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Research Article

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Saliva Diagnostic Tool for Oral Health

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ABSTRACT

The aim of this investigation is to evaluate the values of salivary fluid from biochemical estimation, nitrogen analysis and antioxidants represent promising tool for the research of oral in four groups. The first group contains 25 healthy persons (no history of tobacco and alcohol) considered as control, the second group contains 25 people with oral cancer, the third group contains 25 people with kidney failure or chronic kidney diseases (CKD) and the fourth group contains 25 smoker people.
From the results, it could be noticed that the mean kidney function concentrations in saliva were higher in CKD group followed by oral cancer and smoker groups than healthy control group. The nitrogen analyses were high by 60%, 190%, and 93%, respectively, in the oral cancer patients. Moreover, in the kidney failure patients, these salivary values were increased to 66.9, 207.13 and 117.55%, respectively in comparison to healthy control. Smoker People increased to 19.86, 27.13 and 13.03%, respectively compared healthy control.
The determination of antioxidant capacity (ImAx) had significant reduction by 22, 16 and 3.8% in oral cancer patients, CKD patients and in the smokers, respectively. Whereas, the total antioxidants had 49, 39 and 8.16% reduction in the same groups, respectively. Similarly, the peroxidase activity, glutathione S-transferase (GST) activity and superoxide dismutase (SOD) activity of enzymes as antioxidant in salivary were reduced by 27.46, 30 and 28% in oral cancer patients. Moreover, in kidney failure, patients were reduced by 20.98, 23.9 and 21.6%, respectively. Whereas, in smokers reduced by 8.29, 10.87 and11.2%, respectively than healthy control.
Findings of this investigation with regard to evaluation of the salivary fluid in biochemical estimation, nitrogen analysis and antioxidants in oral carcinoma, chronic kidney disease and smokers could be beneficial to diagnose underlying disease.

Key words: *Salivary, chronic kidney diseases (CKD), smokers, nitrogen analysis, total antioxidants, antioxidant capacity (ImAx)*

INTRODUCTION

Salivary fluid is an important physiologic fluid and it is considered as a diagnostic agent. Saliva fluid in the mouth protects dental caries, erosion, scraping and periodontal diseases. The utilization of salivary fluid provides a substantial addition to the diagnostic as an investigative agent of disease processes and disorders and it is known as a diagnostic agent including its ease of the positive correlation between many parameters in the serum [1].

In the oral, the nitrates of the salivary fluid are turned into nitrites (NO2) that might be caused their reaction with amines and amides to form the carcinogenic nitrosamines [2,3]. Moreover, the production of nitrosamine in salivary

fluid and metabolism are also based on the dietary nitrates (NO3), which are absorbed from the upper gastrointestinal tract and actively from the plasma into the salivary fluid by the salivary glands through an active transport system similar to that for iodide, thiocyanate, and per chlorate [4,5]. Saliva holds a significant function in cigarette related nicotine induced DNA damage.

Salivary fluid is believed to have a significant function in the changes of the mouth medium. The non-protein nitrogen compounds available in salivary fluid such as uric acid appeared to have important effects. Urea is the end product of amino acid metabolism in the blood, which may be changed by several factors such as proteins, diet, and dehydration. There is a positive correlation between blood urea with salivary fluid urea [6,7]. Uric acid is a product from the purines and similar relationship is reported in blood and salivary fluid [8].

Several studies reported the connection of the salivary flow with periodontal, dental and oral status in chronic kidney diseases (CKD) patients [9,10]. Schuller and Holst [11] found that the salivary fluid of CKD people has important protective properties participating in keeping mucosa and hard tissues and oral integrity in the physiological balance with it in normal condition. Any deviation may influence the condition of the tissues in the oral cavity. Lasisi et al. [12] found that the several diseases produce marked and distinguishable changes in salivary secretion. Moreover, the salivary fluid can indicate the levels of creatinine and urea in people with CKD, which are the parameters usually assessed in blood samples and these analyses were caused many advantages that attributed to the use of salivary fluid as a diagnostic fluid [13]. Tomás et al. [14] reported that the salivary fluid composition in people with chronic renal failure is conditioned by the stage of renal failure. According to Van den Velde [15], breath analysis could potentially be used as a detecting some systemic disease such as liver pathologies or kidney failure. Bad breath is caused volatile sulfur compounds as a result of bacterial breakdown of protein and can be quantitatively and qualitatively measured in the expired oral breath [16].

