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# Serum levels of various vitamins in periodontal health and disease- a cross sectional study

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## ABSTRACT

**Objectives:** Vitamins are micronutrients that are required in small or trace amounts. They play an essential role in the metabolism and maintenance of tissue function. This investigation aimed to assess if a deficiency of certain essential vitamins is a risk factor for developing periodontitis.

**Methods:** The subject population consisted of 100 subjects, 50 with generalized chronic periodontitis and 50 periodontally healthy volunteers. The following clinical parameters were measured: Gingival Index, pocket depth (mm); Clinical Attachment Loss (mm). Serum samples were collected and analyzed for levels of *cis*- $\beta$ -carotene,  $\beta$ -cryptoxanthin, vitamin B 12, folate, vitamin D, and vitamin E. Individual data collected was summarized and analyzed using statistical software.

**Results:** All the clinical parameters for periodontal status in the periodontitis group compared to healthy volunteers were highly significant ( $p < 0.0001$ ). The mean levels of all the micronutrients, vitamin A precursors *cis*- $\beta$ -carotene and  $\beta$ -cryptoxanthin, folate, vitamin B 12, D & E were lower in the periodontitis group than the healthy volunteers, although the difference was statistically significant only in case of  $\beta$ -cryptoxanthin, Vitamin B12 and Vitamin D ( $p < 0.05$ ).

**Conclusions:** The findings of our study suggest that serum micronutrient levels especially Vitamin A, Vitamin B 12, and Vitamin D may be modifiable risk factors for periodontal disease. Providing an optimized combination of various vitamins in each meal in combination with sufficient measures of standard oral hygiene care may provide an important role in the prevention of periodontitis.

## 1. Introduction

Periodontitis is a highly prevalent inflammatory ailment of the tooth-supporting tissues, initiated by specific microorganisms ensuing continuous destruction of the periodontal ligament and alveolar bone. An inflated host immune-inflammatory response is responsible for the destruction of periodontal tissues.<sup>1,2</sup> In an estimate, 40%–90% of the worldwide population is affected by periodontitis, qualifying it as one of the most widespread epidemics in the world. Numerous factors influence periodontal health; for instance oral hygiene, environmental factors, genetics, systemic health, and nutrition.<sup>3</sup> The insufficiency of specific vitamins can serve as a contributing factor in the initiation and progression of periodontal disease in certain individuals.<sup>4</sup>

Nutrients from a diet form essential life sources in form of a critical energy resource (macronutrients) and cofactors for enzyme action and

other structural and functional moieties (micronutrients).<sup>5</sup> Diminution of each of these macro- or micronutrients is damaging to the health of tooth-supporting tissues.<sup>6</sup> Micronutrients that exert immunomodulatory or antioxidant effects or those associated with bone metabolism may play an essential role in the prevention and management of periodontal disease.<sup>7</sup> The micronutrients evaluated in this study include Vitamin A precursors (*cis*  $\beta$ -carotene &  $\beta$ -cryptoxanthin), Vitamin B 12, Folate, Vitamin D, and Vitamin E.

Vitamin A refers to a group of fat-soluble compounds that exist in various forms, including retinol, retinal, 3,4-dehydroretinol, and 3-hydroxy retinol. Additionally, there are over fifty types of carotenoids, which are lipid-soluble pigments found in plants that can serve as precursors to vitamin A. Among these,  $\beta$ -carotene and  $\beta$ -cryptoxanthin are the most significant ones. Retinoic acid, which is one of the biologically active forms of vitamin A, plays a critical role in maintaining the

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integrity of mucosal tissues and regulating precise cell differentiation, encompassing immune cells as well.<sup>8</sup> Numerous epidemiological studies have explored the relationship involving vitamin A with periodontal disease, many of which have found an inverse relationship between vitamin A precursors level in blood and periodontal health.<sup>9,10</sup>

