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# The Effect of Nutrients and Their Role in the Progression of Periodontal Disease

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**Abstract**

Periodontal disease is a common disease and may affect 90% of people, and is classified into gingivitis and periodontitis. The objective of this study was if the deficiency of some vitamins and trace elements affects the increase in exposure to periodontal diseases or to find out whether the periodontal disease can cause changes in the blood level of some nutrients and their role in reducing periodontal diseases. For this purpose, samples were taken from the University of Baghdad, College of Dentistry, Department of Periodontics, in the Periodontics clinic, including a group of 40 patients' periodontitis, as well as another 40 patients' gingivitis, and 40 healthy periodontium as control. There was no overall sickness in any of the groups. Periodontal clinical data were recorded, and serum parameter levels were assessed. The findings showed that plaque index, bleeding on probing, probing pocket depth, and clinical attachment loss were significantly greater ($P< 0.01$) in patients compared to the control. Levels of vitamin C and D were significantly decreased in patients compared with control ($P<0.01$), while levels of copper were high significant increase) in the patients compared to the control, and zinc show a highly significant increase in the periodontitis group. A negative correlation was found between vitamin D with BOP and PLI, in conclusion, low levels of C and D are associated with a risk factor for periodontal disease and contribute to the development of the disease, and high levels of copper and zinc play an important function as an indication of periodontal disease.

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## 1. Introduction

Periodontal disease is a serious public health problem [1] including gingivitis and periodontitis [2], gingivitis is an inflammatory disease that only damages the soft tissues of the periodontium [2], on the other hand, periodontitis, is a prolonged inflammatory disease, that damage the soft and hard tissue of the periodontium including gingival tissues, periodontal ligament, cementum, and alveolar bone are all damaged, it will lead to tooth loss if not treated [3[[4], periodontitis affects up to 50 percent of the world population [3], The primary cause of dental diseases is biofilm bacteria [5], bacteria cause a wide range of diseases in humans, including bloodstream infections [6]. Vitamin C, vitamin D, and zinc all these nutrients play a role in the immune system, and their barriers according to European Food Safety Authority (EFSA) [7], vitamin C (ascorbic acid) is water-soluble in addition to being a strong antioxidant [8, 9], and has play reduces reactive oxygen species (ROS) and acts as an anti-senescence agent [10], as a result of inflammation, the tissue's antioxidant rates will be decreased quickly, and the generation of free radicals increases around the inflammatory site in specific inflammatory diseases such as periodontal diseases [11], it is reported to have an adverse effect on periodontal health, with an indirect relationship between periodontitis and plasma vitamin C levels [11], whereas antioxidants lower the risk of all chronic diseases [12]. Vitamin D is a type of secosteroid hormone. [13] has anti-inflammatory and antibacterial properties through Cathelicidin (LL-37) production, and also the innate immune regulator [14, 15], which alters immune cell cytokine secretion and increases macrophage, and monocyte cells which release antimicrobial peptides [14], and has been high cytokine levels have been linked to several chronic inflammatory diseases also with low levels of vitamin D [16, 17], also vitamin D is necessary for bone strength, which promotes bone health and calcification including alveolar bone [14, 18], One billion individuals worldwide are at risk of vitamin D deficient [19]. Zinc (Zn) plays role in the production of DNA, RNA, and proteins [20, 21], and also has an impact on the functional ability of cells in the innate and adaptive immune systems [20], Zn influences the generation of cytokines, the activity of the complement system, and antibody formation [20], as well as catalyzing the activation of over 300 enzymes [21], also has a role in the removal of bacterial toxins, as well as being a key component of periodontal dressings [22], while on the other hand, excess Zn can reduce immune response by reducing T- and B-lymphocyte function and reducing intracellular pathogen elimination in macrophages [23]. Copper (Cu) plays an essential role in DNA integrity preservation by reducing oxidative DNA damage [21]. Cu has a role in maintaining an optimal immunological response in addition to preventing oxidative stress [24]. An unbalance of Zn and Cu in the blood can cause a person to have chronic periodontitis [24], sufficient Cu levels are essential to avoid the development of inflammatory pathways and the production of normal connective tissue [24], while increased Cu levels in periodontitis patients' serum can enhance changes in collagen metabolism and potentially connective tissue damage [24], Cu rise in serum with situations of inflammation could be due to the varied effect of stress states [25]. Zn and Cu are required by many enzymes and proteins in humans. Both Zn and Cu are essential for the functioning of the antioxidant enzyme copper-zinc superoxide dismutase [21]. The purpose of this study is to assess the levels of vitamins C, D, Cu, and Zn with periodontal disease and the extent of their relationship with periodontal disease as an increase or decrease and their effect on the development of periodontal disease, most of the previous studies dealt with the subject from one side, all previous studies include an evaluation just trace elements or just vitamins C or D, in addition to all the previous studies, they found an increase in copper and a decrease in zinc this is a contrary result of previous studies, but the result of the current study founded increase levels in both zinc and copper levels, also in previous studies related to the assessment of vitamin D levels founded to increase and decrease with periodontitis in the same study, but in this study founded all groups have insufficient vitamin D until the healthy control group, in addition, almost studies consist of a periodontitis group with healthy control group, while this study includes all groups of periodontal disease ( periodontitis group, gingivitis group, and healthy control group), the studies that dealt with the evaluation of vitamin C in the blood are limited, most of the studies were evaluated in saliva , tissue, and gingival crevice fluid, Finally, a new classification of the disease was made in 2018 after the disease was divided into chronic and acute. The disease was divided into stages and grad depending on the severity, and this affected the results of the parameters.