Anuradha et al. [17] showed that the potassium levels were significantly higher in the kidney failure people when compared to healthy people and the difference was insignificant in relation to bicarbonate level. The increased levels in dialysis patients correlated with renal disease severity.

The aim of this study is to evaluate chemical analysis, nitrogen analysis, and the antioxidant profile of the saliva in oral carcinoma patients, chronic kidney diseases (CKD) patients and smokers. These parameters might be understanding the relation between saliva and oral cancer pathogenesis, chronic kidney diseases and smokers in comparison with healthy control.

MATERIALS AND METHODS

Patients:

Four groups of people were recorded in the study from the Ibn Sina National College Hospital for Medical Studies-Jeddah, Saudi Arabia. There were 25 people in each group considering 100 subjects under study. The biochemical analyses were determined in all subjects' salivary fluid, which was collected.

The first group was the Control group: 25 healthy subjects without tobacco related habits, alcohol consumption and associated lesions.

The second group was the malignant group: 25 oral cancer patients. All 25 cases were oral cancers and all cases of oral cancers were differentiated.

The third group was Kidney Failure group: 25 subjects' chronic kidney diseases (CKD) patients.

The fourth group was the Smokers group: 25 subjects with history of smoking more than 20 cigarettes per day for a period of 20-30 years with no alcohol consumption and no associated lesions.

Saliva sampling

Saliva collection was undertaken throughout the day (between 9.00 h and 16.00 h) and participants had no meal before salivary fluid collection. Participants were asked to spit (after rinsing the mouth with distilled water) into calibrated universal plastic bottles until about 3.0 ml of saliva was collected. Saliva samples were stored at -20 °C for laboratory analysis. Samples were centrifuged before being used for the analysis in order to remove contaminants, as previously described by Reznick et al. [18].

Measurement of pH in salivary fluid:

PH was measured using PH meter in salivary fluid, which were collected previously.

Urea measuring in salivary fluid:

For urea measurement, diacetylmonoxime colorimetric was applied. Diacetylmonoxime analyzed with the acid gave diacetyl, which reacted with urea and it contained yellow color and urea was measured at 520nm [19].

Uric acid measurement in salivary fluid:

For uric acid measurement, phosphotungstate method was used. In this method, the phosphotungstic acid and sodium carbonate had a blue color, which was measured by colorimeter at 700 nm wavelength [20, 21].

Creatinine measurement in salivary fluid:

For creatinine measurement, Jaffe method was used. Creatinine makes orange complex with bicarbonate (This color relate to creatinine and other nonspecific materials). With acidification, the color created by existence of creatinine would be disappeared.

The difference in color intensity in 520 nm has positive correlation with creatinine concentration [22].

Malondialdehyde MDA in salivary fluid:

Lipid peroxidation product-MDA was analyzed according to the method described by [23,24].

Ascorbic acid in salivary fluid:

The ascorbic acid in the salivary fluid was measured calorimetrically according to the method described by Carl et al. [25].

Salivary nitrogen species analysis in salivary fluid:

Salivary nitrogen species was measured by the Griess method modified by Fiddler [26].

Salivary antioxidant analysis in salivary fluid:

Antioxidant capacity (ImAx) in salivary fluid:

The antioxidants capacity in the salivary fluid sample was eliminated according to Nagler et al. [27].

Total antioxidant (TA) in salivary fluid:

The total antioxidant in the salivary fluid sample was eliminated calorimetrically at 600 nm according to Nagler et al. [27].

