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EVALUATION OF GLUTATHIONE LEVELS IN SALIVA AND GINGIVAL CREVICULAR FLUID IN DIABETIC PATIENTS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY

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ABSTRACT

Objective: the objective of this study was designed to evaluate the effect of non-surgical periodontal therapy on glutathione levels in saliva and gingival crevicular fluid among diabetics with chronic periodontitis. **Subjects and Methods:** A total number of 60 patients were selected consists of three groups: group 1 was 20 patients with healthy periodontal condition, group 2 was 20 patients with chronic periodontitis and group 3 was 20 diabetic patients with chronic. Scaling, root planning and oral hygiene instructions were performed for patients in group 2 and group 3.Saliva and GCF samples were collected from each subjects at baseline (before treatment) , one month and three months after performing the non –surgical periodontal therapy. **Results:** Following non-surgical therapy, glutathione levels in diabetic and chronic periodontitis groups improved significantly when compared to base line levels.No significant correlation between glutathione, age and disease activity in diabetic & periodontitis groups. **Conclusion:** Glutathione levels should be considered a marker for disease and the concentration of reactive oxygen species in human body and an important indicator for the progression of the periodontal treatment in patients with periodontal disease.

KEYWORDS: Glutathione; Periodontitis; Diabetic Patients

INTRODUCTION

Periodontitis is a chronic, irreversible multifactorial inflammatory disease that affects the supporting tooth structures and is triggered and spread by a complex interaction between pathogens and the host's immune system. It begins with microbial infection followed by host-mediated destruction of periodontal tissue caused by excessive leukocyte activity and the formation of cytokines, eicosanoids, and matrix metalloproteinases. Clinically, the disease progresses with loss of the root surface, deep pocket formation, alveolar bone resorption, and subsequent tooth loss [1].

Periodontal disease is often diagnosed according to certain clinical criteria and measures such as probing depth, loss of clinical attachment, bleeding on probing, and bone resorption. These parameters include probing pocket depth according to the Community Periodontal Index of Treatment Needs (CPITN), in which representative teeth are examined. It's usually used to check for periodontal disease [2].

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CPITN was developed and recommended by the World Health Organization (WHO) and is considered the practical standard for routine screening and recording of periodontal disease [3]. One of the advantages of this method is that it is simple, standardized, and easy for dentists to understand and use. It is therefore recommended for use in dental practices, healthcare, and epidemiological surveys [4].

Studies have shown a link between some blood tests and periodontal disease, including C-reactive protein (CRP) seems to show such a link, as patients with periodontal disease have high levels of C-reactive protein (CRP) in their blood (5). However, serum C-reactive protein is not specifically increased by periodontal disease, but it is increased in many inflammatory conditions caused by many systemic diseases. Therefore, when CRP is used to screen for periodontal disease, there is a risk of cross-action against a background of systemic disease. Therefore, no blood parameters are known that have a high specificity for periodontitis.

Gingival crevicular fluid (GCF) can be a strong candidate for screening and testing for periodontal disease. A specific relationship between periodontitis and parameters in the crevicular gingival fluid was shown. However, there are many sampling points available in the oral cavity and differences in results between sampling points should be taken into account [6].

Saliva contains many enzymes, molecules, and some markers of inflammation [7].Serum enzymes were routinely checked for systemic disease. Therefore, the intention has been advanced to apply these traditional laboratory tests to saliva samples and examine their feasibility and reliability for periodontal screening.

Glutathione (GSH) is one of the most important redox regulators that control the inflammatory process. In its reduced form, GSH is an important antioxidant (radical scavenger). GSH is a low molecular weight, important non-protein cell thiol that is found in both eukaryotic and prokaryotic cells and is found in every single cell in the human body [8]. The risk of developing periodontal disease is about three times higher in people with diabetes than in people without diabetes[9].

With this in mind, an assay for the biochemical markers (GSH) in saliva samples and (GCF) for periodontitis in diabetics was carried out in this study.

MATERIAL AND METHODS

A randomized controlled trial was conducted at the Department of Oral Medicine, Periodontology, Oral Radiology and Oral Diagnosis, Boys, Cairo, Al-Azhar University. Sixty patients (37 males & 23 females) with periodontitis were selected from the Outpatient clinic.

The inclusion criteria: Patients with chronic periodontitis and with diabetes mellitus

The exclusion criteria: Patient with systemic condition except diabetes mellitus, history of smoking and history of scaling and root planning during the previous 6 months.

