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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 collected from the University of Baghdad, College of Dentistry, Department of Periodontics, Periodontics Clinic, including a group of 40 patients with periodontitis and another 40 patients with gingivitis and 40 healthy periodontal patients as controls. 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 study was conducted at the University of Baghdad, College of Dentistry, Department of Periodontics, in the Periodontics Clinic from October 2021 to April 2022. In addition, verbal and written informed consent was obtained from all participants in this study. The study was conducted at the Department of Applied

Sciences, University of Technology – Iraq, after receiving approval from the Human Research Ethics Committee (Number: UOT/1250/9-10/2021).

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 smoke or drink alcohol, pregnant and 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 Enzyme-Linked Immunosorbent Assay (ELISA) 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 Fig. 1.

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

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.

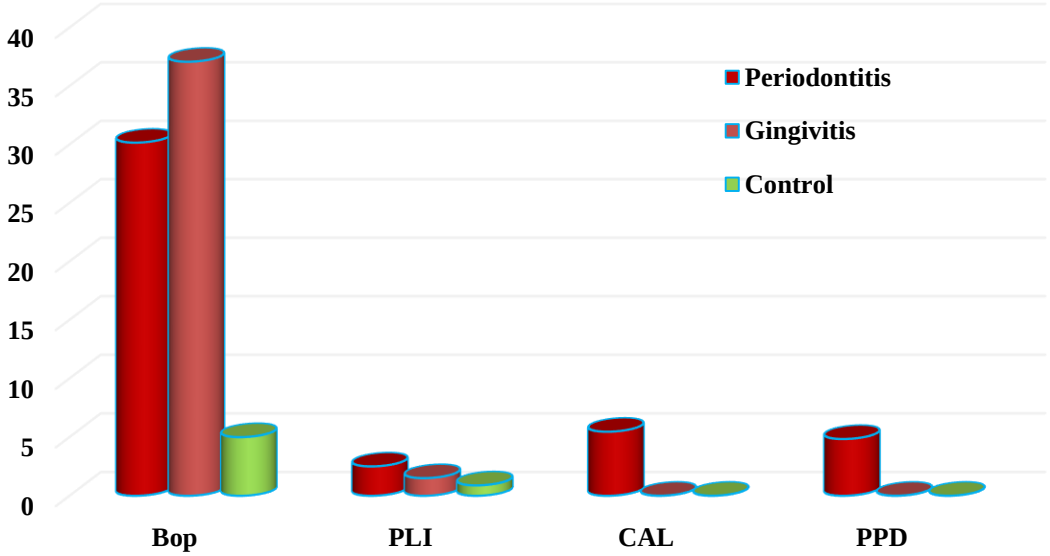

Figure 1: Mean values of PLI, GI, and BOP in periodontal diseases and control groups.

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.

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 Fig. 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.

Parameters	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 (ug/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).

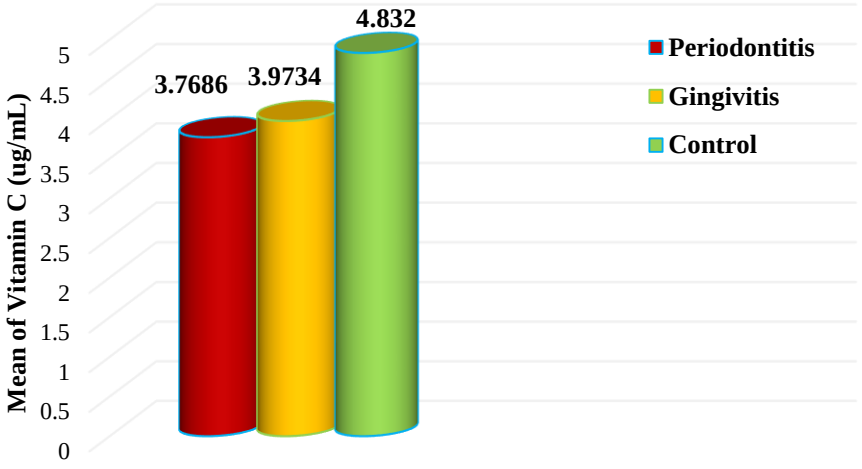

Figure 2: Vitamin C levels in the serum of periodontal disease patients and healthy subjects.

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 Fig. 3.

