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Evaluation of Glutathione Levels in Gingival Cervical Fluid in Periodontitis from Stage 1 – Stage 4 among Type 2 Diabetic Patients

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ABSTRACT

Purpose: This study aimed to evaluate if glutathione could be used as a diagnostic biomarker in diabetic patients with stage 1, stage 2, stage 3, and stage 4 periodontitis who were receiving non-surgical periodontal therapy. **Material and methods**: Twenty-eight participants were included, who were separated into four groups, each with seven members. Seven diabetic patients with stage 1 periodontitis were assigned to Group 1, seven diabetic patients with stage 2 periodontitis were assigned to Group 2, and seven diabetic patients with stage 3 periodontitis were assigned to Group 3 and Seven diabetic patients with stage 4 periodontitis were assigned to Group 4. Gingival crevicular fluid (GCF) samples were taken from every individual at baseline (before non-surgical periodontal therapy) and one month after non-surgical periodontal therapy was completed. **Results**: Glutathione levels in the four groups dramatically improved after non-surgical therapy. **Conclusion:** Glutathione levels should be considered important indicator for prognosis of the periodontal treatment in patients with periodontal disease.

KEYWORDS

Glutathione, Periodontitis, Diabetic Patients

INTRODUCTION

Periodontal disease is a multifactorial chronic non-reversible inflammatory disease that affects the supporting tissues of the teeth and is caused by a complex interplay between periodontal pathogens and the host defensive system. Increased leukocyte activity and the generation of cytokines, chemokines, and matrix metalloproteinases begins with

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a microbial infection and advance to host-mediated destruction of periodontal tissues first [1].

There are several risk factors for periodontal disease that include: Smoking, systemic disorders, medications such as steroids and cancer therapy drugs, ill-fitting bridges and loose fillings, pregnancy, and oral contraceptive [2].

Hyperglycemia caused by abnormalities in insulin secretion, insulin action, or both leads to diabetes mellitus [3]. Chronic hyperglycemia in diabetic patients is linked to long-term organ damage, malfunction, and failure. Diabetes is caused by several pathogenic mechanisms [4].

Gingival crevicular fluid (GCF) is one of the least traumatic exploratory procedures for obtaining information on periodontal tissue problems, such as the state of connective tissue and the extent of hard tissue destruction[5].

Glutathione (GSH) is a significant antioxidant (a radical reduced inflammatory process) and one of the most essential redox regulators that control the shape. GSH is a non-protein cell thiol with a low molecular weight that is found both eukaryotic and prokaryotic cells and in every single cell in the human body. Persons with diabetes are three times liable to develop periodontal disease than people without diabetes [6-7].

In this investigation, a test for the biochemical markers glutathione samples from (GCF) for periodontitis in diabetic patients was performed because there is insufficient information on the role of glutathione in periodontal disease prediction.

MATERIAL AND METHODS

This study included twenty-eight articipants divided into four groups 7 participants of each group and selected from the outpatient clinic in Oral Medicine, Periodontology and diagnosis department Faculty of Dental Medicine for Girls AL-Azhar University.

Ethical Considerations

The Ethics Committee of the Faculty of Dental Medicine, Al-Azhar University for Girls, reviewed the study's procedure. **REC-ME-22-12** All subjects were told about the nature ,benefits and risk of their involvement in this study prior to any procedure. Signed consents were obtained from the twenty-eight patients who agreed to participate in the planned research program and experimental design.

Sample Size Calculation

To study the biochemical markers in periodontitis, ANOVA test will be used for comparison between 4 groups. According to a previous study by Grant et al (2010), concentration of total glutathione was 1497±590 in periodontitis patient, in comparison to 2427±501 in control group. A large effect size of approximately 0.73 is expected. Using an actual power (1-β error) 0.8 (80%) and significance level (α error) 0.05 (5%) for two-sided hypothesis test, the estimated sample size was 28 patients (7 in each group). Sample size calculation was performed using G power Statistical Power Analyses Version 3.1.9.2 [8] .

Inclusion criteria for Patients with diabetes mellitus were diagnosed with stage 1, stage 2, stage 3, and stage 4 periodontitis [21,22]. Exclusion criteria Presence of systemic diseases as: autoimmune diseases, liver diseases , chronic renal failure, blood diseases ,history of periodontal treatment in the last 6 months Pregnancy, history of smoking[21].

Grouping

1. **Group 1 :**Seven diabetic patients with stage 1 periodontitis.
2. **Group 2 :**Seven diabetic patients with stage 2 periodontitis.
3. **Group 3 :**Seven diabetic patients with stage 3 periodontitis.
4. **Group 4 :**Seven diabetic patients with stage 4 periodontitis.

Pre-operative assessment:

All participants underwent a thorough examination, which included a medical and dental history as well as a periodontal examination. All participants' periodontal condition was determined by probing pocket depth with a Williams graduated periodontal probe and clinical attachment loss (CAL) [9].

