ah et al. (1994) have demonstrated a poorer response to periodontal treatment in smokers compared with non-smokers. ***Preber and Bergstrom (1990)***, found that 12 months following surgery, smokers had a statistically significantly reduced probing depth reduction compared with nonsmokers, despite accounting for differences in levels of plaque accumulation.

Preber et al. (1995) studied the clinical and microbiological effects of non-surgical therapy and found that smokers had a less favorable outcome in terms of pocket depth reduction than did non smokers.

Approximately 90% of patients who were categorized as having failed to respond to conventional therapy were smokers ***(MacFarlane et al., 1992, Wolff et al., 1994).***

Colombo et al. (1998), has disagreed with this stated proportion, since these investigators found that only 25% of their patients were current smokers, but that 40% were former smokers. ***Bostrom et al. (1998a)*** suggested that former smokers often begin smoking again, and therefore one must interpret the status of the former smokers cautiously, since self-reporting of smoking status is not reliable.

Kinane and Radvar (1997) found that the response to non-surgical mechanical therapy is particularly poor in deep pockets in smokers.

The results of this study showed that the mean total salivary glutathione level in controls was 2.39 micromole. In periodontitis patients, the mean total salivary glutathione levels were 8.15 micromole, 4.91 micromole in smokers and non smokers respectively. These results were decreased after 2 months of treatment to be 4.7 micromole, 3.79 micromole in smokers and non smokers respectively. After 4 months of treatment the total salivary glutathione levels were 3.55 micromole, 3.05 micromole in smokers and non smokers respectively. There was slight significant difference in pre treatment values where p-value was (0.001). And there was a significant difference 2 months after treatment with p values (0.03) and also there was significant difference 4 months after treatment with p-value (0.001)

Balwant Rai. (2008) showed that the mean salivary total glutathione level in subjects with a clinically healthy periodontium was 3.6+ 1.8 micromole ($p < 0.05$). In periodontitis in smokers and non-smokers involved teeth of hopeless prognosis, the mean total salivary glutathione levels were 5.7+ 1.8 micromole ($p < 0.05$), 4.8 + 1.3 micromole ($p < 0.05$) respectively.

Salivary total glutathione conc. were reduced following therapy in smoker as well as in non-smoker ($P<0.05$).

Number of studies have evaluated the use of various host derived factors in saliva for diagnosis of periodontal disease ***(Takane et al., 2005, Buduneli et al., 2006, Ohshima et al., 2002).***

In the treatment of smokers, the use of non-surgical therapy will generally be effective in terms of probing depth reduction and gingivitis, although to a lesser extent than with non-smokers.

It has been reported that the possible effects of smoking and gingival inflammation on salivary antioxidants in gingivitis patients. They reported that no statistically significant difference was found in any of antioxidant indices between any of the groups ***(Wilton et al., 1989).*** Few authors determined that GCF antioxidant concentration was significantly lower in periodontitis subjects compared to healthy controls. Thus, periodontal disease has been suggested to be associated with reduced salivary antioxidant status and increased oxidative damage within oral cavity ***(Buduneli et al., 2006, Ohshima et al., 2002, Brock et al., 2004, Wilton et al., 1989, Aurer et al., 1999).***

There are many reports that wound healing is poorer among smokers compared to nonsmokers. In a systematic review of the effect of smoking on nonsurgical periodontal therapy ***Labriola et al., 2000*** concluded that there was no evidence of a difference in gain in clinical attachment between smokers and nonsmokers or a reduction of bleeding on probing ***(Labriola et al., 2000).***

Although, smoker and non-smoker patients exhibit more or less the same periodontal pathogens ***(Preber et al. 1992, Buduneli et al. 2005a)*** smokers also tend to respond less favourably to periodontal treatment ***(Ah et al. 1994, Renvert et al. 1998).*** Smoking was suggested to influence host cytokine levels ***(Boström et al. 1999, Buduneli et al. 2005b, Buduneli et al. 2006).*** Furthermore, smoking was reported to reduce salivary osteoprotegerin concentrations in untreated and also treated chronic periodontitis patients ***(Buduneli et al.,2008).***

Since, saliva can be easily collected; measurement of total salivary antioxidant capacity levels may prove to be useful in identifying patients at risk of tooth loss. Moreover, a salivary analysis for periodontal diagnosis may prove a cost effective method for screening large populations. Further, studies involving larger group sizes and analysis of GCF total glutathione are required to address these questions.

Periodontal disease is clearly an important and potentially life-threatening condition, often underestimated by health professionals and the general population. The available evidence implicating inflammatory mediators and cells in the disease process suggests that local antioxidant status may be of importance in determining susceptibility to the disease and its progression following initial bacterial colonization. It is now of importance to determine the possible contribution of diet to salivary antioxidant status.