## 2. Materials and Methods

### 2.1. Study Design

The case-control study comprised 120 patients include with 40 patients 20 males and 20 females in the gingivitis group with a range of 20-45years, 40 patients 20 males and 20 females in the periodontitis group with a range of 20-50 years, 40 healthy periodontium (control group) 20 males and 20 females in the control group with a range of 21-48 years. This research was conducted at the University of Baghdad, College of Dentistry, Department of Periodontics, in the Periodontics clinic from October 2021 to April 2022, Furthermore, a verbal and written agreement was obtained from all participants in this research.

### 2.2. Inclusion Criteria

The cases that have been taken are moderate gingivitis and severe gingivitis, as well as generalized periodontitis, and healthy periodontium.

### 2.3. Exclusion Criteria

The cases that were excluded are a previous history of systemic disease, either smoked or drank alcohol, pregnant or lactating women, menopausal women, anyone who has received Anti-inflammatory or systemic antibiotics in the past 3 months, persons who have received any periodontal therapy such as deep scaling and root planning or local antimicrobial treatment inside the previous last 3 months, stable periodontitis, patients wearing orthodontic appliances, intake of supplements, and mild gingivitis.

### 2.4. Collection and Storage of Blood Samples

Blood samples were obtained from periodontal disease subjects and a healthy control group and centrifuged to isolate them. samples were kept at -40° C till they were tested. Competitive ELISA (Enzyme-Linked Immunosorbent Assay) kits were used to assess the serum concentrations of vitamin C (MyBioSource-USA), this technique is utilized to detect antibodies against a specific antigen by strong antibody-antigen interactions [26], vitamin D (Roche- Germany) Zn, Cu (Spinreact- Span).

### 2.5. Determination of Vitamin C

Vitamin C levels were assessed by using the competitive -ELISA principle.VC-HRP conjugate and polyclonal anti-VC antibody were utilized. The test sample serum and buffer were incubated with the VC-HRP conjugate for one hour in a plate that has been pre-coated, then incubated, the wells were removed and 5 times washed. The wells were then incubated with HRP enzyme substrate. The enzyme-substrate reaction produced a blue chemical, which was subsequently stopped using a stop solution, as a result of which the solution turned yellow. At 450nm, color intensity is evaluated spectrophotometrically in a reader for microplates, Because the number of sites is restricted, the color intensity was negatively proportional to the VC content, as the sample's VC occupied more sites, there were fewer locations available to bind the VC-HRP conjugate. the standard curve was constructed that connects color intensity (OD) to standard concentration.