Grouping

- Group 1: Twenty patients (13 males & 7 females) age (21 to 60) with healthy periodontal condition (Control group).
- Group 2: Twenty patients (13 males & 7 females) age (35 – 66)with chronic periodontitis (periodontitis stage II grade A,B,C and stage III grade A,B,C),but not diabetics.
- Group 3: Twenty (11 males & 9 females) age (45 – 66) diabetic patients with chronic periodontitis (periodontitis stage II grade B, C and stage III grade B, C)

Pre-operative assessment:

All participants received a complete evaluation including medical and dental histories, and periodontal examination. The periodontal status for all individuals was assessed by the following: Plaque index [10], Gingival index [11], Probing pocket depth measured using the Williams graduated periodontal probe and clinical attachment level (CAL) [12]

Radiographically, evidence of bone changes as confirmed by intra-oral periapical radiographs.

Intervention:

Patients within periodontitis group underwent conventional periodontal treatment consisting of oral hygiene instructions, full mouth thorough scaling and root planning. Saliva and GCF samples were collected from each subject at baseline (before treatment), one month and three months after performing the non –surgical periodontal therapy. **(Figure 1).**

Sample collection:

Whole pooled saliva samples were obtained simply by expectorating into polypropylene tubes prior to clinical periodontal measurements or any periodontal intervention. This was performed during morning sessions, following an overnight fast during which subjects were requested not to drink (except water) or chew gum. The individuals were asked to rinse their mouth with tap water, before expectorating whole saliva into sterile 50 ml tubes for 5 min. The saliva samples were placed on ice. Then were centrifuged at 10000 x g for 15 min at 4 °C. The resulting supernatants were immediately aliquoted and frozen (–80 °C), until the analysis. The reduced glutathione was measured coulimetrically using spectrophotometer. GCF is removed using strips of paper placed in the crevice until slight resistance is felt (intra-crevicular method) and left in place for 30 seconds. Strips contaminated with blood or saliva were discarded. Each removed strip was placed in a disposable tube and stored at -40 ° C until analysis. For in laboratory analysis, 200 μl phosphate buffered saline (pH 7.4) was added to each tube with a sample strip. Centrifugation was performed at 10,000 × g for 5min, then the supernatant was used to determine reduced glutathione levels [13].