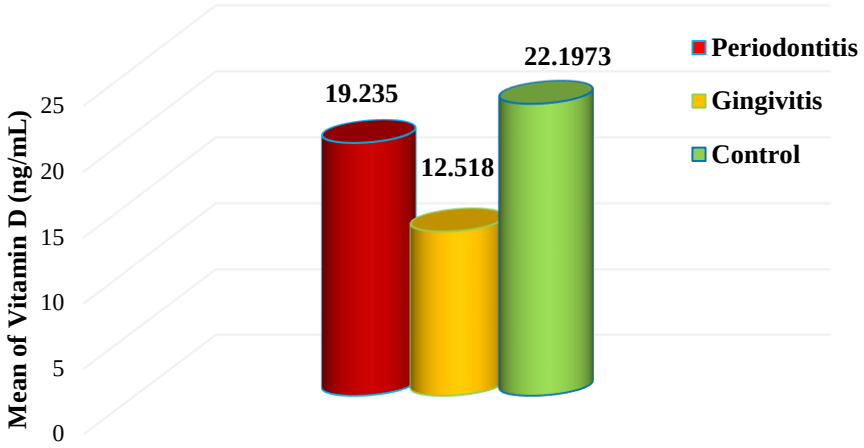

Figure 3: Vitamin D concentration in the serum of periodontal disease patients and healthy subjects.

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 Fig. 4.

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

Parameters	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).

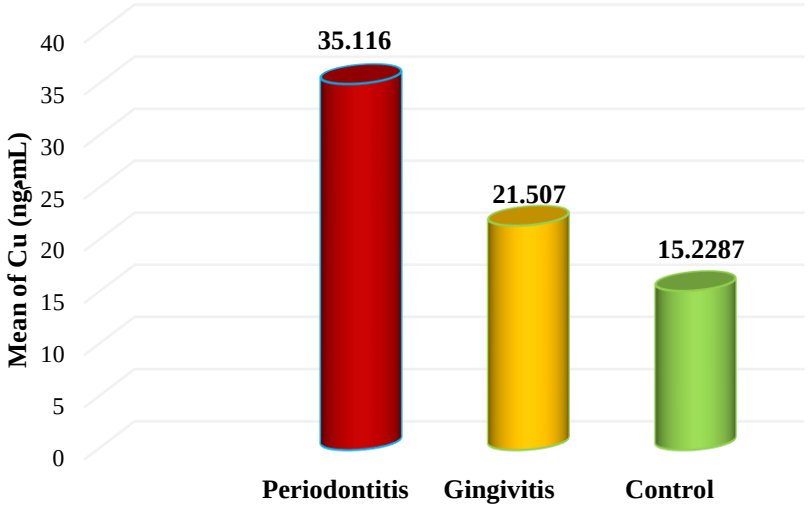

Figure 4: Copper levels in the serum of periodontal disease patients and healthy subjects.

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].

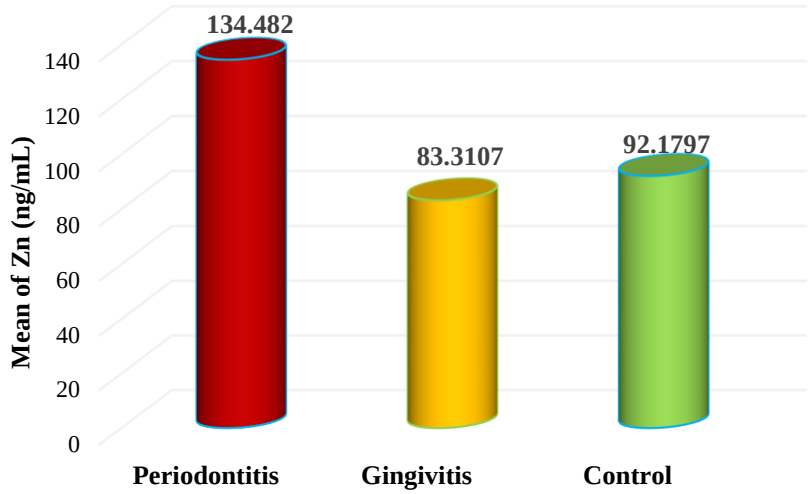

Figure 5: Zinc levels in the serum of periodontal disease patients and healthy subjects.

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

5. 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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Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] J. Gamonal, J. Bravo, Z. Malheiros, B. Stewart, A. Morales, F. Cavalla, et al., "Periodontal disease and its impact on general health in Latin America. Section I: Introduction part I," *Brazilian Oral Research,* vol. 34, no. 1, pp.1-5, 2020.

[2] M. Zhao, W. Dai, H. Wang, C. Xue, J. Feng, Y. He, et al., "Periodontal ligament fibroblasts regulate osteoblasts by exosome secretion induced by inflammatory stimuli," *Archives of oral biology*, vol. 105, no. 9, pp. 27–34, 2019.

