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Original Article

Effect of Psychological Stress on Salivary Cortisol and Trace Elements of Copper, Iron and Manganese in Patients with Periodontitis

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Abstract: Periodontitis can be described as chronic multifactorial inflammatory disease which can be modified by genetic and environment risk factors such as stress. Trace minerals may impact periodontal tissue health by influencing both locally, the hard and soft tissues, as well as systemically, the immune and inflammatory processes throughout the body. This study aimed to assess salivary levels of cortisol and trace elements between psychologically stressed and non-stressed individuals having healthy periodontium and periodontitis. In this study, eighty adult participants were included. Patients completed a stress self-assessment questionnaire by Perceived stress scale and un stimulated saliva was collected to test cortisol levels by ELISA and trace elements levels of copper (Cu), iron (Fe) and manganese (Mn) by inductively coupled plasma mass spectrometry. The study involved four groups: group 1: non stress with healthy periodontium (NSH), group 2: non stress with periodontitis (NSP), group 3: subjects with stress and healthy periodontium (SH), and group 4: subjects with stress and periodontitis (SP). The result showed that SP group had the highest salivary cortisol level (40.56±4.99), followed by SH (39.75±6.28), NSP (15.22±3.09) and NSH (13.66 ± 3.17) in nmol. Moreover, salivary concentrations of Fe (0.549±0.385mg^{L-1}) and Cu (172±85.447μg^{L-1}) were lowest in SP in comparison with other groups, and Mn (45.032±18.565μg^{L-1}) was significantly reduced in SP group compared to NSP group. It was concluded that the impact of psychological stress on trace elements can result in a significant decrease in all elements in saliva and oral health is greatly influenced by dietary practices and an adequate intake of vital vitamins and minerals.

1. Introduction

Periodontitis is an inflammatory disease caused by dental biofilm dysbiosis leading to progressive damage to the periodontal tissues with probing pocket formation, clinical attachment loss, or both [1]. Untreated condition will lead to tooth loss [2].

Dental biofilm is considered as the main cause of periodontitis, while its risk factors (modifiable or non-modifiable) are associated with progression and severity of the disease. The non-modifiable risk factors, also known as determinants, are not easily changed, including genetic factors, host response, osteoporosis, aging, and other systemic diseases [3]. However, modifiable risk factors are typ-

ically behavioral or environmental, for example, smoking, nutrition, alcohol intake, socioeconomic status, and stress [4, 5].

Psychological stress is described as a condition of physiological or psychological tension caused by conflicting stimuli, including emotional, mental, physiological, external, or internal factors, which disrupt the normal functioning of an organism and the organisms typically attempt to avoid [6]. Anxiety or stress is frequently an objective stimulus that might instantly enhance periodontal diseases through various behavioral or biological processes if they become severe and long lasting [7].

Despite numerous experimental, clinical, and epidemiological studies on the relationship between stress and periodontal disease, the exact mechanisms linking these two phenomena remain largely unknown [8], these studies have shown the indirect impact of stress on the periodontium through changes in behavior and lifestyle (food and oral hygiene, smoking, parafunctions, etc.) [9]. More recently, the progress psycho-immuno-endocrinology and the growing interest in the study of stress and its medico psychosocial consequences have made it possible to demonstrate a direct, biological impact of stress on the periodontium by biological mechanisms that will result in a decrease in the patient's immune response and chronic inflammation [10].

Nutrition significantly affects the inflammatory processes and the humoral and cellular immune mechanisms. The nutritional state and the body defense mechanism against the microbial load could be related to the advancement of periodontal diseases [11]. Nutrients can be classified into two main categories: 'macronutrients' (carbohydrates, fats and proteins), which are needed in large quantities from the diet to provide a vital energy source. Whereas, 'micronutrients' (vitamins, minerals, trace elements, and amino acids) are dietary aggregates that do not produce energy but are needed by living organisms and are vital for optimal health, metabolism and proper growth, being crucial cofactors required for the functioning of numerous enzyme systems, such as DNA polymerase, RNA polymerase, superoxide dismutase, alkaline phosphatase, and catalase [12].