### 2.6. Statistical Analyses

The SPSS statistical program (statistical package for social sciences) software, version (26) to analyze the data. The outcomes of this investigation were evaluated using one-way ANOVA. after normally distributed results were as mean ±SD. Pearson's correlation (r-correlation) was utilized to compare vitamin C levels., vitamin D, Zn, Cu, and periodontal disease. Data were considered significant at $P < 0.05$.

## 3. Results and Discussion

### 3.1. Demographic Features in the Current Study

The findings found no statistically significant differences in age, and body mass index (BMI) between periodontal disease patients and healthy control groups as shown in Table 1.

**Table 1:** Characteristics features of periodontal disease and healthy subjects.

| *Parameters* | *Healthy group mean ± SD* | *Gingivitis group mean ± SD* | *Periodontitis group mean ± SD* | *p-value* | *Sig.* |
|---|---|---|---|---|---|
| *Age (Year)* | 32.13±7.219 | 31.8±7.014 | 35.8±9.065 | 0.95 | NS |
| *BMI (Kg/m$^2$)* | 23.6320±2.02851 | 25.0027± 3.32157 | 25.0163±4.07623 | 0.173 | NS |

SD: standard deviation, NS: Non-significant.

### 3.2. Demographic Features of Periodontal Parameters

The result showed highly significant elevation in all periodontal parameters between the periodontitis group compared to the gingivitis group, and also as between the periodontitis group with the healthy control group, and showed highly significant elevation between gingivitis group with the healthy control group in all periodontal parameters, the periodontal parameters include bleeding on Probing (BoP), Plaque Index (PLI), Clinical Attachment Loss (CAL), and Probing Pocket Depth (PPD) as mention in Table 2 and Figure 1.

**Table 2:** Clinical features of periodontal disease and healthy subjects.

| *Periodontal Parameters* | *Healthy group mean ± SD* | *Gingivitis group mean ± SD* | *Periodontitis group mean ± SD* | *p-value* | *Sig.* |
|---|---|---|---|---|---|
| *Bop* | 4.995±2.00025 | 37.075±10.93896 | 30.155±11.6829 | 0.01** | HS |
| *PL I* | 0.883±0. 3052 | 1.500±0.5085 | 2.500±0.6823 | 0.01** | HS |
| *CAL* | - | - | 5.4575±0.9018 | 0.01** | HS |
| *PPD* | - | - | 4.8350±0.8480 | 0.01** | HS |

SD: standard deviation, ** High significance at P≤ 0.01 level.

The results in Table 2 agree with these results [27-29], the report of this study was PLI and BOP reduced after treatment [30], also displaying significant differences in the mean of BOP, PLI, PPD, and CAL between gingivitis, periodontitis, and healthy control group [31], which is PPD, and CAL occurs with only periodontitis patients found in the clinical diagnosis.