[3] I. Scherbaum, H. Heidecke, K. Bunte, U.Peters, T. Beikler, and F. Boege, "Autoantibodies against M5-muscarinic and beta1-adrenergic receptors in periodontitis patients," *Aging*, vol. 12, no. 16, pp. 16609–16620, 2020.

[4] W. J. M. Lobão, R. C. C. Carvalho, S. A. M. Leite, V. P. Rodrigues, J. E. Batista, I. S. Gomes-Filho, et al., "Relationship between periodontal outcomes and serum biomarkers changes after non-surgical periodontal therapy," *Anais da Academia Brasileira de Ciencias,* vol. 91, no. 2, pp. 1–10, 2019.

[5] T. Larsen and N.E. Fiehn, "Dental biofilm infections–an update," *Journal of Pathology, Microbiology and Immunology - the APMIS journal*, vol.125, no. 4, pp. 376-384, 2017.

[6] H. F. Rafeeq, and Z. A. Sharba, "Study the Effect of Cinnamon and Tea Tree Oils on Biofilm Formation of Klebsiella Pneumoniae," *Journal of Applied Sciences and Nanotechnology,* vol. 2, no. 2, pp.16-26, 2022.

[7] M. Rondanelli, A. Miccono, S. Lamburghini, I. Avanzato, A. Riva, P. Allegrini, et al., "Self-Care for Common Colds: The Pivotal Role of Vitamin D, Vitamin C, Zinc, and Echinacea in Three Main Immune Interactive Clusters (Physical Barriers, Innate and Adaptive Immunity) Involved during an Episode of Common Colds-Practical Advice on Dosages and on the Time to Take These Nutrients/Botanicals in order to Prevent or Treat Common Colds*," Evidence-based complementary and alternative medicine,* vol. 2018, no. 5813095, pp.1-37, 2018**.**

[8] A. M. AL-Gebori, "Interleukine-6, Vitamins A, E, and C Levels in Serum of Iraqi Patients with Fibromyalgia Syndrome (FMS)," *Engineering and Technology Journal*, vol.31, no.1, pp.49-57, 2013.

[9] R. Alyami, F. A. Alshehri, R.Al Jasser, S. Shaheen, A. Mahmood, and M. A. Elsafadi, "Vitamin C stimulates RNA expression of human gingival fibroblasts proliferation and adhesion in cigarette smokers: An in vitro study," *The Saudi dental journal*, vol. 34, no. 4, pp. 98–305, 2022.

[10] J. Pizzicannella, G. D.Marconi, S. Guarnieri, L. Fonticoli, Y. Della Rocca, F. Konstantinidou, et al., "Role of ascorbic acid in the regulation of epigenetic processes induced by Porphyromonas gingivalis in endothelial-committed oral stem cells," *Histochemistry and cell biology*, vol.156, no. 5, pp. 423–436, 2021.

[11] A. Toraman, T. Arabaci, Z. Aytekin, M. Albayrak, and Y. Bayir, "Effects of vitamin C local application on ligature-induced periodontitis in diabetic rats," *Journal of applied oral science,* vol. 28, no. e20200444, pp.1-12, 2020.

[12] R.A. Al-Qaisi, A. M. Al-Gebori, and M. H. M. Alosami, "Superoxide Dismutase and Vitamin E Levels in Serum as Indicators in Patients with Acute and Chronic Leukemia," *Journal of Applied Sciences and Nanotechnology*, vol. 2, no. 3, pp.95-105, 2022.

[13] G.M. Sulaiman1, R.F. Al-Ansari, A.M. AL-Gebori, K.A.A. Khalil, S. Albukhaty, E.M. Ahmed, et al. "Serum Levels of Interleukin 10, Interleukin 17A, and Calcitriol in Different Groups of Colorectal Cancer Patients," *Jordan Journal of Biological Sciences*, vol. 15, no. 3, pp. 75 – 81, 2022.

[14] S. M. Mishra, P. L. Ravishankar, V. Pramod, P. B. Rajula, K. Gayathri, M. K. Alam, et al., "Effect of Supplementation of Vitamin D in Patients with Periodontitis Evaluated before and after Nonsurgical Therapy,". *BioMed research international*, vol. 2022, no. 5869676, pp.1-5, 2022.

[15] J. Tokajuk, P. Deptuła, E. Piktel, T. Daniluk, S. Chmielewska, T. Wollny, et al. "Cathelicidin LL-37 in Health and Diseases of the Oral Cavity," *Biomedicines*, vol. 10, no. 5, pp. 1-30, 2022.

[16] A. S. Mohammed, A. A. Al-Janabi, "Computational Analysis of Interleukin 17A Activity in Breast Cancer Patients Using Bioinformatics Methods," *Journal of Applied Sciences and Nanotechnology*, vol. 2, no. 3, pp.79-85, 2022.

