Estimation and Comparison of Salivary PH and Salivary Oxidative Stress in Diabetic Smokers, Diabetic Nonsmokers and Controls

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ABSTRACT

Introduction: Saliva serves as an important diagnostic medium, reflecting systemic conditions and oxidative stress, which are associated with several oral and systemic diseases. This study aims to estimate and compare salivary pH and salivary oxidative stress (measured by malondialdehyde [MDA] levels) among diabetic smokers, diabetic non-smokers, and healthy controls. Understanding these factors could provide insights into oxidative damage in diabetic and smoking-affected populations.

Materials and Methods: A total of 102 male subjects, aged over 30 and with diabetes and/or smoking history of 5-10 years, were categorized into three groups: diabetic smokers (n=34), diabetic non-smokers (n=34), and healthy controls (n=34). Salivary samples were collected using a standardized protocol and analyzed for pH using a pH meter and oxidative stress (MDA) levels using spectrophotometry. Statistical analyses, including ANOVA, t-tests, and Pearson correlation, were conducted to identify differences and associations between groups.

Results: Salivary pH was significantly lower in diabetic smokers (mean pH 5.91 ± 0.45) compared to diabetic non-smokers (6.79 ± 0.29) and controls (7.14 ± 0.20) ($p<0.001$). MDA levels were significantly higher in diabetic smokers (1.539 ± 0.233 mmol/L) than in diabetic non-smokers (0.977 ± 0.102 mmol/L) and controls (0.407 ± 0.095 mmol/L) ($p<0.001$). A positive correlation was observed between MDA and pH levels across all groups.

Discussion: The study highlights the combined impact of diabetes and smoking on salivary oxidative stress, as shown by elevated MDA levels and reduced pH in diabetic smokers. These findings suggest that oxidative stress exacerbates salivary alterations in diabetic smokers, potentially contributing to oral and systemic pathologies.

1. Introduction

Saliva has attained a greater diagnostic height in the past two decades. Saliva, with the qualities of being colorless, odorless, and mirroring the components of blood in the oral cavity[1]. About 600mL of saliva is been produced in a day. The pH in the saliva plays an important role in the life, growth, and multiplication of oral bacteria. One of the obstacles that hinders the wider use of saliva for diagnosis

and monitoring of systemic diseases is its composition, which is affected by local oral status.[2] However, this makes saliva very interesting for the clinical biochemistry of oral diseases. Periodontitis, caries, oral precancer, and other local oral pathologies are associated with oxidative stress. Saliva is the first biological fluid that is exposed to cigarette smoke, which contains numerous toxic compositions responsible for structural and functional changes in saliva.[3] A higher incidence of dental caries, oral mucositis, dysphagia, oral infections, and altered taste has been reported in individuals with reduced salivary flow. In long-term smoking, the taste receptors, a primary site for salivary secretion, are repeatedly exposed to tobacco for a long time thus presumably affecting the salivary reflex.[4-6]

Oxidative stress can be defined as an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage (Sies, 1991). According to Halliwell, oxidative stress refers to a serious imbalance between reactive species production and antioxidant defense.[4] Almost many of the systemic and oral pathologies are attributed to the increase in oxidative stress. The entire body function is associated with the constant balance of neutralizing the reactive oxidative species and repair of damaged DNA through apoptosis and physiological DNA repair. The effect of smoking on oral and general health has been studied through vast research. Smoking may enhance oxidative stress not only through the production of reactive oxygen radicals in smoke but also through the weakening of the antioxidant defense systems.[7,8]. Diabetes mellitus (DM) alters the oxidative stress. Diabetes overloads glucose metabolic pathways, resulting in excess free radical production and oxidative stress. With the increasing prevalence of diabetes mellitus oxidative stress peaks and causes continuing damage at cellular and molecular levels[9-11]. Even though oxidative stress is studied as a reason for the development of cancer, the high concentrations of oxidative stress during lipid peroxidation can cause various ranges of damage from periodontitis to general tissue damage and cancer. MDA is the most studied product of lipid peroxidation. However, MDA is only one of many products formed during lipid peroxidation. TOS assay developed by Erel provides a possibility to measure the additive effects of oxidants. Akalin et al. utilized a TOS assay to measure oxidants in the saliva of chronic periodontitis patients. Higher MDA and TOS levels were observed in saliva and also gingival crevicular fluid of chronic periodontitis patients.[6] The importance of lipid peroxidation in saliva was confirmed in another showing that the lipid peroxidation in saliva of patients who smoke and suffer from periodontitis is higher when compared to healthy propends. Increased oxidative damage of DNA, lipids, and proteins was observed in periodontitis patients.[12-14]