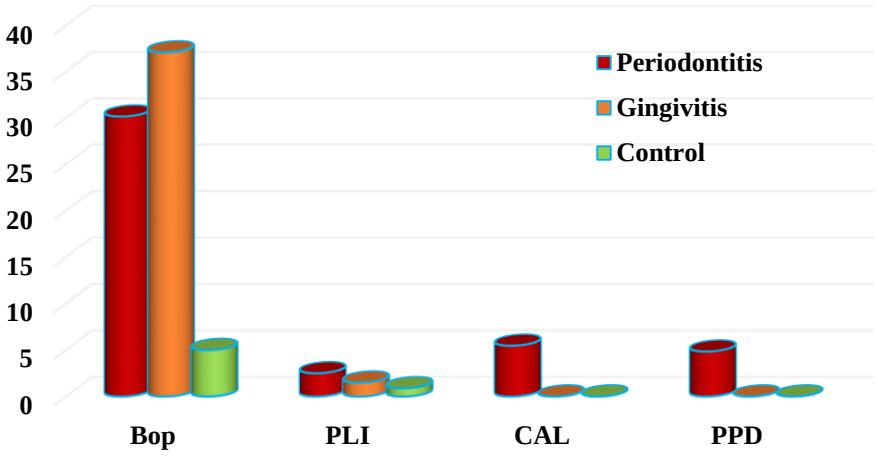

**Figure 1:** Mean values of PLI, GI, and BOP in periodontal diseases and control groups.

### 3.3. Vitamin C and D Levels in Periodontal Disease Patients and Healthy Group

This result found in the levels of vitamin C shows a highly significant decrease ($p < 0.01$) between periodontitis groups (3.7686±0.5968 ng/mL) compared with the healthy control group (4.8320 ±1.0166 ng/mL), and between the gingivitis group (3.9734±0.6575 ng/mL) to the healthy control group (4.8320 ±1.0166 ng/mL), but they found was no significant difference ($p > 0.05$) between periodontitis group (3.7686±0.5968 ng/mL) with the gingivitis group (3.9734±0.6575 ng/mL) as shown in Table 3 and Figure 2. Vitamin C is an important vitamin play has many functions in immunity, as well as important it is located within the components of the gingiva tissue, so it is necessary to protect the tissues and give them strength in the fight against pathogens of periodontal disease.

**Table 3:** Serum levels of Vitamin C and D (Mean± SD) between periodontal disease and control group.

| *Parameter* | *Healthy group mean ± SD* | *Gingivitis group mean ± SD* | *Periodontitis group mean ± SD* | *P &H (LSD)* | *G &H (LSD)* | *P & G (LSD)* |
|---|---|---|---|---|---|---|
| *Vitamin C (ug/mL)* | 4.8320 ±1.0166 | 3.9734±0.6575 | 3.7686±0.5968 | 0.001** | 0.01** | NS |
| *Vitamin D (ng/mL)* | 22.1973±5.0970 | 12.5180±3.5325 | 19.2350±6.5057 | NS | 0.01** | 0. 01** |

SD: standard deviation, ** High significance at P≤ 0.01 level, NS: Non-significant, P (Periodontitis group), G (Gingivitis group), H healthy (Control group).

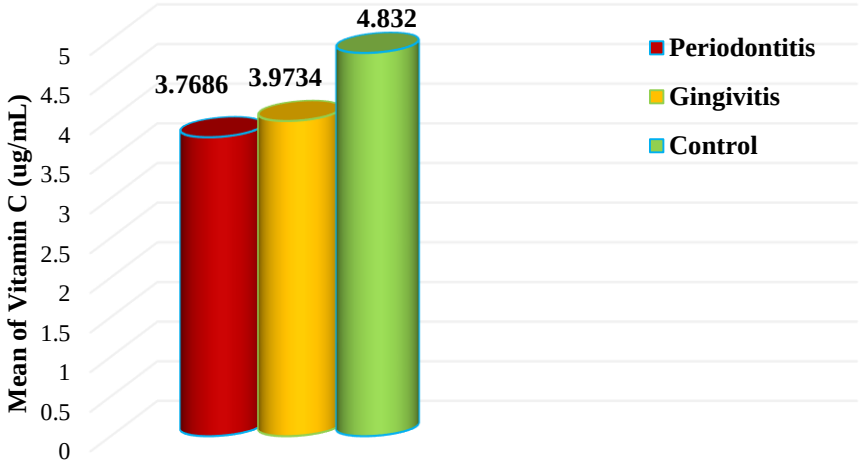


**Figure 2**: Vitamin C levels in the serum of periodontal disease patients and healthy subjects.

Periodontitis is associated with oxidative stress, and antioxidants like ascorbic acid scavenge ROS, which may be useful for periodontal disease prevention [32], whereas low vitamin C levels are linked to advanced periodontal disease [33], hence reduces vitamin C levels, which is considered a risk factor for periodontitis [34], the report of this study agree with our results was periodontitis patients had reduced serum and salivary vitamin C levels than healthy controls [34], in previous studies, vitamin C intake and level in blood were founded to be linked with periodontal disease, with patients with lower dietary consumption or blood levels of vitamin C progressing quicker than the healthy control group[35], this study looked at vitamin C levels in the