The major minerals are calcium (Ca), magnesium (Mg), and sodium (Na). The trace minerals include iron (Fe), copper (Cu), fluoride (F), chromium and manganese (Mn) [13]. Inductive coupled plasma mass spectrometry (ICP-MS) has been used to measure the level of minerals in biological samples such as saliva, providing a quick and precise method for routine multielement analysis with enhanced sensitivity [14]. Stress due to changes in the environmental or working conditions may result in changes in the absorption of trace elements (Fe, Cu, Mn) levels [15].

The trace elements Mn, Cu and Fe are key elements of different antioxidant activity. These micronutrients are crucial for various regenerative processes in the body, helping to combat oxidative stress and support a proper immune response. Deficiency, imbalance, or excess of these micronutrients can lead to a range of diseases. Due to their influence on the functioning of the immune response, these micronutrients have been reported to participate in the interaction between the microbiome and the host. To date, very few studies have analyzed the alterations in salivary micronutrients and the relationship between stress and micronutrients in periodontitis. This study explored the possibility of a potential link between the two in periodontitis patients. Therefore, it is important to explore the impact of stress on salivary trace element concentrations in subjects with varying periodontal conditions to determine if there is a correlation between certain trace elements level in saliva and periodontal health status [16]. The current study was performed to compare salivary levels of cortisol and trace elements (Fe, Cu, Mn) between psychologically stressed and non-stressed individuals with healthy periodontium and periodontitis.

2. Materials and Methods

2.1 Study Design

The study proposal received approval from the Scientific Committee of the College of Dentistry, University of Sulaimani (ethical approval reference number: 59 on 9 /11/2021). Subjects attending Ali Kamal Hospital (psychiatric department) in Sulaymaniyah city, Iraq from November 2021 to June 2022 were recruited. Stress evaluation was performed and study participants were screened and assessed for periodontal disease and inclusion criteria before being invited to join the study. A patient

information sheet explaining the detail of the study was provided before obtaining written informed consent from volunteers.

The inclusion criteria were: subjects with stress and aged 20-45 years old, with or without periodontitis [17], systemically healthy and presence of ≥20 teeth. Periodontitis was defined according to the newest classification of periodontal and peri-implant diseases and conditions, patients involved in this study were stage II periodontitis, either with stress or without stress. The exclusion criteria were: patients with a history of systemic disease or drugs that could affect the periodontal condition, patients with immune suppressant medications, smokers, pregnant, lactating and post-menopausal females, patients with history of antibiotics in the last three months and supplements like Ca, zinc, and vitamins that alter salivary composition [18], patients taking drugs that affect salivary secretion, such as antihypertensive drugs (beta-blockers, calcium channel blockers, diuretic) [19], anticonvulsants (Phenytoin), antihypertensive example (Nifedipine) [19], and receiving periodontal treatment in the prevous 6 months. Subjects were divided into the following four groups:

- No stress and healthy periodontium (NSH): 20 subjects.
- No stress with periodontitis (NSP): 20 subjects.
- Subjects with stress and healthy periodontium (SH): 20 subjects.
- Subjects with stress and periodontitis (SP): 20 subjects.

2.2 Psychosocial Stress Measurement

Questionnaires and cortisol levels were used to evaluate the stress level of both diseased and control subjects. The Cohen's Perceived Stress Scale (PSS) measures subjects' perceived stress in the prior months and ability to cope with it. It consists of a 10-question inventory, with each question rated from 1 to 5 points as outlined below. The higher the score, the higher the level of stress [20]. Scores on the PSS can vary from (0 to 40), as outlined below:

- Scores ranging from 0-13 were considered low stress.
- Scores ranging from 14-26 were considered moderate stress.
- Scores ranging from 27-40 were considered high perceived stress.