Malondialdehyde (MDA) assay is the most widely used lipid peroxidation technique due to its simplicity. The determination of oxidative stress requires sometimes invasive techniques such as taking blood samples. Whole saliva is an important physiologic fluid that contains a highly complex mixture of substances. Variable amounts of serum products are present in whole saliva. Exploring saliva as a diagnostic tool for the assessment of oxidative stress and antioxidant markers could be of significant clinical interest. Hence, an attempt was made to analyze the levels of salivary pH and salivary oxidative stress in diabetic smokers and diabetic non-smokers in comparison with healthy controls[15,16].

2. Materials And Methods

The study commenced after obtaining approval from the Scientific Committee of the study institute and from the Institutional Review Board at the study institute. The patients for this study were selected from the outpatient section of our Institution. The present study is a non-invasive method designed with the main objective of determining the salivary pH and salivary oxidative stress (malondialdehyde) in diabetic smokers, diabetic non-smokers, and healthy individuals. A total of 102 subjects were evaluated in the study and those above 30 years and Duration of diabetes and smoking was 5±10 years were categorized into following groups.

- Group 1: 34 subjects with diabetic smokers.
- Group 2: 34 subjects with non-smoker diabetics.

- Group 3: 34 subjects with healthy individuals (non-smoker non-diabetic).

After obtaining informed consent from the patients, the complete history was taken on a proforma for the study including the details of their habits, especially of smoking were specifically required. All participants included in the study were asked to complete a proforma with a questionnaire that elicited the demographic data (age, gender), medical status, deleterious habit history, prior or current exposure to medication, etc. Whole unstimulated saliva (5 ml) was taken for 5 min and was collected by drooling the saliva into the vial (Figure-1).

Samples taken were transported in ice bags at a temperature range of 0°C–4°C to the laboratory designated for the study. Saliva samples were cold centrifuged at 3000 rpm for 5 min. The supernatant was aspirated and was stored at −20°C until analyzed. The clear supernatant was used for the biochemical analysis of MDA, using a spectrophotometer. A spectrophotometer is an instrument that measures the amount of photons (the intensity of light) reflected from a sample object or the amount of light that is absorbed by the same object.

Biochemical Measurements:

A thorough general and oral examination was carried out blood samples were collected fasting blood glucose was estimated and salivary samples were collected from each subject. Unstimulated saliva samples were collected and centrifuged for 5 min at 3000 rpm to obtain a clear supernatant fluid. The clear supernatant saliva was analyzed for the salivary pH using a pH meter.

All participants were advised to:

- Avoid eating, drinking (except for water), or smoking for one hour before the start of the test.
- Rinse their mouths with water several times (minimum 3 times) at the beginning of the test.
- Swallow any remaining saliva before taking sample collection.
- Unstimulated saliva was collected in a disposable cup
- Avoid speaking or swallowing during the test.

Oxidative parameters Malondialdehyde (MDA)

- Biochemical procedure - For estimation oflipid peroxidation product malondialdehyde we used the method by Hogberg et al (1974). MDA is formed from the breakdown of polyunsaturated fatty acids and serves as a convenient index for the determination of the extent of peroxidation reaction. MDA, a product of lipid peroxidation, reacts with thiobarbituric acid (TBA) to give a pink-colored product, having absorption maxima at 535 nm, measured using a spectrophotometer (Figure 2 and 3).

3. Results

Data were analysed using both descriptive and inferential statistics. Student t test were used to find out the significant difference between two groups. ANNOVA was used to find out the significant difference between three groups. Multiple comparisons were carried out using post hoc Bonferroni. Pearson correlation was used to find out the significant relationship between MDA and pH in three groups. Statistical significance was observed at $p < 0.05$.

In the present study, the levels of salivary pH and malondialdehyde (MDA) were assessed in diabetic smokers, diabetic non-smokers and control groups. There is a positive significant correlation between salivary MDA and pH in diabetic smokers, diabetic non-smokers and healthy individuals. MDA showed a statistically significant increase among the diabetic smokers group when compared with diabetic non-smokers and control groups (Table 1,3). pH showed a statistically decrease among the diabetic smokers group when compared with diabetic non-smokers and control groups (Table 2,4).