Peroxidase activity in salivary fluid:

Peroxidase activity in salivary fluid was an assay [27] and it was read at a wavelength of 412 nm for 20 seconds.

Glutathione S-transferase (GST) activity in salivary fluid:

The glutathione S-transferase (GST) enzyme activity determined salivary fluid through the method described by Sundberg et al. [28].

Superoxide dismutase (SOD) activity in salivary fluid:

The superoxide dismutase (SOD) enzyme activity in salivary fluid determined salivary fluid through the method described by Nagler et al. [27].

Statistically analysis:

The data obtained from the obviously results were analyzed by ANOVA. For all analyses, a significant variation ($p< 0.05$) was detected in some differences. The data were applied and analyzed with the aid of the Windows software [29].

RESULTS AND DISCUSSION

Biochemical analysis in salivary fluid:

Biochemical analysis as pH, urea, uric acid, creatinine, malondialdehyde (MDA) and ascorbic acid was determined in salivary oral cancer people, chronic kidney diseases patients (CKD) and smokers than healthy saliva; and the data are reported in table (1). The mean results of pH saliva of oral cancer and CKD, smokers and healthy group showed that the pH in salivary fluid was decreased in smokers than healthy group, whereas, it was not significant in oral cancer and CKD patients. From the results, the healthy group showed that the pH of salivary fluid was 7.1. Therefore, the pH of saliva parallels the extracellular fluid pH. These results were achieved by Scott [30] who found that pH excessive acidity in the body is associated with degenerative diseases including cancer, kidney, gall stones and tooth decay. Therefore, the saliva shows pH balance in our bodies. Salivary buffer capacity is an important parameter in maintaining pH of saliva, thereby, reflecting the integrity of soft and hard tissue in the oral [31, 32].

From the results, it could be noticed that the mean uric acid in salivary fluid was 3.70 mg/dl in CKD group followed by oral cancer patients and smokers (2.45 and 2.32 mg/dl, respectively) but in healthy people contained 2.27 mg/dl. The differences between four groups were statistically significant at $p< 0.05$. Urea in salivary fluid was 3.19 mg/dl in CKD group followed by oral cancer and smokers that were 2.79 and 2.65 mg/dl, respectively; but in healthy people was 2.60 mg/dl. Saliva creatinine concentration was measured in both groups that the results are reported in table (1), the mean creatinine in saliva was the highest decrease in CKD group, 0.67 mg/dl, followed by oral cancer patients and smoker groups were 0.96 and 0.98 mg/dl, respectively and 0.98 mg/dl was in healthy people.

Uric acid could be found in saliva in concert with its blood concentration [33]. From the results, it could be noticed that the salivary fluid of uric was increased to 43.17, 27.31 and 6.61% in CKD, oral cancer and smokers' groups, respectively than healthy people and also, the salivary fluid of urea was increased to 41.92, 22.69 and 5.77%, respectively in CKD, oral cancer and smokers' groups in comparison to healthy people.

Creatinine produced from keratin daily, its density consisted of an individual muscle mass, and any decrease in creatinine caused the kidney disease and carcinoma. Moreover, the evident results showed that the salivary fluid was decreased to 31.63, 18.37 and 9.18% in creatinine for people with CKD, oral cancer and smokers, respectively than healthy people. The reason of this creatinine decrease in CKD, oral cancer and smokers occurs when urea and uric acid concentration increased in salivary fluid that may cause the hydrogen ion absorption by creatinine compounds, which would decrease in salivary fluid [34, 35].

Dahlberg et al. [36] showed that the salivary fluid is used to observe the dialysis process. The urea and uric acid in salivary fluid are common in plasma and it reflects the blood levels [37, 38]. Belazelkovska et al. [39] reported that the uric acid in salivary fluid was increased in patient renal failure and it was reduced salivary fluid flow rate, so an increase of the presence of uremic smell might occur.