Intervention:

Patients with periodontitis got standard periodontal treatment, which comprised oral hygiene instruction, whole-mouth scaling, and root planing. GCF samples were obtained from each individual at baseline (before treatment) and one month after non-surgical periodontal therapy was finished.

Sample collection:

Contaminated strips were discarded. But strips that ready for laboratory examination was placed in a disposable tube and stored at -40 °C 200uL ,phosphate buffered saline (PH7.4) was added to each tube. The supernatant was utilised to measure reduced glutathione levels after centrifugation at 10,000 x g for 5 minutes (fig. 1).

RESULTS

Statistical analysis was performed by applying One-way ANOVA followed by Post Hoc test for multiple comparisons between different groups, and T test was used to compare the two time intervals within the same group.

The percentage of change was calculated by the following equation:

$$\textit{Percentage of change (\%)} = \frac{\textit{Baseline value - the value after time t}}{\textit{Baseline value}}$$

The negative value of the percentage change means the baseline value changed to a higher value after time t, while the positive value of the percentage change means the baseline value changed to a lower value after time t.

P-value ≤ 0.05 was considered statistically significant (95% significance level), and P-value ≤ was considered highly statistically significant (99% significance level).

Shapiro Wilk test was used for testing the normality of data.

Data were analyzed using the statistical software SPSS (version 23, IBM Co. USA).

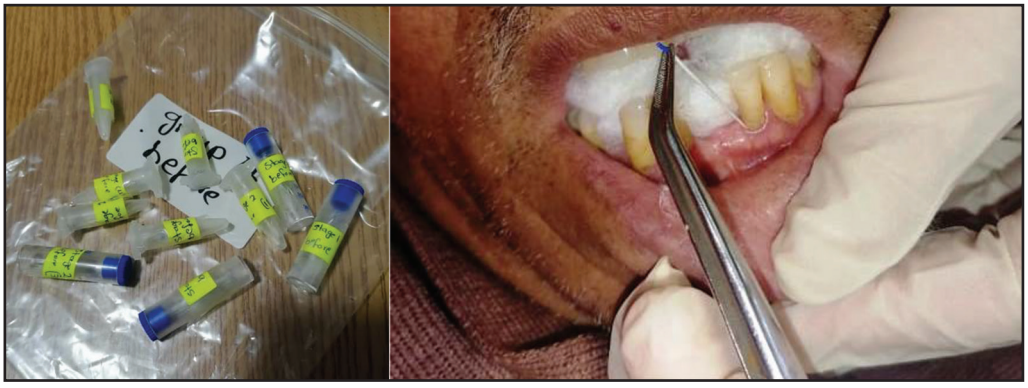

Figure (1): Show strips in disposable tube and how it were taken inside the clinic.

The effect of time on the amount of glutathione (GSH) in the same group (Intra-group comparison):

The First Group before starting therapy, the mean GSH level was (47.12±2.88), which increased to (70.68±1.8) following one month of typical nonsurgical therapy. This rise was highly significant at the 0.001 level (P ≥ 0.001) according to the T-test results. The second Group before starting therapy, the mean GSH level was (48.15±7.88) and increased to (67.01±5.16) after one month of standard non-surgical therapy. This rise was significant at the 0.05 level (P ≥ 0.05), according to the T-test results.

The third Group Before starting therapy, the mean GSH level was (47.33±2.2) and climbed to (63.85±5.37) after one month of standard non-surgical therapy. This rise was very significant at the 0.001 level (P ≥ 0.001), according to the T-test results. The fourth Group Before starting therapy, the mean GSH level was (38.52±4.32), and after one month of typical non-surgical therapy, it was (48.3±1.93). This rise was significant at the 0.05 level (P ≥ 0.05), according to the T-test results.

Comparison of the mean of glutathione (GSH) level in the groups at same time interval (Inter-group comparison) tab (1)

Before therapy (Baseline) The mean GSH level was (47.12±2.88) in group 1, (48.15±7.88) in group 2, (47.33±2.2) in group 3, and (38.52±4.32) in group 4**.**

Although the fact that group 2 has the highest mean GSH level (51.4±8.55), the Tukey post hoc test revealed that there was no statistically significant difference between the groups before starting therapy (the means have the same superscript letter((A)). According to the results of the ANOVA test, the overall p- value for inter-group comparison was not significant before starting medication.

After one month of therapy

The mean GSH level was (70.68±1.8) in the group 1, (67.01±5.16) in the group 2, (63.85±5.37) in the group 3, and (48.3±1.93) in the group 4**.** Even though the fact that group 2 has the highest mean GSH level (73.93±5.12), the Tukey post hoc test found no statistically significant difference between groups one, two, or three (the means all have the same superscript letter (A)), but there was a significant difference between group 4 and the other three groups (the means have different superscript letters). The total p-value for intra-group comparison was 0.001 (P 0.001), suggesting a very statistically significant difference in mean GSH across the four groups, especially between group 1 (highest mean GSH) and group 4 (lowest mean GSH) (the lowest mean of GSH).