blood of people who had periodontitis [36], periodontitis patients had considerably lower plasma levels than healthy controls, and eating grapefruit improves sulcus bleeding scores and elevates plasma vitamin C levels [36], while patients with periodontitis had higher blood vitamin C levels than a healthy control group in two investigations, these findings support the idea that vitamin C from the diet is supplied to periodontal tissue via blood circulation, lowering the risk of periodontal disease [34]. Otherwise, according to the above table, there was no significant difference ($p > 0.05$) in the concentration of vitamin D in the periodontitis group (19.2350±6.5057 ng/mL) to with the healthy control group (22.1973±5.0970 ng/mL). There was a highly significant decrease ($p < 0.01$) in the concentration of vitamin D in the gingivitis group (12.5180±3.5325ng/mL) with a healthy control group (22.1973±5.0970 ng/mL), but the results have shown a highly significant elevation ($p < 0.01$) in levels of vitamin D in the periodontitis group (19.2350±6.5057 ng/mL) compared with the gingivitis group (12.5180±3.5325 ng/mL) as shown in Table 3 and Figure 3.

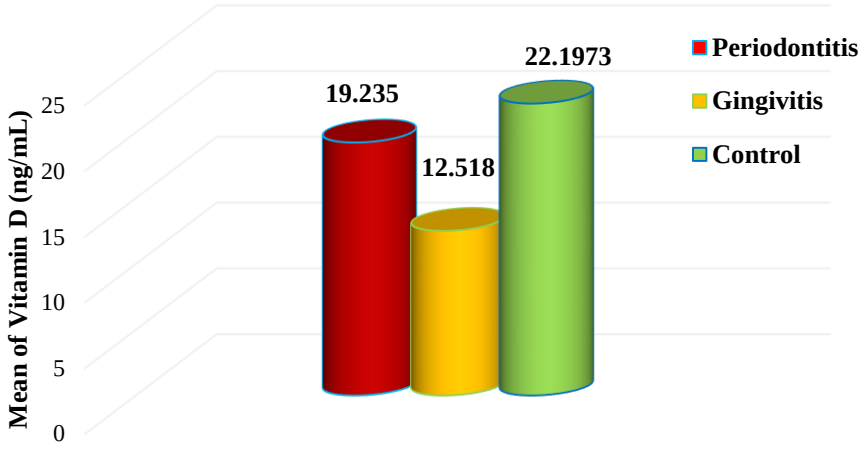


**Figure 3:** Vitamin D concentration in the serum of periodontal disease patients and healthy subjects.

The current of this study found the level of vitamin D does not strongly affect to development of periodontal disease because all groups have suffered from Insufficient Vitamin D, although vitamin D has play role in immunity this result leads to a non-strong effect on the development of periodontal disease. Several studies have connected low vitamin D levels to periodontal disease [37], and this research found a negative relationship between serum vitamin D concentrations and case gingivitis, indicating that increasing vitamin D plasma concentrations could be beneficial in reducing gingivitis [38], the finding of this study agrees with this study's findings that the variance in reduced plasma vitamin D concentrations between the periodontitis and healthy control groups was not statistically significant [37][39,40], it was also recently discovered that higher vitamin D consumption may protect against the progression of periodontal disease in older males [14], and that people suffering from periodontal disease could benefit from a variety of functions of vitamin D by avoiding alveolar bone deterioration, and attachment loss [14], also in this study discovered no differences in serum vitamin D levels between chronic periodontitis and healthy groups, and vitamin D levels and chronic periodontitis were not connected [41].

### 3.4. Copper and Zinc Levels in Periodontal Disease Patients and Healthy Subjects

The result of the copper level was a highly significant elevation ($p < 0.01$) between periodontitis groups (35.1160±7.5546 ng/mL) with the healthy control group (15.2287±2.57160 ng/mL), and between the gingivitis group (21.5070±6.6286 ng/mL) to the healthy control group (15.2287±2.57160 ng/mL), also between periodontitis group (35.1160±7.5546ng/mL) with the gingivitis group (21.5070±6.6286 ng/mL) as mention in Table 4 and Figure 4.

**Table 4:** Serum levels of zinc and copper (Mean± SD) between periodontal disease and healthy control group.

| *Parameter* | *Healthy group mean ± SD* | *Gingivitis group mean ± SD* | *Periodontitis group mean ± SD* | *P&H (LSD)* | *G&H (LSD)* | *P& G (LSD)* |
|---|---|---|---|---|---|---|
| *Cu(ng/mL)* | 15.2287±2.5716 | 21.5070±6.6286 | 35.1160±7.5546 | 0. 01** | 0.01** | 0.001** |
| *Zn(ng/mL)* | 92.1797± 23.450 | 83.3107±28.1183 | 134.4820± 42.4909 | 0.01** | NS | 0.001** |

SD: standard deviation, ** High significance at P≤ 0.01 level, NS: Non-significant, P (Periodontitis group), G (Gingivitis group), H healthy (Control group).