Salivary MDA highly increased in CKD, oral cancer and smokers that were 0.98, 0.51 and 0.79nmol/l, respectively, while normal group was not detected. Ascorbic acid in salivary fluid was significantly decreased in CKD and oral cancer people since its amount was 0.47 and 0.46 mg/dl compared to healthy people (0.48nmol/l) and in smokers, the decrease in ascorbic acid was not highly significant.

The malondialdehyde is increased in the salivary fluid of oral cancer and CKD people and smokers. The ascorbic acid and total antioxidant activity in salivary fluid are significantly reduced in oral cancer and smokers. Increase in MDA indicates that there is an increase in lipid peroxidation and free radical generation. Oxidative damage might cause disorders, carcinogenesis and aging [40, 41].

Table 1. Salivary biochemical analysis between healthy people and patients:

Chemical analysis	Healthy control	Oral cancer	CKD	Smokers
pH	7.1±0.14	6.0±0.12	6.0±0.12	5.0±0.11
Uric acid mg/dL	2.27±1.72	2.45±1.84	3.70±1.89	2.32±1.53
Urea mg/dL	2.60±1.12	2.79±1.34	3.19±1.85	2.65±1.62
Creatinine mg/dL	0.98±0.14	0.96±0.13	0.67±0.11	0.98±0.14
MDA nmol/L	0.00±0.00	0.51±0.09	0.98±0.12	0.79±0.09
Vit C mg/dL	0.48±0.05	0.45±0.02	0.47±0.04	0.48±0.03

Nitrogen species analysis:

Nitrogen species were determined in salivary oral cancer patients, chronic kidney diseases patients (CKD) and smokers than in healthy saliva that the data are tabulated in table (2). From the results, it could be observed that the analysis of total nitric oxide, nitrites and nitrates in salivary healthy control concentrations demonstrated 72.0mmol/L, 80.0mmol/L, and 37.6mmol/L, respectively. In the oral cancer, patients were higher by 60%, 190%, and 93%, respectively, in comparison to the healthy control. Moreover, in the CKD patients, these salivary values were the highest (120.2, 145.7 and 81.8 mmol/L) by increasing to 66.9, 207.13 and 117.55%, respectively than healthy control. Whereas, the smokers increased to 19.86, 27.13 and 13.03%, respectively than control and the salivary values from the smokers were lower than oral cancer and CKD. The results are in agreement with Balwant et al. [42] who reported that the salivary fluid is a diagnostic agent for many oral and systemic diseases. Moreover, Xia et al. [5] showed that the oral nitrates switched to nitrites (NO_2^-) in saliva, which play an important role in reaction with amines and amides to form the carcinogenic nitrosamines, thus initiate and promote oral cancer. Moreover, the ROS and RNS play a key role in human cancer development in the form of nitrosamines (NO_3 and NO_2) [43]. Reactive free radicals are able to damage chemical components and nucleotides in the tissue. ROS can cause DNA base alterations, strand breaks, damage tumor suppressor genes and enhance expression of proto-oncogenes [44].

The measurement of uric acid concentrations along with NO2–, as suggested by Levin et al. [45] who reported that not only people with other oncological and oxidative related disorders undergo dialysis but also patients with end stage renal disease.

Levine et al. [46] reported that ascorbic acid might act as a cofactor for enzymes involved in collagen hydroxylation, biosynthesis of carnitine and norepinephrine, tyrosine metabolism, and peptide hormones. Moreover, Saral et al. [47] found that ascorbic acid might be directly as a free radical scavenger in watery environment of the cells and interact with vitamin E in the lipid rich areas of the cells. Mayland et al. [48] showed that ascorbic acid in plasma is declined in chronic or acute oxidant states. Few studies have monitored concentrations and supplementation ascorbic acid in salivary fluid [49, 54]. Human requirements for ascorbic acid is from satisfied diet [51].