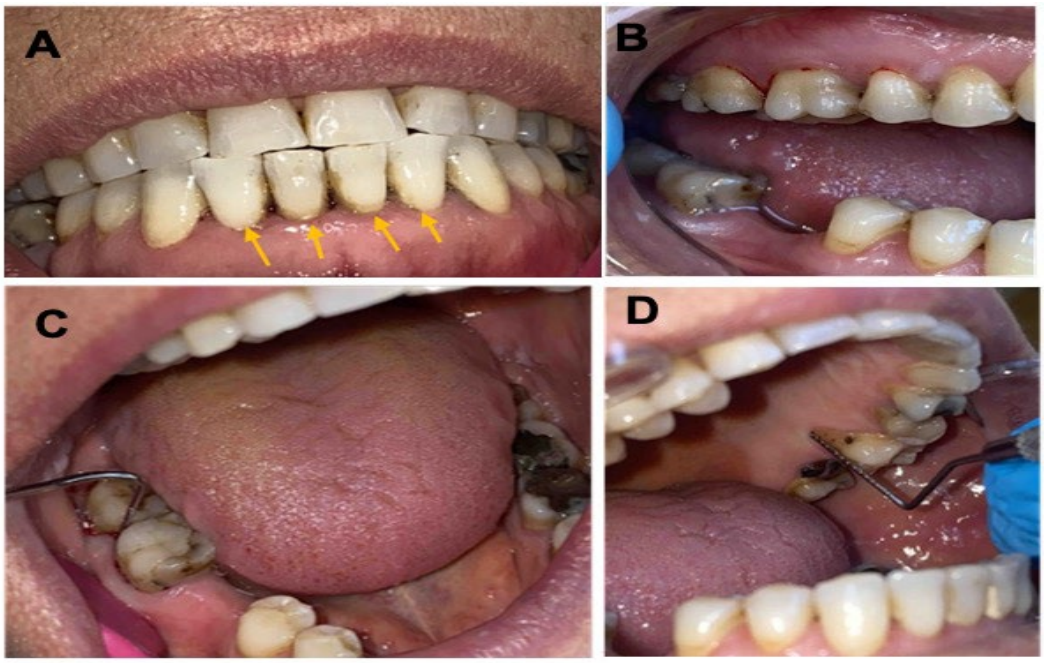

Figure 1: Periodontal examination. **A**: Dental Plaque, **B**: bleeding index of buccal surface of upper teeth, **C**: probing pocket depth measurement and **D**: CAL measurement.

2.3 Periodontal Clinical Parameters

William's periodontal probe was used to perform the full mouth periodontal examination. The periodontal clinical parameters of pocket probing depth (PPD) [21], clinical attachment level (CAL) [22], bleeding index (BI), and plaque index (PI) [23] were recorded as shown in figure 1. PPD was determined by the distance from the gingival margin to the base of the pocket and the distance from the cemento-enamel junction to base of pocket was used to determine CAL. The PI and BI were recorded as present or absent.

2.4 Saliva Collection and Storage

For analyis of the levels of trace elements and salivary cortisol, samples consisting of 5 mL of unstimulated whole saliva were obtained from each subject, using the spitting technique, between 9 am and 11am [24]. Before salivary sample collection, patients were instructed to avoid alcohol consumption for 12 hours prior to the sample collection, and to refrain from consuming food, sugary drinks, and caffeine in the morning of collecting the samples. They were also instructed to refrain from consuming seafood, beverages high in Cu, Mn, and Fe, as well as nutritional supplements and vitamins for 48 hours before the day of the examination. Moreover, enrolled patients were advised to avoid brushing their teeth and using mouth rinses containing Cu, Mn, or iron-based products the night before the sample collection [25]. The subjects were then asked to allow saliva to pool in the floor of their mouth while sitting upright, and to expel saliva into a graduated plastic sterile tube for approximately 5–10 minutes. The collected samples were promptly centrifuged at 4000 rpm for 20 minutes, then frozen at −80 in College of Veterinary Medicine, University of Sulaimani until analysis.

2.5 Measurement of Salivary Cortisol and Trace Elements

The quantitative measurement of salivary cortisol in all the study groups was conducted by ELISA(Teco diagnostics, USA [17] in accordance with the manufacturer's directions. Further, ICP-MS was used to measure the trace elements of Mn, Fe, Cu in saliva [18] in the chemistry lab., College of Science, University of Sulaimani, as described below.