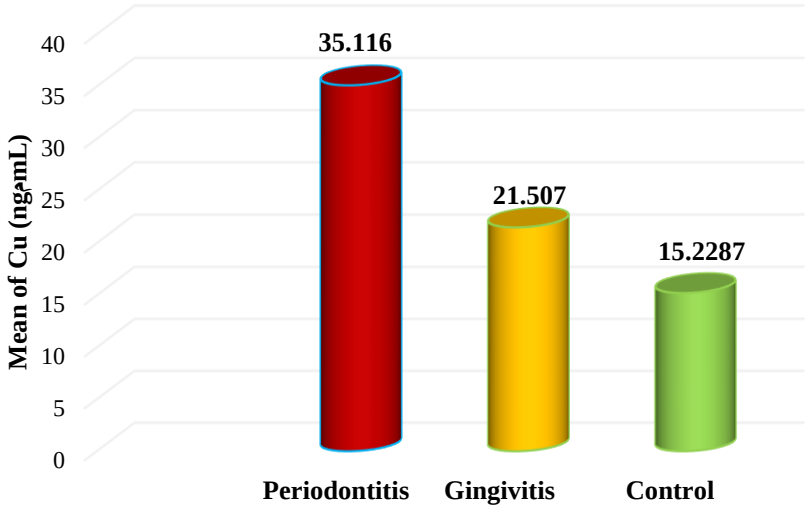


**Figure 4:** Copper levels in the serum of periodontal disease patients and healthy subjects.

The high level of each Cu, and Zn level is due to tissue destruction, and since the trace elements are present within the components of the tissues, this explains the reason for their high levels in the blood. Few studies have discovered that higher copper concentration can influence immune function, including neutrophil proliferation, lymphocyte proliferation, and antigen-specific antibody generation [25], our results agree with this study include compared between the healthy control group and periodontitis, the result was levels of copper were significantly higher in chronic periodontitis [42], which is high copper levels were also connected to the periodontal deterioration [43], following inflammation, serum copper levels are seen to be higher [42]. This one is thought to also be linked to an endogenous leukocyte mediator at the site of inflammation working as a feedback controller to recruit copper from the hepatic. That technique could describe the increased plasma copper concentration observed in chronic periodontitis [42], as per Freeland et al., higher serum copper levels disrupt collagen metabolism, enhancing the individual's risk of chronic periodontitis [42], elevated copper concentration in plasma have been linked with weakened immunity in animal research. That's also due to its actions on the complement system, lower neutrophil counts, lymphocyte proliferation, and antigen-specific antibody generation [42], in this research, there was a rise in serum copper levels in periodontitis, which will reduce following therapy (nonsurgical treatment) [25]. Otherwise, the result of the zinc level was a highly significant elevation ($p < 0.01$) between periodontitis groups (134.4820± 42.4909 ng/mL) with the healthy control group (92.1797± 23.4504 ng/mL), and between the gingivitis group (83.3107±28.1183 ng/mL) to the healthy control group (92.1797± 23.4504 ng/mL), also between periodontitis group (134.4820± 42.4909