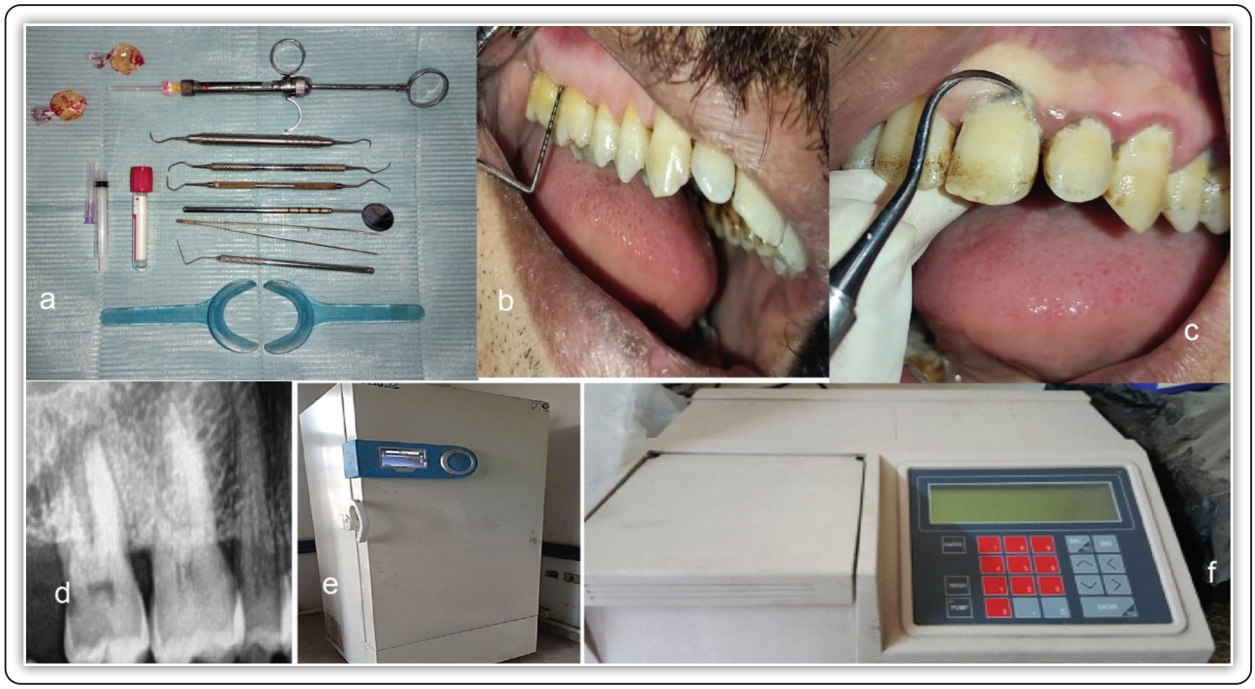

FIG (1) a; Armamentarium used, b; photo showing initial situation of a periodontal pocket, c; Full mouth scaling, d; X-ray showing bone resorption, e; Transport of samples for storage in very cold temperature -80°C, f; Spectrophotometry by autoanalyzer.

RESULTS

The study included 3 groups, twenty patients ranged in age between 21.0 – 60.0 years with a mean age of 40.60 ± 13.36 years for Group 1, twenty patients ranged in age between 35.0 – 66.0 years with a mean age 51.0 ± 7.71 years for Group 2, and twenty patients ranged in age between 45.0 – 66.0 years with a mean age 54.55 ± 5.62 years for Group 3

Results showed that following non-surgical therapy, glutathione levels in diabetic and chronic periodontitis groups improved significantly when compared to base line levels. No significant correlation between glutathione, age and disease activity in diabetic & periodontitis groups.

Table No. (1) Summarizes the comparison between the two groups examined according to loss of attachment. Baseline: There was a statistically significant difference in the mean loss of attachment in the two groups. Group 2 showed lower loss of attachment than group 3. After 1 month: There was a statistically significant difference in the mean loss of attachment in the two groups. Group 2 showed lower loss of attachment than group 3. After 3 months: There was a statistically insignificant difference in the mean loss of attachment in the two groups. Both groups showed lower loss of attachment.

TABLE (1) Comparison between the two studied groups according to attachment loss

Attachment loss	Group 2 (n = 20)	Group 3 (n = 20)	t	p
Baseline	3.40 ± 0.50	4.0 ± 0.65	3.269*	0.002*
1 month	2.0 ± 0.65	2.60 ± 0.50	3.269*	0.002*
3 months	1.60 ± 0.50	1.60 ± 0.50	0.000	1.000

t: Student t-test

p: p value for comparing between the studied groups

**: Statistically significant at p ≤ 0.05*

TABLE (2A) Descriptive statistics of GSH Saliva in each studied groupsv

Groups	Time	GSH Saliva					
		Min.	Max.	Mean ± SD	Median	95% CI	
						LL	UL
Group 1	**Baseline**	50.68	78.98	66.88 ± 7.87	67.03	63.20	70.56
Group 2	**Baseline**	11.56	27.54	18.53 ± 3.79	18.58	16.76	20.31
	1 month	49.18	61.53	55.16 ± 4.05	54.23	53.27	57.05
	3 months	46.51	79.51	58.84 ± 8.28	58.11	54.97	62.72
Group 3	**Baseline**	10.13	25.92	17.57 ± 4.97	18.96	15.24	19.90
	1 month	32.61	77.52	50.35 ± 11.57	49.28	44.94	55.77
	3 months	42.68	63.52	52.36 ± 6.08	50.97	49.51	55.20

Group 1: *Healthy control*

Group 2: *Chronic periodontitis*

Group 3: *Diabetic with Chronic periodontitis*

Table (2b) summarizes comparison between the different time periods in each group according to GSH Saliva. Both group showed a statistically significant increase in mean GSH Saliva measurements at 1 and 3 months.

TABLE (2B) Comparison between the different time periods in each group according to GSH Saliva

	GSH Saliva			p
	Baseline	**1 month**	**3 months**	
Group 2	18.53±3.79	55.16±4.05	58.84±8.28	<0.001*
p_0		<0.001*	<0.001*	
Group 3	17.57±4.97	50.35±11.57	52.36±6.08	<0.001*
p_0		<0.001*	<0.001*	

**: Statistically significant at $p \leq 0.05$*

Table (2c) summarizes comparison between the three studied groups according to GSH in Saliva. At Baseline: there was a statistically significant difference in mean GSH in Saliva in the three groups. Group 1 group showed a higher GSH in Saliva than Group 2 and 3. At 1 months: there was a statistically significant difference in mean GSH in Saliva in the three groups. Group 1 group showed a higher GSH in Saliva than Group 2 and 3. At 3 months: there was a statistically significant difference in mean GSH in Saliva in the three groups. Group 1 group showed a higher GSH in Saliva than Group 2 and 3. Group 2 group showed a higher GSH in Saliva than Group 3.

