

## **Assessment of Salivary Micronutrient Levels in Healthy and Chronic Periodontitis**

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### **ABSTRACT:**

**Introduction:** Periodontal disease is an immune inflammatory disease of the periodontal tissue which is characterised by the destruction of bone and connective tissue attachment loss. The risk factors include diabetes, smoking, diet and host response. Adequate nutrition is required for maintaining periodontal health as well as for various biochemical activities. Deficiency of these micronutrients like iron, copper, zinc etc, could severely affect the periodontal health. Saliva on the other hand can be used as a resourceful material in obtaining clinical information related to oral and systemic health as it contains various biomarkers of inflammation and bone loss in periodontitis.

**Aim:** To assess the salivary micronutrient levels in healthy and chronic periodontitis

**Materials and methods:** The study was a case control study comprising 20 subjects, 10 in each group. Group 1 comprises 10 systemically healthy and without chronic periodontitis and group 2 comprises 10 systemically healthy and with chronic periodontitis and the salivary micronutrient zinc, copper, iron levels were evaluated by standard methods as per the manufacturer's instructions.

**Results:** The salivary micronutrient zinc, copper, iron levels are significantly reduced in chronic periodontitis than in healthy individuals.

**Conclusion:** Within the limitations of the present study, it can be concluded that there is a significant reduction in salivary micronutrient levels of iron, copper and zinc in chronic periodontitis when compared to healthy individuals

**Key words :** Chronic Periodontitis, Salivary micronutrients , Zinc , Copper, Iron, ELISA, Novel method, Innovative method.

### **INTRODUCTION:**

Chronic periodontitis is an inflammatory disease of teeth and it's supporting tissue which is caused by microorganisms which can progressively cause destruction of periodontal ligament, alveolar bone and also results in the formation of pocket and also recession of bone [1]. It is very commonly seen in the general population which affects the quality and systemic health of life. This happens when the bacterial mass tries to accumulate over the tooth surface and even below the level of gingiva [2]. The thin film of bacteria which forms plaque which advances and becomes harder extends below the gingiva which can cause injury to the soft tissues and periodontium [3].

Tissue destruction in periodontitis causes breakdown of the collagen fibres of the periodontal ligament, leading to the formation of periodontal pockets between the gingiva and the tooth [4][5]. Periodontal disease occurs in childhood, adolescent, early adulthood where the destruction of tissue and the simultaneous loss of tooth increases when there is an progression of age. Factors affecting periodontitis are the environmental conditions of periodontium and the system factors such as age, smoking, genetics, medications such as steroids contribute to it [6]. The commonly found symptoms could be swollen, red,

tender gingiva and bleeding of the along with gingival recession, drifting of teeth, periodontal pockets, tooth loss, pus and foul breath smell [7].

Nutrition has a significant role in the process of inflammation and also in cell mediated and humoral mediated immune mechanisms. The main factor in the progression of periodontal disease could be the interactions between the immune response of our body to the bacteria and nutritional status [8]. Normal diet contains both micro and macronutrients. Micronutrients are required in milligram to micrograms which broadly includes vitamins and minerals. The micronutrients have a diversified action in metabolism and functions for human health and also helps in proper functioning of enzymes like DNA polymerase, RNA polymerase [9].

Saliva on the other hand can be used as a resourceful material in obtaining clinical information related to oral and systemic health as it contains various biomarkers of inflammation and bone loss in periodontitis [10]. When there is a qualitative and quantitative change in the composition of these biomarkers like enzymes, immunoglobulin, ions etc., all these could indicate the beginning of periodontal disease and a proper diagnosis and treatment of it must be done [11]. And the components from blood pass through active transport and passive diffusion into saliva, and can be used up easily for the analysis. In this study, the micronutrients zinc, copper, iron are analyzed because they are components of the various antioxidant enzyme systems. These micronutrients are important in the regenerative process in our body which also helps to maintain a balanced immune response. Our team has extensive knowledge and research experience that has translated into high quality publications[12–24].[12–24],[25–29] [30] [31].