Table 1: Mean comparison of MDA among three groups

Parameter	Groups	N	Mean	SD	F value	P value
MDA	Diabetic Smoker	34	1.539	0.233	439.48	0.000**
	Diabetic Non-Smoker	34	0.977	0.102		
	Healthy Individuals	34	0.407	0.095		

**Significant p<0.001

Table 2: Mean comparison of PH level among three groups

Parameter	Groups	N	Mean	SD	F value	P value
pH	Diabetic Smoker	34	5.911	0.453	123.38	0.000**
	Diabetic Non-Smoker	34	6.791	0.293		
	Healthy Individuals	34	7.138	0.197		

**Significant p<0.001

Table 3: Multiple comparisons of groups in MDA level

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diabetic Smoker	Diabetic Non smoker	.56206*	.03817	.000	.4691	.6550
	Healthy Individual	1.13176*	.03817	.000	1.0388	1.2247
Diabetic Non smoker	Diabetic Smoker	-.56206*	.03817	.000	-.6550	-.4691
	Healthy Individual	.56971*	.03817	.000	.4767	.6627
Healthy Individual	Diabetic Smoker	-1.13176*	.03817	.000	-1.2247	-1.0388
	Diabetic Non smoker	-.56971*	.03817	.000	-.6627	-.4767

*. The mean difference is significant at the 0.05 level.

Dependent Variable: MDA Bonferroni

Table 4: Multiple comparisons of groups in pH level

Dependent Variable: pH Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Diabetic Smoker	Diabetic Non smoker	-.87941*	.08049	.000	-1.0754	-.6834
	Healthy Individual	-1.22647*	.08049	.000	-1.4225	-1.0305
Diabetic Non smoker	Diabetic Smoker	.87941*	.08049	.000	.6834	1.0754
	Healthy Individual	-.34706*	.08049	.000	-.5431	-.1510
Healthy Individual	Diabetic Smoker	1.22647*	.08049	.000	1.0305	1.4225
	Diabetic Non smoker	.34706*	.08049	.000	.1510	.5431

* The mean difference is significant at the 0.05 level.

4. Discussion

Oxidative stress status may lead to a large number of diseases, including precancerous and neoplastic lesions of the oral cavity which may be due to the altered levels of the salivary antioxidant system that fails to cope with the altered level of oxidative stresses originating due to cigarette smoke.[17] This study focused on two of the common sources of oxidative stress in our region currently. In the present study, the mean and standard deviation of pH for Group 1; diabetic smokers were 5.9 (±0.45), Group 2; diabetic non-smokers 6.7 (±0.29) and Group 3; healthy individuals 7.1 (±0.13). A significant correlation was obtained, a lower salivary pH was observed in Groups 1 and 2 compared to controls (Group 3). Salivary pH was the lowest in Group 1 compared to Group 2 and Group 3 because the diabetic condition of altered salivary function and reduced salivary secretion and use of smoking form, which can react with bicarbonate buffering system by the loss of bicarbonate, turning saliva more

acidic. The alteration in electrolytes and ions alters the pH as they interact with the buffering systems of saliva. Khan et al.[18] also observed a lower salivary pH in smokers than in non-smokers which was consistent with the findings of the present study.

A comparison of mean pH among diabetic smokers and diabetic non-smokers showed that there was a statistically significant difference found using t-test, $p<0.001$.(table1) A comparison of mean pH among diabetic smokers and Healthy Individuals showed that there was a statistically significant difference found using the test, $p<0.001$. (table 2) Comparison of mean pH among diabetic non-smokers and Healthy Individuals showed that there was a statistically significant difference found using t-test, $p<0.001$(table 3) Our study reveals that the mean salivary pH of Group 1; 5.9 (0.45), Group 2; 6.7 (±0.29), and Group 3; 7.1 (±0.13) which is by the study conducted by Fenoll Palomares et al.[9] in which the mean salivary pH was lower in diabetic smokers that is, 6.7 ± 0.27 as compared to diabetic non-smokers that is, 6.8 ± 0.29. Similarly, Rooban et al.10 also observed a lower salivary pH in diabetic smokers that is, 6.48 ± 0.36 in comparison to 6.59 ± 0.56 in diabetic non-smokers. The difference was statistically significant (P=0.03). In uncontrolled diabetics changes in the metabolic process lead to a decrease in pH level and smoking form which can react with the bicarbonate buffering system by the loss of bicarbonate, turning saliva more acidic. The alteration in electrolytes and ions alters the pH as they interact with the buffering systems of saliva.