ng/mL) with the gingivitis group 83.3107±28.1183 ng/mL) as shown in Table 4 and Figure 5. The levels of serum zinc in the healthy controls group were significantly higher than in the periodontitis groups [42]. According to studies, zinc shortage in gingiva enhances the sensitivity of the gingival epithelium to bacteria and has an opposite relationship with the alveolar bone loss [42]. According to investigations, a lowering in plasma zinc concentration can lead to reduced phagocytosis by macrophages and neutrophils, lowered NK cell function, and lowered oxidative burst formation, as well as stimulating the significant interaction with cytokines and proteases, thus indirectly influencing immune system function [42]. Copper and Zinc activities are known to be tightly connected, as they are important elements of antioxidant enzymes such as copper-zinc superoxide dismutase (SOD) [43], according to the data of various research, there is an inclination for an accumulation of Cu and zinc to reducing in the saliva of periodontitis patients. Increasing copper levels may change the permeability of the gingival epithelium and limit zinc mucosal absorption [43], this study showed a reduction in serum zinc levels in the periodontitis group, which will rise after nonsurgical therapy [25]. In one research, smokers with periodontitis had decreased zinc values and increased Cu values in their saliva [44], in some investigations, excessive copper levels were connected to periodontal destruction. copper and zinc metabolism are tightly related. It has been theorized that high copper levels inhibit zinc absorption. Its levels may be raised in gingiva with zinc deficiency, resulting in increased permeability of gingival epithelium against period onto pathogens [44].

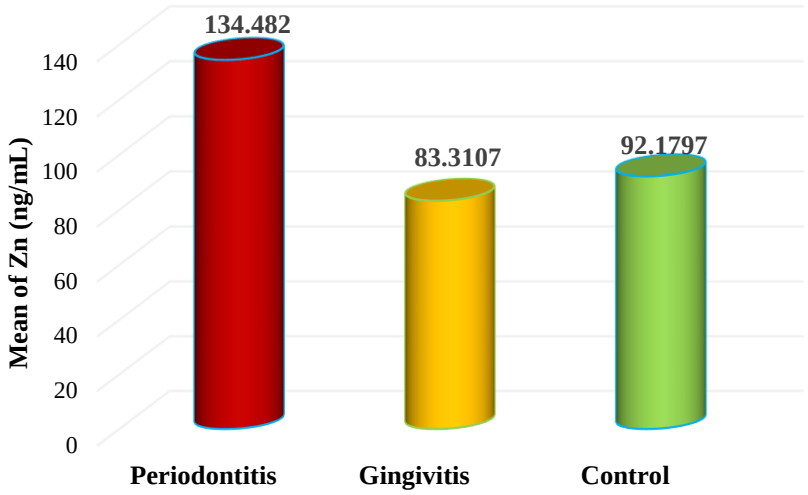


**Figure 5.** Zinc levels in the serum of periodontal disease patients and healthy subjects.

### 3.5. Correlations between Parameters in Gingivitis and Periodontitis Patients with Periodontal Parameters

As indicated in Table 5, the results revealed a non-significant (P > 0.05) link among parameters in the gingivitis group and parameters of periodontal disease.

**Table 5:** Correlations among parameters in gingivitis patients with periodontal parameters.

| Correlation between | Pearson correlation (r) | P-value | Sig. |
|---|---|---|---|
| **Vitamin c and bop** | 0.095 | 0.617 | NS |
| **Vitamin c and pli** | -0.150 | 0.429 | NS |
| **Vitamin d and bop** | 0.015 | 0.937 | NS |
| **Vitamin d and pli** | -0.028 | 0.885 | NS |

| | | | |
|---|---|---|---|
| **Cu and bop** | 0.196 | 0.300 | NS |
| **Cu and pli** | 0.213 | 0.259 | NS |
| **Zn and bop** | 0.004 | 0.985 | NS |
| **Zn and pli** | -0.226 | 0.230 | NS |

BOP (bleeding on Probing), PLI (Plaque Index).

As indicated in Table 6, the results revealed a non-significant (P > 0.05) link between parameters in the periodontitis group and parameters of periodontal disease, just found a negative correlation strong between Vitamin D and BOP (r = -0.528P < 0.01) and a negative correlation medium between Vitamin D and PLI (r = -0.407 P <0.05). Agrawala et al found vitamin D is inversely correlated to probing pocket depth and clinical attachment loss, which may contribute to an enhancement in periodontal [45], furthermore, serum vitamin D levels were not related to other periodontal indicators as PPD CAL or BOP [39], while the report of this study was vitamin D levels were negatively correlated with all periodontal parameters [46], vitamin D levels were found to have a statistically significant relationship with periodontal parameters such as GI, PPD, and CAL, while was no correlation between vitamin D levels and PI.