Zinc is primarily chosen as it is required for synthesis of various proteins and proliferation of cells, deficiency of zinc will cause reduced collagen synthesis which will disrupt cellular integrity. Copper is a known member of iron and lipid metabolism, connective tissue synthesis and immunological reactions. So any change in the normal range can cause increased oxidative stress and can increase the periodontal destruction. And iron which is present in haemoglobin can cause anemia which could deteriorate periodontal health. Thus the aim of the study was to compare the salivary micronutrients levels between chronic periodontitis and in healthy individuals.

## **MATERIALS AND METHODS:**

The study was a case control study comprising 20 subjects, 10 in each group.

Group 1: 10 subjects was healthy controls

Group 2: 10 subjects with chronic periodontitis

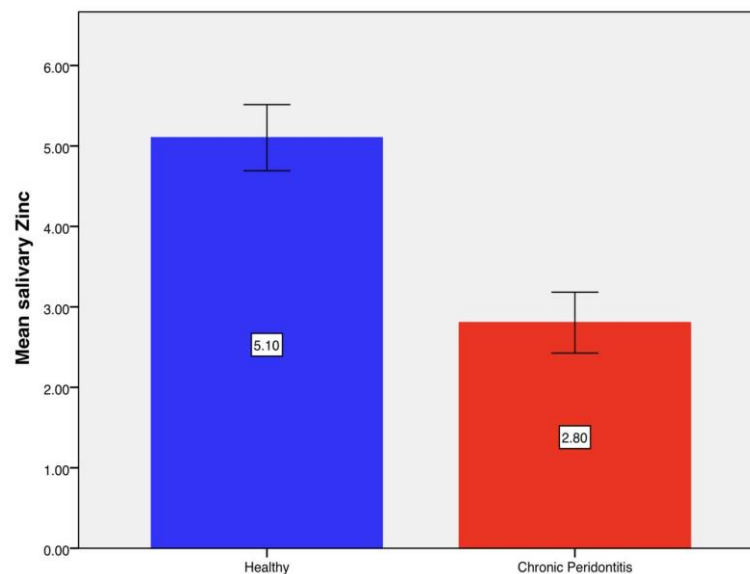
Inclusion criteria were subjects with chronic periodontitis with bleeding on probing, probing depth > 4mm, clinical attachment loss was found.

Gender, pregnant and lactating women, systemic disease and immunocompromised were excluded from the study.

Evaluation of zinc, copper and iron in saliva:

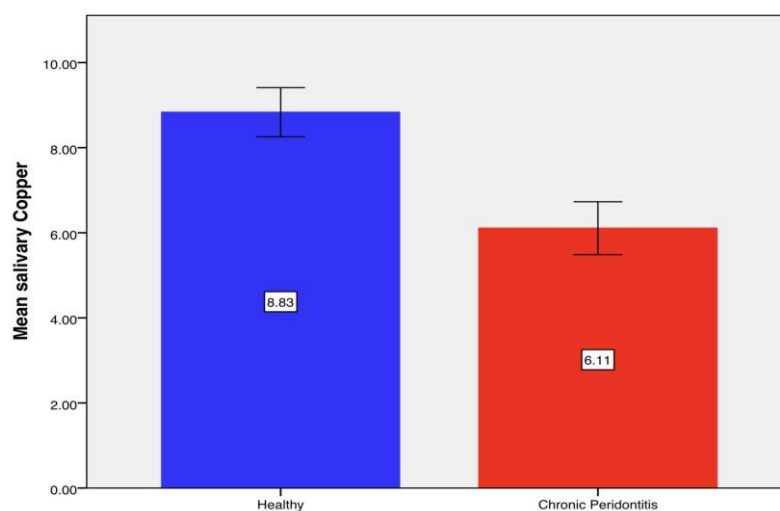
5 ml of unstimulated saliva sample was taken from the subject. The sample was centrifuged at 3000 rpm for 15 min and the supernatant serum was collected. Estimation of micronutrients levels were done using standard methods as per the manufacturer instructions. The collected data was tabulated in Microsoft Excel and imported into SPSS software version 2.0. Chi-square test and Pearson correlation analysis were used for the data analysis, with a p-value less than 0.05 to be statistically significant.