TABLE (2C): Comparison between the three studied groups according to GSH in Saliva

GSH Saliva	Group 1 (n = 20)	Group 2 (n = 20)	Group 3 (n = 20)	p
Baseline	66.88 ± 7.87	18.53 ± 3.79	17.57 ± 4.97	<0.001*
Sig	p_1<0.001*,p_2<0.001*,p_3=0.860			
1 month	66.88 ± 7.87	55.16 ± 4.05	50.35 ± 11.57	<0.001*
Sig	p_1<0.001*,p_2<0.001*,p_3=0.176			
3 months	66.88 ± 7.87	58.84 ± 8.28	52.36 ± 6.08	<0.001*
Sig	p_1=0.003*,p_2<0.001*,p_3=0.022*			

**: Statistically significant at $p \leq 0.05$*

TABLE (3A) Descriptive statistics of GSH GCF in each studied groups

Groups	Time	GSH GCF					
		Min.	Max.	Mean ± SD	Median	95% CI	
						LL	UL
Group 1	**Baseline**	90.54	135.9	109.5 ±14.28	111.6	102.85	116.22
Group 2	**Baseline**	39.65	55.56	47.79 ± 5.43	47.07	45.25	50.33
	1 month	54.51	89.77	78.91 ± 7.94	79.03	75.19	82.63
	3 months	71.58	108.93	98.26 ± 8.26	98.58	94.39	102.12
Group 3	**Baseline**	30.59	58.92	45.20 ± 9.81	44.22	40.61	49.79
	1 month	50.98	79.56	68.39 ± 7.74	68.60	64.77	72.01
	3 months	65.97	99.57	90.75 ± 8.31	92.56	86.86	94.64

TABLE (3B) Summarizes comparison between the different time periods in each group according to GSH GCF. Both group showed a statistically significant increase in mean GSH GCF measurements at 1 and 3 months.

Table (3c) summarizes comparison between the three studied groups according to GSH in GCF. At Baseline: there was a statistically significant difference in mean GSH in GCF in the three groups. Group 1 group showed a higher GSH in GCF than Group 2 and 3. At 1 months: there was a statistically significant difference in mean GSH in GCF in the three groups. Group 1 group showed a higher GSH in GCF than Group 2 and 3. Group 2 group showed a higher GSH in GCF than Group 3. At 3 months: there was a statistically significant difference in mean GSH in GCF in the three groups. Group 1 group showed a higher GSH in GCF than Group 2 and 3.

TABLE (3B): Comparison between the different time periods in each group according to GSH GCF

	GSH GCF			**F**	**p**
	Baseline	**1 month**	**3 months**		
Group 2	47.79 ± 5.43	78.91 ± 7.94	98.26 ± 8.26	479.154*	<0.001*
p_0		<0.001*	<0.001*		
Group 3	45.20 ± 9.81	68.39 ± 7.74	90.75 ± 8.31	185.606*	<0.001*
p_0		<0.001*	<0.001*		

**: Statistically significant at p ≤ 0.05*

TABLE (3C): Comparison between the three studied groups according to GSH GCF

GSH GCF	**Group 1 (n = 20)**	**Group 2 (n = 20)**	**Group 3 (n = 20)**	**F**	**P**
Baseline	109.5 ± 14.28	47.79 ± 5.43	45.20 ± 9.81	241.421*	<0.001*
Sig	p_1<0.001*,p_2<0.001*,p_3=0.717				
1 month	109.5 ± 14.28	78.91 ± 7.94	68.39 ± 7.74	83.876*	<0.001*
Sig	p_1<0.001*,p_2<0.001*,p_3=0.006*				
3 months	109.5 ± 14.28	98.26 ± 8.26	90.75 ± 8.31	15.726*	<0.001*
Sig	p_1=0.004*,p_2<0.001*,p_3=0.075				

**: Statistically significant at p ≤ 0.05*

DISCUSSION

Oxidative stress is a condition of altered physiological balance within a cell or tissue / organ, defined as "a condition that occurs when there is a serious imbalance between the level of free radicals in the cell and its antioxidant defense [14]. It is estimated that 1-3 billion reactive species are generated / cell / day Given this fact, the importance of the body's antioxidant defense systems in maintaining good health becomes clear [15]. Therefore, the present study determined salivary and GCF levels of one of the main antioxidant enzymes found in the extracellular fluids of the body, (glutathione), in patients suffering from periodontal disease, before and after non-surgical therapy, and compared them with control subjects.