A 200µL aliquot from the supernatant of each sample was diluted at 10mL using a 2.5% v/v nitric acid solution. PerkinElmer Optima 2100 DV ICP-MS was used to determine the levels of Mn, Fe, and Cu. Radio-frequency power was applied at 1300 W, 0.2 L/min, along with 0.8 L/min flow of auxiliary and nebulizer gasses, respectively, while plasma gas was flowing at 15 L/min. The original concentration of each mineral was calculated from the diluted solution using the following equation:

$$C_i = \frac{C_f \times V_f}{V_i}$$

Where Ci and Cf were start and final concentrations, respectively, and Vi and Vf were initial and final volumes.

Ci: Concentration in the supernatant of the sample.

Cf: Concentration measured from ICP-MS.

Vi: Volume taken from the supernatant of the sample (200µL).

Vf: Final volume (10mL).

Element concentrations in saliva were recorded in parts per billion and converted to parts per million.

2.6 Statistical Analysis

The Shapiro–Wilk test was conducted to assess the normality of the data. The differences within variables were assessed by Kruskal Wallis test and the Mann-Whitney U test was employed to find the differences between the two independent groups. Spearman's test was applied to determine the associations between the variables. The significance level was established at $p<0.05$. The SPSS software package (version 22; SPSS Inc., Chicago, IL, United States) was used to analyze the data.

3. Results

Eighty subjects (41 male and 39 female) were recruited amongst 300 subjects screened, according to the inclusion and exclusion criteria. PSS indicated that stressed participants had high perceived stress, either with healthy periodontium and periodontitis, and their mean scores were 34 for SH group and 35 for SP group.

The clinical measures of periodontal disease for all study groups are presented in table 1. The SP patients recorded the greatest PI (0.26±0.05), BI (0.17±0.03), PPD (4.99±0.53) and CAL (3.72±0.49) levels, followed by NSP group (PI= 0.22±0.07, BI=0.13±0.06, PPD=4.41±0.49 and CAL=2.89±0.81). Meanwhile, the healthy periodontitis groups (NSH and SH) recorded low levels of these clinical parameters. Statistically significant differences between the values were observed in the four studied groups for PI (p = 0.000), BI (p = 0.000), PD (p = 0.000), and CAL (p = 0.000).

Table 1: Periodontal clinical parameters of the study groups.

Clinical parameters	Group	Mean	SD	Mean Rank	P value (Kruskal Wallis test)
PI	NSH	0.14	0.02	28.28	0. 0001
	NSP	0.22	0.07	50.25	
	SH	0.15	0.02	20.88	
	SP	0.26	0.05	62.6	
BI	NSH	0.05	0.02	20.42	0. 0001
	NSP	0.13	0.06	51.4	
	SH	0.06	0.01	25.28	
	SP	0.17	0.03	64.9	
PPD (mm)	NSH	1.67	0.40	13.05	0. 0001
	NSP	4.41	0.49	54.48	
	SH	2.44	0.53	27.95	
	SP	4.99	0.53	66.52	
CAL (mm)	NSH	0	0	20.5	0.0001
	NSP	2.89	0.81	54.62	
	SH	0	0	20.5	
	SP	3.72	0.49	66.38	

Cortisol levels were shown to be higher in the SP (40.56±4.99nmol) and SH (39.75±6.28 nmol) groups compared to NSP (15.22±3.09 nmol) and NSH (13.66±3.17 nmol) groups and the differences were shown to be statistically significant between the studied groups (P=0. 0001). Whereas the level of Mn was revealed to be highest in NSP group (192±91.08ug^{-1}), followed by NSH (73.89±9.5 ug^{-1}) and SP (45.03±18.56 ug^{-1}). Similarly, the differences among the studied groups were revealed to be statistically significant (P=0. 0001) (Table 2).

Regarding Cu and Fe, the highest level was detected in NSP group (Cu: 1780±1105ug^{-1}), (Fe: 16.61±8.95 mg^{-1}). Whereas the lowest levels of Cu and Fe were detected in NSH group (45.4±23.52 ug^{-1}) and SP (0.54±0.38 mg^{-1}), respectively. Comparison between the studied groups showed statistically significant differences regarding the levels of both Cu and Fe (p=0.0001) (Table 2).

Table 2: Cortisol and trace element levels among the study groups.