## RESULTS:



**Figure 1:** Graphical representation of salivary micronutrient zinc distribution of the study participants

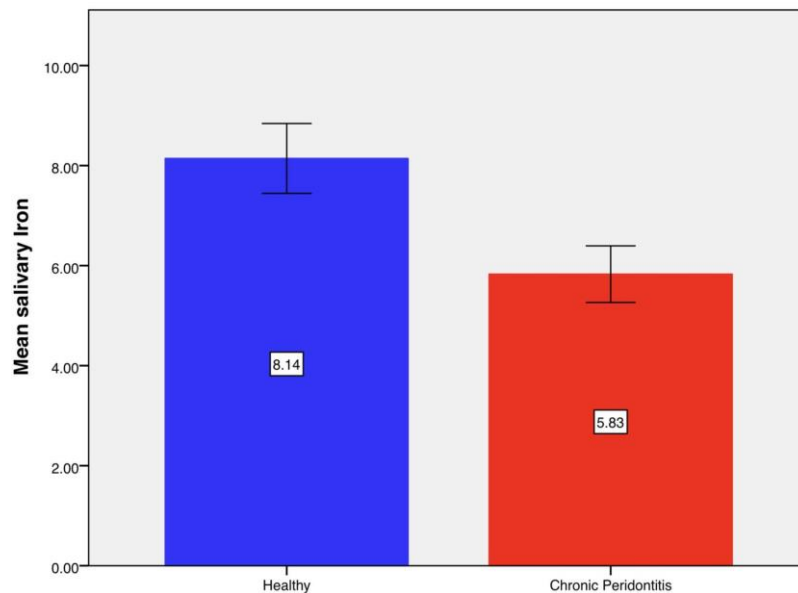
Figure 1 represents the salivary micronutrient nutrients levels in healthy and chronic periodontitis. The X-axis represents the healthy and chronic periodontitis population and Y-axis represents the salivary micronutrient zinc levels. Blue colour represents healthy individuals and Red colour represents chronic periodontitis. With the mean score of salivary micronutrient zinc 5.10 in healthy individuals and 2.80 in chronic periodontitis pcos. However this is statistically not significant with chi-square value- 5.103 and p-value =0.0013 (p-value > 0.05) hence significant.



**Figure 2:** Graphical representation of salivary micronutrient copper distribution of the study participants

Figure 2: Represents salivary micronutrient nutrients copper levels in healthy and chronic periodontitis. The X-axis represents the healthy and chronic periodontitis population and Y-axis represents the

salivary micronutrient copper levels. Blue colour represents healthy individuals and Red colour represents chronic periodontitis . With the mean score of salivary micronutrient copper 8.83 in healthy individuals and 6.11 in chronic periodontitis. However this is statistically not significant with chi-square value- 8.834 and p-value =0.02 (p-value > 0.05) hence significant.



**Figure 3 :** Graphical representation of salivary micronutrient iron distribution of the study participants

Figure 3: Represents salivary micronutrient nutrients iron levels in healthy and chronic periodontitis. The X-axis represents the healthy and chronic periodontitis population and Y-axis represents the salivary micronutrient iron levels. Blue colour represents healthy individuals and Red colour represents chronic periodontitis . With the mean score of salivary micronutrient copper 8.14 in healthy individuals and 5.83 in chronic periodontitis. However this is statistically not significant with chi-square value- 8.301 and p-value =0.0021(p-value > 0.05) hence significant.

## DISCUSSION:

From the above graphs it's clear that there is a significant reduction in salivary micronutrients copper, iron and zinc levels in chronic periodontitis individuals than in healthy individuals. Micronutrients like zinc, copper and iron play a major role in cell regeneration and also to cope up with oxidative stress and also in immune mediated response. So these minerals are essential for maintaining a healthy life. These micronutrients can cause diseases through deficiency, imbalance, or toxicity. The results of the present study shows that mean salivary zinc, copper, iron levels were reduced in individuals with chronic periodontitis. Similar results were obtained in a study done by Inonu E 2020 et al, where there was a significant reduction in the mineral content [32].