[17] E.K. Weir, T. Thenappan, M. Bhargava, and Y. Chen, "Does vitamin D deficiency increase the severity of COVID-19?, " *Clinical medicine (London, England)*, vol.20, no. 4, pp.e107-e108, 2020.

[18] H. R. Alkabi, W. R. Alfatlawi, M. A. H. Aldabagh, "Impact of Vitamin D Elements and Osteoporosis Factors in Postmenopausal Iraqi Women with T2DM," *Journal of Applied Sciences and Nanotechnology*, vol. 2, no. 3, pp.137-146, 2022.

[19] M. Madi, V. Pavlic, M. S Alammar, M.L. Alsulaimi, S. R. Alotaibi, G.M. Alotaibi, et al. "The association between vitamin D level and periodontal disease in Saudi population, a preliminary study," *Saudi Dental Journal*, vol.33, no. 7, pp.595-600, 2021.

[20] C. Weyh, K. Krüger, P. Peeling, and L. Castell, "The Role of Minerals in the Optimal Functioning of the Immune System," *Nutrients*, vol. 14, no. 3, pp.1-15, 2022.

[21] R. F. Al-Ansari, A.M. AL-Gebori, G. M. Sulaiman, "Serum levels of zinc, copper, selenium and glutathione peroxidase in the different groups of colorectal cancer patients," Caspian J Intern Med, vol.11, no. 4, pp. 384-390, 2020.

[22] S. Najeeb, M.S. Zafar, Z. Khurshid, S. Zohaib, and K. Almas, "The Role of Nutrition in Periodontal Health: An Update," *nutrients*, vol.8, no. 9, pp.1-18, 2016.

[23] J. P. Mossink, "Zinc as nutritional intervention and prevention measure for COVID–19 disease," *BMJ nutrition, prevention & health*, vol.3, no. 1, pp.111-117, 2020.

[24] C. A. Mohammad, "Efficacy of Curcumin Gel on Zinc, Magnesium, Copper, IL-1β, and TNF-α in Chronic Periodontitis Patients," *BioMed research international*, vol. 2020, no. 8850926, pp. 1-11, 2020.

[25] G. Sundaram, T. Ramakrishnan, H. Parthasarathy, J. Moses, and T. Lalitha, "Evaluation of Micronutrient (Zinc, Magnesium, and Copper) Levels in Serum and Glycemic Status after Nonsurgical Periodontal Therapy in Type 2 Diabetic Patients with Chronic Periodontitis," *Contemporary clinical dentistry*, vol. 8, no. 1, pp. 26–32, 2017.

[26] N. E. Hassan, and A. A. Mohammed, "Determination of Interferon Gamma Protein in Serum of Breast Cancer Patients Using the ELISA," *Journal of Applied Sciences and Nanotechnology*, vol.2, no.1, pp.37-48, 2022.

[27] L. Levin, A. Zini, J. Levine, M. Weiss, R. Lev, D. C. Taub, et al., "Demographic profile, Oral Health Impact Profile and Dental Anxiety Scale in patients with chronic periodontitis: a case-control study," *International dental journal* vol. 68, no. 4, pp. 269-278, 2018.

[28] E. K. Kalala1, F. B. Nyimi, B. K. Ngamala, J.-P. I. Sekele, A.K. Nyengele, P. M. Muyembi, et al., "Frequency and Profile of Severe Periodontitis in Kinshasa Dental Hospitals, DR Congo," *Scientific Research Publishing*, vol. 10, no. 4, pp. 396-402, 2018.

[29] H. Dukka, M. H. A. Saleh, A. Ravidà, H. Greenwell, H. L. Wang, "Is bleeding on probing a reliable clinical indicator of peri-implant diseases?, " *Journal of periodontology*, vol. 92, no. 12, pp.1-6, 2021.

[30] H. J. Talib, H. A. Mousa, and A. A. Mahmood, "Assessment of the Plaque-Induced Gingivitis Patient with and Without Hyaluronic acid and Xylitol Toothpaste," *Journal of International Society of Preventive & Community Dentistry*, vol. 11, no.2, pp. 138–143, 2021.

[31] C. J. Ali, and M. A. Ahmed, "Evaluation of Hematocrit Level, Red Blood Cells and White Blood Cells Counts in Blood from Patients with Different Severities of Periodontal Diseases," *Journal of Baghdad College of Dentistry*, vol. 30, no. 3, pp. 1-6, 2022.