Salivary parameters	Group	Mean	SD	Mean Rank	P value (Kruskal Wallis test)
Cortisol (nmol)	NSH	13.66	3.17	17.8	0.0001
	NSP	15.22	3.09	23.2	
	SH	39.75	6.28	59.62	
	SP	40.56	4.99	61.38	
Mn (µg -1)	NSH	73.89	9.50	29.08	0.0001
	NSP	192	91.08	49.62	
	SH	0	0	0	
	SP	45.03	18.56	12.8	
Cu (µg -1)	NSH	45.4	23.52	11.45	0. 0001
	NSP	1780	1105	70.35	
	SH	218	43.09	44.3	
	SP	172	85.44	35.9	
Fe (mg-1)	NSH	1.77	1.80	33.1	0. 0001
	NSP	16.61	8.95	70.35	
	SH	2.01	1.03	41.45	
	SP	0.54	0.38	17.1	

Table 3 shows the mean differences across all examined variables between each pair of groups. For cortisol, statistically significant differences between all paired groups were noticed ($p < 0.05$), apart from NSH and NSP groups, as well as between the SH and SP groups. Regarding Mn, statistically significant differences among all paired groups were identified ($p<0.05$). Further, Cu concentrations were shown to be statistically significant at level ($p< 0.05$) between all studied groups. Finally, for Fe, the differences among paired groups were statistically significant ($p<0.05$), apart from the NSH and SH groups.

Table 3: Comparison in cortisol and trace element levels between the study groups.

Parameters	Group comparison	Mean Difference	p-value
Cortisol	NSH-NSP	-1.56	0.14
	NSH-SH	-26.08	0.001
	NSH-SP	-26.90	0.001
	NSP-SH	-24.52	0.001
	NSP-SP	-25.34	0.001
	SH-SP	-0.81	0.63
Mn	NSH-NSP	1.30	0.001
	NSH-SH	NA	0.001
	NSH-SP	-3.21	0.001
	NSP-SH	NA	0.001
	NSP-SP	-4.51	0.001
	SH-SP	NA	0.001
Cu	NSH-NSP	-1730.62	0.001
	NSH-SH	-172.78	0.001
	NSH-SP	-126.23	0.001
	NSP-SH	1557.84	0.001
	NSP-SP	1604.39	0.001
	SH-SP	46.54	0.04
Fe	NSH-NSP	-14.84	0.001
	NSH-SH	-0.24	0.17
	NSH-SP	1.23	0.001
	NSP-SH	14.59	0.001
	NSH-SP	16.07	0.001
	SH-SP	1.47	0.001

NA: Not applicable.

The correlations between clinical measures and salivary levels of cortisol and trace elements are summarized in table 4. No statistically significant associations between the clinical and salivary parameters were detected in NSP group. Whereas in SP group, PI and PPD showed statistically significant correlation with Mn and Cu. In NSH group, cortisol level was found to have statistically significant correlation with PI (r = 0.545) and BI (r = 0.516); on the other hand, PPD and Fe also correlated significantly (r = 0.573). Lastly, in SH group, only BI was found to have a significant correlation with Cu (r = -0.491) and Fe (r = -0.539). It is important to acknowledge that as clinical parameters such as CAL were zero in NSH and SH groups, the correlation test could not be performed.

Table 4: Correlation between clinical variables and biochemical parameters of saliva among the different groups.

Study groups	Clinical parameters	Cortisol	Mn	Cu	Fe
NSP	PI	0.14	0.236	-0.304	-0.175
	BI	-0.09	0.219	-0.22	0.212
	PPD	-0.142	0.074	0.232	-0.163
	CAL	-0.202	-0.119	0.274	0.088
SP	PI	0.408	-.546*	-.621**	-0.353
	BI	-0.195	0.273	0.336	0.024
	PPD	-0.028	-.478*	-.449*	-0.15
	CAL	0.281	0.193	0.121	-0.095
NSH	PI	0.545*	-0.085	-0.205	0.172
	BI	0.516*	-0.378	-0.301	0.31
	PPD	0.224	-0.26	0.091	.573**
	CAL	NA	NA	NA	NA
SH	PI	0.017	NA	-0.295	-0.011
	BI	0.162	NA	-.491*	-.539*
	PPD	0.242	NA	-0.17	-0.11
	CAL	NA	NA	NA	NA

**: Correlation was significant at the level 0.01. *: Correlation was significant at the 0.05 level. NA: Correlation could not be computed because CAL was zero.