Zinc has antioxidant properties as it stabilizes the cell membrane structure, contributing to the structure of the superoxide dismutase and maintaining the metallothionein tissue concentrations. Hence long term deficiency of zinc can promote pathological tissue injury due to oxidative stress. (Figure 1) The results of the present study shows that mean salivary zinc levels were reduced in individuals with chronic periodontitis. Similar results were obtained in a study done on assessing the micronutrient zinc levels in healthy and chronic periodontitis individuals where there was an decreased serum zinc levels in individuals with periodontitis when compared to healthy individual[33,34] but another research with

similar parameters had a contrasting result of no change in the micronutrient levels[9]. Studies have shown that increased copper and iron levels and decreased zinc levels can act as contributing elements in causing inflammatory reactions[35]. So reduced intake of zinc may lead to suppressed immunity with an increase in oxidative stress and poor regenerative capacity in people which can predispose periodontitis. The micronutrient levels in saliva in patients with chronic periodontitis was compared with pre and post non-surgical periodontal therapy, the results were obtained by comparing the baseline parameters where there was a significant reduction in micronutrient zinc levels when compared to the test and control group and there was a significant increase in zinc post treatment[36]

(Figure 2) The results of the present study shows that mean salivary copper levels were reduced in individuals with chronic periodontitis. Similar results were found in a study done by Freeland 1976 et al where the salivary micronutrient copper was reduced in patients with chronic periodontitis[9,37]. Certain studies have shown an increase in serum copper levels in individuals with periodontitis when compared with healthy individuals. A study also showed increased serum copper levels in individuals with periodontitis. Copper can modulate immune function and antioxidant status. Studies on animal models have shown that increased serum copper levels reduce several aspects of immune response, including neutrophil numbers, lymphocyte proliferation, and antigen-specific antibody production. It has also been reported that elevated serum copper levels alter collagen metabolism and hence can promote periodontitis[38]. Excessive copper and iron in serum may promote development and progression of oxidative stress, altered immunity and altered insulin secretion or its action. [39,40]

(Figure 3) The results of the present study shows that mean salivary iron levels were reduced in individuals with chronic periodontitis. Similar results were found in a study done where there is an increased iron levels in patients with chronic periodontitis [41] and they suggested that increased iron concentrations were caused by increased hemoglobin levels with dental plaque and inflamed periodontal tissue. Difference in salivary iron levels can be useful in identifying the severity of periodontal disease. But salivary iron levels can be affected by gingival bleeding due to gingival inflammation [42].

The study was geographically limited and predominantly consisted of the South Indian population. So the limitation of the study is uni centric with a limited demographic area of smaller sample size. By investigating the cause and prevalence of chronic periodontitis and its association with micronutrients and also its association with the pathological conditions as this might help in broadening the existing knowledge about chronic periodontitis and its association with micronutrients. The present study shows the importance of salivary micronutrients in periodontal disease and his study is primarily limited to particular cross sectional areas so a larger sample size could have been taken to assess the relationship between the salivary micronutrients in healthy and periodontal individuals.

## **CONCLUSION:**

Based on the present study, it could be concluded that there was a significant reduction in salivary micronutrient levels of iron, copper and zinc among chronic periodontitis when compared to healthy individuals. zinc, copper, iron are analyzed because they are components of the various antioxidant enzyme systems. In this study the zinc values were found to have increased in healthy when compared to chronic periodontitis. Similar results were found in iron and copper where the values were increased in healthy individuals. This cross sectional study shows that these micronutrients play a significant role in maintaining periodontal health. Further longitudinal study has to be conducted to evaluate the future scope in having pre and post surgical and non surgical therapy.

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**CONFLICT OF INTEREST:**

The authors declare no conflict of interest.

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