[32] J. H. Lee, M. S. Shin, E. J. Kim, Y. B. Ahn, and H. D. Kim, "The association of dietary vitamin C intake with periodontitis among Korean adults Results from knhanesIV," *PloS one*, vol. 12, no. 5, pp. 1-14, 2017.

[33] M. R. Munday, R. Rodricks, M. Fitzpatrick, V. M. Flood, and J. E. Gunton, "A Pilot Study Examining Vitamin C Levels in Periodontal Patients," *Nutrients*, vol. 12, no.8, pp. 1-9, 2020.

[34] G. Isola, A. Polizzi, S. Muraglie, R. Leonardi, and A. L. Giudice, "Assessment of Vitamin C and Antioxidant Profiles in Saliva and Serum in Patients with Periodontitis and Ischemic Heart Disease," *Nutrients*, vol. 11, no. 12, pp. 1-13, 2019.

[35] A. Tada, and H. Miura, "The Relationship between Vitamin C and Periodontal Diseases: A Systematic Review," *International journal of environmental research and public health*, vol. 16, no. 14, pp. 1-15, 2019.

[36] H. Staudte, B. W. Sigusch, and E. Glockmann, "Grapefruit consumption improves vitamin C status in periodontitis patients," *British dental journal*, vol. 199, no. 4, pp. 213–210, 2005.

[37] V. Machado, S. Lobo, L. Proença, J. J. Mendes, and J. Botelho, "Vitamin D and Periodontitis: A Systematic Review and Meta-Analysis, "*Nutrients*, vol. 12, no. 8, pp. 1-18, 2020.

[38] T. Dietrich, M. Nunn, B. Dawson-Hughes, and H. A. Bischoff-Ferrari, "Association between serum concentrations of 25-hydroxyvitamin D and gingival inflammation," *The American journal of clinical nutrition*, vol. 82, no. 3, pp. 575-580, 2005.

[39] H. Kim, M. H. Shin, S. J. Yoon, S. S. Kweon, Y. H. Lee, C. K. Choi, et al. "Low serum 25-hydroxyvitamin D levels, tooth loss, and the prevalence of severe periodontitis in Koreans aged 50 years and older," *Journal of periodontal & implant science*, vol. 50, no. 6, pp. 368-378, 2020.

[40] V. S. Patil, R. S. Mali, and A. S. Moghe, "Evaluation and comparison of Vitamin D receptors in periodontal ligament tissue of Vitamin D-deficient chronic periodontitis patients before and after supplementation of Vitamin D3," *Journal of Indian Society of Periodontology*, vol. 23, no.2, pp. 100-105, 2019.

[41] S. Pradhan, and S. Agrawal, "Serum Vitamin D in Patients with Chronic Periodontitis and Healthy Periodontium," *Journal of Nepal Health Research Council*, vol. 18, no. 4, pp. 610-614, 2021.

[42] B. Thomas, B. R. Prasad, N. S. Kumari, V. Radhakrishna, and A. Ramesh, "A comparative evaluation of the micronutrient profile in the serum of diabetes mellitus Type II patients and healthy individuals with periodontitis," *Journal of Indian Society of Periodontology*, vol. 23, no.1, pp. 12–20, 2019.

[43] G. Baima, G. Iaderosa, M. Corana, F. Romano, F. Citterio, A. Giacomino, et al. "Macro and trace elements signature of periodontitis in saliva: A systematic review with quality assessment of ionomics studies," *Journal of periodontal research*, vol. 57, no. 1, pp. 30–40, 2022.

[44] S. Gaur, and R. Agnihotri, "Trace Mineral Micronutrients and Chronic Periodontitis-a Review," *Biological trace element research*, vol. 176, no. 2, pp. 225–238, 2017.

[45] A. A. Agrawala, A. P. Koltea, R. A. Koltea, S. Charib, M. Guptab, and R. Pakhmodea, "Evaluation and comparison of serum vitamin D and calcium levels in periodontally healthy, chronic gingivitis and chronic periodontitis in patients with and without diabetes mellitus – a cross-sectional study," *Acta Odontologica Scandinavica*, vol. 77, no. 8, pp. 1-8, 2019.

[46] G. Isola, A. Alibrandi, E. Rapisarda, G. Matarese, R. C. Williams, and R. Leonardi, "Association of vitamin D in patients with periodontitis: A cross-sectional study," *Journal of periodontal research*, vol. 55, no.5, pp.1-11, 2020.

[47] A. Bhargava, P. Rastogi, N. Lal, R. Singhal, S. Khatoon, and A. A. Mahdi, "Relationship between VITAMIN D and chronic periodontitis," *Journal of Oral Biology and Craniofacial Research*, vol. 9, no. 2, pp. 177-179, 2019.