**Table 6:** Correlations among parameters in periodontitis patients with periodontal parameters.

| *Correlation between* | *Pearson Correlation (r)* | *P-value* | *Sig.* |
|---|---|---|---|
| *Vitamin C and BOP* | -0.227 | 0.227 | NS |
| *Vitamin C and PLI* | 0.043 | 0.824 | NS |
| *Vitamin C and PPD* | 0.105 | 0.580 | NS |
| *Vitamin C and CAL* | 0.232 | 0.217 | NS |
| *Vitamin D and BOP* | -0.528** | 0.003 | HS |
| *Vitamin D and PLI* | -0.407* | 0.026 | S |
| *Vitamin D and PPD* | -0.120 | 0.527 | NS |
| *Vitamin D and CAL* | 0.047 | 0.804 | NS |
| *Cu and BOP* | -0.003 | 0.986 | NS |
| *Cu and PLI* | -0.072 | 0.705 | NS |
| *Cu and PPD* | -0.168 | 0.375 | NS |
| *Cu and CAL* | -0.164 | 0.385 | NS |
| *Zn and BOP* | -0.038 | 0.843 | NS |
| *Zn and PLI* | 0.119 | 0.530 | NS |
| *Zn and PPD* | -0.182 | 0.337 | NS |
| *Zn and CAL* | -0.085 | 0.655 | NS |

### 3.6. Correlations among Parameters in Periodontal Disease

As indicated in Table 7, the results revealed a non-significant (P > 0.05) correlation among parameters in the gingivitis patients. As indicated in Table 8, the results revealed a non-significant (P > 0.05) correlation among parameters in the periodontitis patients.

**Table 7:** Correlations among parameters in gingivitis patients.

| *Correlation between* | *Pearson Correlation (r)* | *P-value* | *Sig.* |
|---|---|---|---|
| *Vitamin C and Vitamin D* | 0.155 | 0.414 | NS |
| *Vitamin C and Cu* | -0.106 | 0.576 | NS |
| *Vitamin C and Zn* | -0.184 | 0.329 | NS |
| *Vitamin D and Cu* | 0.325 | 0.080 | NS |
| *Vitamin D and Zn* | -0.175 | 0.356 | NS |
| *Cu and Zn* | -0.169 | 0.372 | NS |

**Table 8:** Correlations among parameters in periodontitis patients.

| *Correlation between* | *Pearson Correlation (r)* | *P-value* | *Sig.* |
|---|---|---|---|
| *Vitamin C and Vitamin D* | -0.009 | 0.961 | NS |
| *Vitamin C and Cu* | -0.148 | 0.435 | NS |
| *Vitamin C and Zn* | -0.102 | 0.592 | NS |
| *Vitamin D and Cu* | -0.095 | 0.616 | NS |
| *Vitamin D and Zn* | 0.119 | 0.533 | NS |
| *Cu and Zn* | 0.282 | 0.132 | NS |

## 4. Conclusions

The finding of this research is that reduced levels of vitamin C, and D are linked to increased levels of oxidative stress and increased inflammation in a patient with periodontal disease which increases the development of this disease because of their role in the immunity system Thus, other body infections will increase and the ease of exposure to other systemic diseases due to high inflammatory mediators, and the high levels of copper and zinc that result from periodontal disease because the destruction of periodontal tissue, that an indication of periodontal disease, and cause toxicity in the body and weaken the immune response. As a result of this, antioxidants, and nutrients are suggested to be assessed as the limitation of the development of the inflammatory mediators and therefore reduce to development of periodontal disease. We suggest that the level of other trace elements be evaluated because it has an important role in the development of periodontal disease and indicates to degradation of the periodontal tissue, as well as evaluate trace elements with other immune parameters, or the level of vitamin C be evaluated, along with an evaluation of the level of tissue components.

## Limitations of the Study

The limitations of this study include the small sample size because the disease is complex and varies according to different sampling methodologies, disease diagnosis, and measurement methods, periodontal disease criteria, and study socioeconomic context population, in addition to finding appropriate samples for the study has become more difficult as a result of the exclusions in this study, and the study will take longer to complete.

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## Conflicts of interest

The authors declared that there are no conflicts of interest.

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