4. Discussion

This study shows that relation of stress with cortisol and clinical periodontal parameters and the salivary levels of metal ions could reflect on the health and disease status of periodontal tissues. Additionally, it shows how psychological stress can alter the salivary content of micronutrients in periodontitis patients. This might provide new insights in pathogenesis of periodontitis and provide a tool for diagnosis of periodontal disease, which may represent the most important points of the current study.

Stress and anxiety have been recognized as risk factors for several diseases, including those that can alter inflammatory reaction and result in inflammatory diseases [26]. Psychosocial stress, both short-term and long-term, seems to have an impact on low-grade inflammation in humans. There are possibly associations between the Immune reaction to short-term stress and chronic diseases like cardiovascular diseases, type 2 diabetes mellitus, and periodontal disease [27].

The present results revealed statistically significant differences in BI and PI between all studied groups except for groups NSH and SH. SP group, which included subjects having periodontitis (stage II) with stress, showed the highest BI and PI levels in comparison to the other groups. This result was in line with another study [28], and this could be explained by the association between plaque level

and stress and cortisol [29]. It has been observed that stress has a key role in impacting plaque amount and periodontal diseases [30].

Regarding PPD and CAL, a statistically significant difference was noticed among all groups, which is commensurate with another study [31] that reported a good reproducibility for both PPD and CAL, as Psychological stress has been associated with periodontitis and is known to elevate interleukin-1 and matrix metalloproteinase levels in subjects with both diseases [32]. This imbalance in cytokines disrupts the host's immune response and resistance to infection, exacerbating tissue destruction in chronic diseases such as periodontitis [33].

A study indicated that the cortisol level in saliva reflects the 'free' biologically active cortisol and thus it is not influenced by the flow rate of saliva. Ultimately, the salivary cortisol level can be used as reliable source reflecting the free serum cortisol levels. Therefore, it is considered a more convenient tool for assessment in stress research compared to venipuncture, as it can trigger spurious elevation in cortisol secretion, indicating a 'hyper-stress' component [34]. This study's results on comparison of the cortisol levels in saliva across different groups demonstrated statistically significant differences across the groups. However, comparison between NSH and NSP groups or between SH and SP groups showed no statistically significant differences, with the highest level found in SP group. This could be due to the co-occurrence of both diseases (psychological stress and periodontitis), as they might both affect cortisol levels [35]. The high level of cortisol corresponded positively with high scores from the stress scale questionnaires gained, in which the SP group recorded the highest score, followed by SH, NSP, and NSH group. This study agrees with the finding by Obulareddy *et al.* [36] that periodontitis subjects had greater mean salivary cortisol levels, thus justifying the exploration of saliva as a prospective medium for evaluating biomarkers related to periodontal disease and stress.

Mn is crucial for maintaining bone health, including the development and upkeep of bone structure [11]. It is also incorporated into antioxidant superoxide dismutase enzyme, which protects tissue against free radicals that contribute to periodontal tissue destruction in periodontitis [37]. Elevated levels of Mn were identified in group NSP in this study, which could potentially be the cause of bone resorption found in periodontitis patients. Even though there is a lot of Mn in bone tissue, only a limited amount is immediately exchanged in the extracellular compartment. Thus, the level of Mn can detected in the gingival crevicular fluids and the extracellular space following destruction of the alveolar bone tissue [38]. Additionally, it has been noted that Mn amount tends to rise in proportion to the severity of the inflammatory disease. Higher salivary Mn content may show the destructive inflammatory action within the periodontal tissue since the volume of gingival crevicular fluid increases as inflammation intensifies and periodontal damage worsens [39]. The finding in the current study is in agreement with another study [16]. Meanwhile, in the stressed groups, the Mn levels were decreased, which may have been caused by the effect of stress and this is in agreement with other studies [40, 41].