Summary

Periodontitis is a complex disease in which disease expression involves intricate interactions of the biofilm with the host immune-inflammatory response and subsequent alterations in bone and connective tissue homeostasis.

In the past 20 years, there has been an increasing awareness of the role of tobacco use on the prevalence and severity of periodontal diseases. Smoking meets the majority of criteria given for causation of a disease.

Saliva in humans is a mouth fluid possessing several functions involved in oral health and homeostasis with an active protective role in maintaining oral healthiness.

Similar to other biological systems, the salivary antioxidant system includes various molecules and enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), etc., which can be used as biomarkers for diagnosis of various periodontal diseases.

Glutathione is a low molecular weight antioxidant, which can directly scavenges free radicals or acts as a substrate for glutathione peroxidases and glutathione S-transferases during the detoxification of hydrogen peroxide, lipid hydroperoxides, and electrophilic compounds.

In the present study, we are testing the hypothesis that total glutathione of saliva is higher in subject of periodontitis in smoker as compared to non-smoker and control, and salivary total glutathione concentrations before and after scaling and root planning in smoker as well as in non smoker.

The present study was performed on a total of 40 medically healthy individuals divided into three groups. The first group was composed of 15 non-smoking patients with generalized moderate to severe chronic periodontitis.

The second group was composed of 15 smoking patients with generalized moderate to severe chronic periodontitis. According to Buduneli et al. (2006) smokers are defined as those who smoked $\geq$10 cigarettes per day for more than 5 years.

These two groups were subjected to clinical examination, radiological examination and laboratory investigations before supragingival scaling and subgingival clinical debridement in 3-4 sessions and 2 & 4 months after the non-surgical periodontal treatment.

The third group was a control group which was composed of 10 non-smoker subjects (controls) with clinically healthy periodontium. This group was also subjected to clinical examination, radiological examination and laboratory investigations.

Levels of total salivary glutathione level were determined using kinetic enzymatic recycling assay according to manufacturers' instructions.

The 40 included individuals presented the following results, within their specific groups. The control group consists of 10 periodontally healthy subjects. The subjects included in this group have mean age of 30.3 (3.6) years, mean weight 68.73 (13.01) Kgs, mean height 163.06 (11.45) cm, and mean BMI 25.85 (3.71) kg/m^2. These patients showed zero mean plaque index and gingival index.

The chronic periodontitis group consists of 30 patient subdivided into 2 subgroups: smokers and nonsmokers. . The subjects included in this group have mean age of 40.6 (10.43) years, mean weight 73.46 (13.4) Kgs, mean height 164.46 (9.1) cm, and mean BMI 27.24 (3.11) kg/m^2. These patients showed zero mean plaque index and gingival index.

The plaque index of the smokers group was 2.42±0.27 which decreased to be 0.79±0.24 after treatment. While the gingival index of this group was 2.124±0.299 which decreased to be 0.7533±0.184 after treatment. The PPD of this group was 2.99±0.977 which decreased to be 1.62±0.429 after treatment. The CAL was 4.98 ± 1.24, which decreased to be 3.17 ±0.68 after treatment. The mean recession of this group was 2.109 ±0.866, which decreased to be 1.976±0.947.

The plaque index of the non smokers group was 1.62±0.32 which decreased to be 0.70±0.27after treatment. While the gingival index of this group was 1.706± 0.559 which decreased to be 0.806±0.338 after treatment. The PPD of this group was 2.42± 0.373, which decreased to be 1.57± 0.178 after treatment. The CAL was 3.54± 0.897, which decreased to be 2.57 ± 0.525after treatment. The mean recession of this group was 1.41 ± 0. 682, which decreased to be 1.26± 0.854.

There was a significant difference in the Mann Whitney test between chronic periodontitis patients and healthy control subjects as the mean value glutathione in chronic periodontitis patients were (**6.5± 1.9**) and from healthy control subjects were (**2.39±.465**) where the P-value was 0.0001 (≤0.05).

The Mann Whitney test results for the glutathione levels pre and post treatment between smoker and non smokers with chronic periodontitis showed that there was slight significant difference in pre treatment values where p-value was (0.001). And there was a significant difference 2 months after treatment with p values (0.03) and also there was significant difference 4 months after treatment with p value (0.001).

Since, saliva can be easily collected; measurement of total salivary antioxidant capacity levels may prove to be useful in identifying patients at risk of tooth loss. Moreover, a salivary analysis for periodontal diagnosis may prove a cost effective method for screening large populations. Further, studies involving larger group sizes and analysis of GCF total glutathione are required to address these questions.