Table 2. Mean of nitrogen components in salivary fluid (µ mol/l):

Nitrogen analysis	Control	Oral cancer	CKD	Smoker
Total nitric (No) (µ mol/l)	72.0±1.23	115.2±3.11	120.2±2.04	86.3±1.52
Nitrite (No2) (µ mol/l)	80.0±1.57	232.0±3.82	245.7±3.29	111.7±2.01
Nitrate (No3) (µ mol/l)	37.6±1.02	72.7±1.54	81.8±1.76	42.5±0.98

Antioxidant analysis

Antioxidant capacity (ImAx), total antioxidant (TA), peroxidase, glutathione S-transferase(GST) and superoxide dismutase(SOD) enzymes were assayed in salivary oral cancer people, chronic kidney diseases patients (CKD) and smokers than in healthy salivary fluid and the results are illustrated in Table (3). Results from antioxidant capacity of the salivary showed that the oral cancer, KCD and smokers were reduced in comparison to the control. The antioxidant capacity were reduced by 22%, i.e. from 320 to 251 mmol/L in the oral cancer patients, reduced 16% i.e. from 320 mmol/L to 270 mmol/L in the CKD patients and in the smokers reduced 3.8%. Whereas the TAS assay was reduced from 0.49 mmol/L to 0.25 mmol/L that was found in the oral cancer patients, reduced 39% from 0.49 mmol/L to 0.30 mmol/L which was recorded in the CKD patients and in the smokers was reduced 8.16 %.

The peroxidase, glutathione S-transferase and superoxide dismutase enzymes were reduced as antioxidants in salivary by 27.46, 30 and 28%, respectively, i.e. from 386 to 280 mU/mL, from 230 to 161 ng/mL and from 1.25 to 0.90 U/mL, which were observed in the OSCC patients. The same salivary-specific antioxidants in the CKD patients were reduced by 20.98, 23.9 and 21.6%, respectively, from 386 to 305 mU/mL, from 230 to 175 ng/mL and from 1.25 to 0.98 U/mL. In smokers the previous salivary specific antioxidants were reduced by 8.29, 10.87 and 11.2%, respectively, from 386 to 354 mU/mL, namely, from 230 to 205 ng/mL and from 1.25 to 1.11U/mL. A greater decrease in salivary-specific antioxidants may be due to lesser antioxidant buffering capacity of saliva. It may attribute to smaller volume of saliva secreted, which represents the systemic circulation directly in contact with the necrotic tissue and has a greater amount of available antioxidants [52].

Ujhelyi et al. [53] suggested that the decreased antioxidant capacity of plasma ultrafiltrate was observed after a hemodialysis session may cause the removal of several proteins bound uremic toxins, including p cresol and also usually retained in people with CKD.

Akalin et al. [41] showed that in association with oral and dental diseases (especially periodontitis), oxidative stress markers were changed in saliva. Saliva allows monitoring general health and disease in children, elderly people, and non-collaborative subjects [54].

Table 3. Antioxidant components in salivary fluid:

Antioxidants components	Healthy control	Oral cancer	CKD	Smoker
Antioxidant capacity µmol/L	320±3.41	251±2.43	270±2.75	308±3.22
TA mmol/L	0.49±0.02	0.25±0.01	0.30±0.01	0.45±0.02
Peroxidase mU/ml	386±4.11	280±2.54	305±2.84	354±3.28
GST ng/ml	230±2.18	161±1.58	175±2.01	205±2.57
SOD U/ml	1.25±0.45	0.90±0.21	0.98±0.23	1.11±0.14

TA: Total Antioxidant GST: Glutathione S-transferase SOD: Superoxide dismutase

CONCLUSIONS

Salivary diagnostic tools monitor various oral diseases ranging from periodontal diseases, dental caries to infections. Consequently, we probably consider the increased utilization of salivary fluid as a diagnostic fluid. As a result, dentists would have greater involvement in the identification and monitoring of certain non-oral disorders. From the obviously results, it could be recommended that the salivary fluid is a biological fluid that offers several opportunities in diagnosis. Furthermore, salivary fluid offers great potential in clinical as well as in public health studies.

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