Gingival crevicular fluid (GCF) has been extensively investigated in periodontal disease for the release of host response factors. It has been considered as the main site where oxidant-antioxidant interaction occur [16]. GCF Samples were obtained using filter paper strips inserted into crevice or pocket only for 30 seconds as it was reported that any longer time stimulates GCF flow resulting in dilution of the residual fluid in the gingival crevice [17]. Saliva sampling is simple and non-invasive method of collection, salivary diagnostic tests can provide clear data reflecting the periodontal pathogenesis process.

The reduced and oxidized glutathione levels in GCF of periodontitis patients, can provide evidence that non-surgical therapy was successful in reducing PD significantly when compared to baseline levels [18]. Not only PD, but also PI, GI and CAL were significantly improved in both patients' groups after thorough scaling and root planning. Conventional non-surgical mechanical therapy performed in a quadrant with a time gap of 1-2 weeks between appointments seems to be effective in reducing the bacterial load which leads to significant clinical improvements [19].

The GCF levels of glutathione between the groups, control group showed the statistically significant highest mean of its level. There was no statistically significant difference between diabetic and chronic periodontitis groups; both showed the statistically significant lowest mean glutathione level [20]. The significant difference noted between chronic periodontitis group and control group was similar to that observed by another study [21]. However, in their study, chronic periodontitis patients showed higher glutathione levels when compared to the control which didn't match our results of present study as the control group showed the highest glutathione levels [22]. One possible explanation for the different responses is that patients with periodontal disease can be in different stages of the disease. In this regard, it is known that the antioxidant reactions present in various diseases depend on the severity or the course of the disease in the patient and that chronic long-term diseases can impair the antioxidant defenses [23].

It has been demonstrated that individuals with periodontal disease exhibited a statistically significant increase in glutathione activity [24]. The difference between studies may be attributed to methodology evaluated glutathione activity, while in the present study, total amount of glutathione was estimated. However, relationship between total amount and the enzymatic activity of glutathione needs further investigation [25].

The lower mean glutathione level in the periodontal disease group observed in the current study is consistent with the results of previous studies which showed that patients with periodontal disease had a decreased total antioxidant capacity (TAOC) in the blood and locally within the GCF [26]. In addition, large-scale association studies [27] have shown that patients with periodontal disease have decreased plasma TAOC, which supports the concept that oxidative stress is an important factor in the pathogenesis of periodontal disease. The available data may suggest that compromised local

antioxidant levels in GCF are due to periodontal disease rather than a predisposition to conventional non-surgical treatments such as plaque removal and reducing inflammation, restores the TAOC of GCF to levels comparable with those of healthy control patients. [28]

In the current study, non-surgical therapy resulted in significant improvement in mean levels of glutathione levels in diabetic and periodontitis groups. This is consistent with the previous work [29] evaluated GCF and plasma total antioxidant capacity and found that following reductions in periodontal inflammation with successful non-surgical therapy there was significant improvement in the total antioxidant capacity in chronic periodontitis patients. However, in contrast to their findings mean glutathione levels didn't reach control levels. Similarly, another study[31]found that scaling and root planing increased levels of GSH and total glutathione to levels that were still lower than controls[31]and reported that nonsurgical treatment provided full GSH -Concentrations in GCF does not restore, but does. Restoration of the redox equilibrium (GSH: GSSG ratio), indicating that these changes are secondary to oxidative stress from periodontal disease. However, GSH, GSSG and total glutathione concentration in GCF remain lower than in control patients, which implies a lower buffer capacity against ROS activity in periodontal disease even after successful treatment. [32]

CONCLUSION

Following non-surgical therapy, glutathione levels in diabetic and chronic periodontitis groups improved significantly when compared to base line levels. No significant correlation between glutathione, age and disease activity in diabetic & periodontitis groups. Glutathione levels should be considered a marker for disease and the concentration of reactive oxygen species in human body and an important indicator for the progression of the periodontal treatment in patients with periodontal disease.

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