Fe plays a key role in erythropoiesis and hemoglobin production; maintaining proper levels of this mineral is essential for health of periodontium [42]. Elevated concentrations of free Fe might play a role as pro-oxidants and in catalyzing Fenton reaction, resulting in the generation of reactive oxygen species by neutrophils and macrophage cells. This, in turn, leads to stimulation of matrix metalloproteinase and the secretion of pro-inflammatory mediators that are related to alveolar bone resorption and periodontal tissue destruction [11].

In this study, the highest level of Fe was found in NSP group in comparison to other groups and this is in line with another study [43] which found an elevated level of Fe in the extracellular environment. Furthermore, Fe has been reported to take part in regulation of the maturation and pathogenicity of anaerobic bacteria that are considered as key pathogens associated with periodontal diseases, such as *Treponema denticola, Porphyromonas gingivalis* and *Prevotella intermedia* [44, 45]. Meanwhile, a positive association has been demonstrated between increased concentration of hemoglobin in saliva and extent of inflammation of gingiva, periodontal clinical parameters, and bone destruction [46]. Due to the effect of physiological stress, salivary level of Fe was elevated in the SH group compared to NSP group, but despite that increase, the difference between them was statistically insignificant. The reabsorption of water and excretion of a higher levels of Fe in saliva might be behind the

elevated level of minerals [47]. On the other hand, the SP group recorded the lowest levels of Fe, which aligns with other results that have also showed reduced ferritin levels in patients with anxiety and depression disorders [48, 49].

Cu is a component of various enzymes and proteins that act on regulation of lipid and Fe metabolism, as well as connective tissue formation. Cu is an essential mineral in immune function and antioxidant control. Since it converts superoxide to hydrogen peroxide and hydroxyl radicals, elevated Cu level is associated with oxidative burst and consequently leading to damage of periodontium [50]. Increased serum Cu level disrupts collagen turnover and impairs immune mechanisms against infections by affecting neutrophil function, proliferation, and antigen-specific antibody synthesis, thereby raising the risk of periodontal disease [51].

The present study's results on salivary levels of Cu were in line with other studies [52, 53] that observed the highest level of Cu in group NSP compared to other groups. This finding might point to a protective reaction against the oxidative damage brought on by periodontal inflammation [18]. Comparison of this result with SP group showed that Cu level was decreased more than in NSP group, which was in agreement with another study that analyzed levels of Cu in patients with depression in comparison to healthy people and reported low levels of Cu [54].

Regarding correlations between clinical periodontal examinations and salivary markers, there were positive correlations between cortisol level and periodontal clinical parameters among the studied groups, which again emphasizes the role of cortisol in periodontal diseases. It is important to state that the reason for the reduction of mineral levels in subjects with stress is that stress might cause stomach acid to decrease, which can result in ineffective nutritional absorption, low levels of vitamin B12, vitamin C, Mg, folate, and other micronutrients, as well as Fe absorption problems [55]. Additionally, the levels of trace elements can be affected by diet, place of residence, lifestyle changes, time, and sampling method [56].

This study did not assess salivary flow rate or salivary pH which are factors that might affect saliva secretion. At the same time, psychological stress has been considered as negatively influencing the health of periodontium. Other limitations of this study, such as the number of patients, follow up after complete periodontal treatment, including gingivitis patients, duration of stress, quality of the patient life, jobs, sex, and etc…. can be considered in future studies. However, possible mechanisms for clarifying the connection between periodontal disease and stress have yet to be examined. Nevertheless, a strong point of this study is its focus on exploring the impact of stress on trace elements in patients with periodontitis.

5. Conclusions

The result of this study suggests a strong association between periodontitis and psychological stress, as reflected by the higher level of salivary cortisol in periodontitis and stress groups. In addition, psychological stress decreases Cu, Mn and Fe levels in saliva of stressed patients and these changes in inorganic trace elements due to stress may impact the periodontal tissue health and oral mucosal tissue. Future clinical longitudinal studies are necessary to find the impact of periodontal treatment on cortisol, Mn, Cu and Fe levels and explore their roles in both periodontal health and psychological stress.

Author contributions: Nakhshaw Ahmed: Conceptualization, methodology, project administration, **Shahram H. Aziz**: Software, validation, visualization, **Rebwar Hama Gharib:** Investigation, methodology, supervision., **Shokhan H. Azeez:** Writing – review & editing, supervision

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