In the present study mean salivary MDA in diabetic smokers, diabetic non-smokers, and healthy individuals was (1.539±0.233mmol/l), (0.977±0.102mmol/l) and (0.407±0.095 mmol/l) The mean salivary MDA levels were significantly higher in diabetic smokers than diabetic non-smokers and healthy individual ($p < 0.001$) respectively. The present study is consistent with the studies by Kalaivanam et al and Peerapath et al where the salivary levels of MDA were significantly higher in diabetic smoker patients in comparison to the normal controls. Diabetes mellitus is altered immune cell functions coupled with defective neutrophil apoptosis, systemically hyper-responsive monocytes, and macrophages with the resultant excessive production of oxidative stress and one of the possible reasons for the increase of salivary MDA may be a result of oxidative damage of the salivary glands. Possibly, continuous local irritation by tobacco can lead to OS. In addition, an increase in salivary OS may be related to the alteration of salivary secretion and qualitative changes in salivary proteins. Therefore, the mean salivary level of MDA in diabetic patients was 0.977 mmol which was significantly higher than the healthy controls, which was by the studies conducted by Suryawanshi et al,[19] Kumari et al[20] and Natheer H Al-Rawi[21] thereby establishing that heightened susceptibility and predisposition of cells to lipid peroxidation and inflammation due to oxidative stress plays a prime role in the pathogenesis of diabetes mellitus and its complications.

The present study conforms with the study by Natheer H Al-Rawi[21] where MDA levels were elevated in the salivary samples of diabetic patients. The study stated that the salivary MDA level was significantly increased in the diabetic group which mirrored the high oxidative stress levels. Increased oxidative stress was communicated by an enhanced production of free radicals, peroxidation of lipids, and reduction in antioxidant status. The present study is also by the study conducted by Mahadevan et al[22] where high levels of MDA were observed in diabetics as compared to controls validating the role of oxidation of free radicals in the pathogenesis of diabetes mellitus. The results established that salivary MDA is the indicator of oxidative stress in subjects with diabetes mellitus.

The significance of this study suggests the harmful effect of smoking on diabetes and the role of saliva as an adjunctive tool to monitor the prognosis of diabetes mellitus. This study suggests that exploring saliva for oxidative stress may have boundless clinical importance. So examining the salivary pH and oxidative stress in diabetic smokers, diabetic non-smokers, and healthy individuals the pH level was less and the oxidative stress level was higher amongst the diabetic smoker population when compared with the diabetic non-smokers and healthy individuals. Peroxidation of lipids in patients with smoker diabetes that accurately reflects the severity of the oxidative stress is worthy. The limitation of this present study is that we considered only male patients, so gender distribution is not possible in the assessment of salivary pH and salivary oxidative stress. This study evaluated only the diabetic smokers

and did not compare the oxidative stress level between the diabetic smokers and the diabetic tobacco chewers.

Smoking produces large amounts of reactive oxygen species (ROS), which have an influence on normal cellular function and cause changes in the inflammation markers. Oxidative stress, which is produced through a serious imbalance between the generation of reactive oxygen species and antioxidant protection, is effective in the pathogenesis of inflammatory conditions, such as periodontal diseases. On the other hand, periodontitis is a chronic infective disease, in which local and systemic factors, including diabetes and smoking, change the response of the immune system to local agents, such as dental plaque, and consequently affect the progression of the disease. Excess production of reactive oxygen species and an impaired antioxidant defence mechanism led to increased oxidative stress in diabetes. Reactive oxygen species induces membrane lipid peroxidation and the generated fatty acid peroxides cause cell malfunction. In diabetes mellitus, abnormally increased levels of lipids, lipoproteins, and lipid peroxides in plasma may be due to abnormal lipid metabolism atherogenesis. One of several byproducts of lipid peroxidation processes is MDA which can be used as an indicator for oxidative stress. Decreased levels of pH and increased levels of oxidative stress in the oral cavity cause periodontitis, dental caries, oral pre-cancer, and other oral pathologies.

5. Conclusion

To conclude we observed that the pH level was low and the oxidative stress level has become more amongst the diabetic smoker population when compared with the diabetic non-smokers and healthy individuals. In this study, an attempt was made to emphasize the harmful application of smoking in diabetes patients and the role of saliva as a prognostic marker of diabetes.

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Conflict Of Interest: Nil.

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