Table (1): *Inter-group comparison of glutathione level for all groups before and after one month of therapy.*

	Baseline	1 Month
Group 1	47.12 ± 2.88^{A}	70.68 ± 1.8^{A}
Group 2	48.15 ± 7.88^{A}	67.01 ± 5.16^{A}
Group 3	47.33 ± 2.2^{A}	63.85 ± 5.37^{A}
Group 4	38.52 ± 4.32^{A}	48.3 ± 1.93^{B}
P-value**	0.053^{NS}	0.000^{HS}

-NS =Non significant (P > 0.05

-HS =highly significant (P ≤ 0.001)

*- ** Overall p value for Inter group comparison (ANOVA).*

- Capital letters for Inter-group comparison and the means with different superscript are statistically significant different at P ≤ 0.05 (Tukey Post Hoc test).

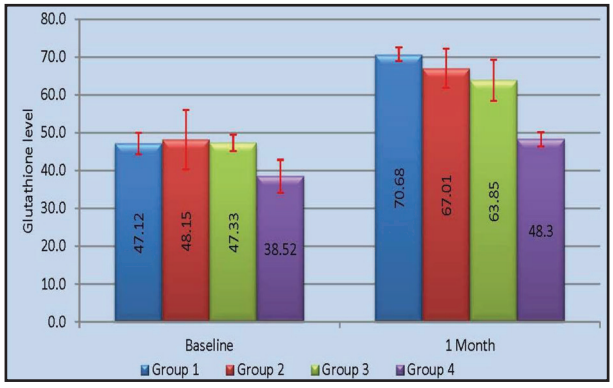

Figure (2): Bar chart shows the mean and SD glutathione level for all groups at different time intervals.

DISCUSSION

The disturbance in the redox balance of the cells and tissues of the innate immune system causes oxidative stress, which leads to periodontal disease. The master antioxidant glutathione is the most significant redox regulator that controls inflammation and thus periodontal disease [11].

As a result, the current study measured GCF levels of one of the most important antioxidant enzymes found in the body's extracellular fluids (glutathione) in periodontal disease patients before and after nonsurgical therapy.

In periodontal disease, the release of host response components from gingival crevicular fluid (GCF) has been widely researched. The key site of oxidant-anti-oxidant interaction is assumed to be here [12].

GCF samples were acquired by inserting filter paper strips into the pocket for only 30 seconds, as any longer period increases GCF flow, resulting in dilution of the leftover fluid in the gingival crevice[13].

The reduced and oxidized glutathione levels in GCF in patients with periodontal disease can provide evidence that non-surgical therapy was successful in reducing periodontal disease significantly when compared to baseline levels [14].

Not only PD but also probing depth and CAL were significantly improved in both patients' groups after thorough scaling and root planning.

Between the groups before and after treatment, the GCF levels of glutathione exhibited the statistically significant greatest mean level. Before therapy, there was no statistically significant difference between groups; both had the statistically lowest mean glutathione level [15].

Another study found a substantial difference between groups before and after therapy, which was identical to this one [16].

However, in their study, glutathione levels were lower following nonsurgical therapy since there was no significant difference between groups before and after treatment, which contradicted our findings, as groups after treatment had high glutathione levels[17].

Their findings are explained in detail. The first is concerned with the amount of proteolytic activity in the gingival crevice/periodontal pocket. In vitro investigations of epithelial cell cultures show that proteolytic enzymes cause GSH to be released from its intracellular position, where it can be catabolized or oxidized to oxidized glutathione. Reduced protease activity in periodontal tissues as a result of biofilm clearance and resolution of periodontal inflammation may lead to lower GSH levels [18].

The current study findings were patients with periodontal disease have a lower (total antioxidant capacity) in the blood and locally within the GCF are consistent with previous findings that showed patients with periodontal disease have a lower (total antioxidant capacity) in the blood and locally within the GCF. Furthermore, large-scale association studies [19].have revealed that individuals with periodontal disease had lower plasma TAOC, supporting the idea that oxidative stress is a key element in periodontal disease etiology. The existing evidence suggests that low local antioxidant levels in GCF are caused by periodontal disease rather than a genetic predisposition to non-surgical treatments such as plaque removal and inflammation reduction, which restores total antioxidant capacity [20].

CONCLUSION

Glutathione levels in the four groups improved significantly after non-surgical therapy compared to baseline levels. In individuals with periodontal disease, glutathione levels should be considered an indication for therapy prognosis.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no conflict of interest.

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