Cobalamin, also known as Vitamin B12, plays a prominent role in various processes such as collagen formation, metabolic activities, synthesis of red blood cells, and development of the nervous system.<sup>11</sup> Low serum B12 levels have previously been related to increased clinical attachment loss and subsequent tooth loss in a previous study.<sup>12</sup> Folic acid (folacin) and the naturally occurring form folate are forms of the water-soluble vitamin B9. Oral manifestations of folic acid deficiency include generalized stomatitis, ulcerative glossitis, and cheilitis.<sup>13</sup> Folic acid is associated with the proliferation of rapidly dividing epithelial cells, therefore it is reasonable that folic acid deficiency may affect the junctional epithelium. With an expeditious turnover rate, junctional epithelium holds the key to preventing as well as halting the progression of periodontal disease. Folic acid insufficiency has previously been associated with chronic periodontitis in smokers.<sup>14</sup> There is presently inadequate understanding available concerning vitamin B and folate supplements in the prevention of periodontal disease and post-periodontal therapy.

Vitamin D comprises several forms of cholecalciferol, which primarily regulates calcium and phosphorus levels for bone development and metabolism. However, cholecalciferol is also important for cell development and neuromuscular function.<sup>15</sup> Additionally, vitamin D may impact immune response and inflammation by inhibiting the release of pro-inflammatory cytokines and T-lymphocyte proliferation.<sup>16</sup> These effects of Vitamin D on the immune and skeletal system might have a consequence on periodontal disease preclusion and treatment since it may help maintain tooth-supporting bone and soft tissues. A relationship between serum vitamin D3 and periodontitis has been described, which was not linked to bone mineral density demonstrating the significance of the anti-inflammatory characteristics of vitamin D.<sup>17</sup>

Among the various forms of Vitamin E, alpha-tocopherol is the most abundant and possesses the highest biological activity, effectively preventing Vitamin E deficiency in humans. It is an essential lipid-soluble antioxidant that prevents the lipid peroxidation of polyunsaturated fatty acids present in the cell membrane of cells of the immune system in addition to preventing protein glycation.<sup>18</sup> Vitamin E employs several mechanisms to exert an anti-inflammatory effect on tissues. It is also found to have an anti-thrombotic effect.<sup>19</sup> Previous studies investigating the link between vitamin E and periodontal disease have yielded either non-significant associations between vitamin E levels and periodontal disease or an inverse relationship between vitamin E and the severity of periodontitis.<sup>20,21</sup>

Conventionally, malnutrition has been associated with poor economic background and inadequate diet. During the early decades of India's independence, insufficient access to food grains and financial limitations were contributing factors to poor nutrition. At present, though, it is the quality of diet deficient in micronutrients; which is of foremost concern. Vitamin deficiency is a chief source of malnutrition caused by improper dietary habits. Studies investigating the possible relationship connecting periodontal disease and serum micronutrient levels, especially in Indians are lacking. There could be potential protective effects of micronutrients in preventing periodontal disease. Therefore, this study intends to assess whether the deficiency of six major human micronutrients (*cis*- $\beta$ -carotene,  $\beta$ -cryptoxanthin, Vitamin B12, folate, vitamin D, and vitamin E) is a risk factor for developing periodontitis. For this serum *cis*- $\beta$ -carotene,  $\beta$ -cryptoxanthin, Vitamin B 12, Folate, vitamin D, and vitamin E levels were assessed and compared in subjects with generalized periodontitis and periodontally healthy controls.

## 2. Material and methods

### 2.1. Subject population

The study included one hundred individuals, consisting of fifty participants with generalized periodontitis and fifty periodontally healthy volunteers. The groups were matched for gender distribution. All the participants were 20 years or more of age with a minimum of 20 natural teeth, had a willingness and ability to sign a consent form, and had overall good systemic health with the absence of any diseases or conditions affecting the periodontium.

Generalized periodontitis for this study was defined as follows: more than 30% of sites with pocket depth  $\geq 5$  mm and/or 30% of sites with clinical attachment loss (CAL)  $> 4$  mm. The healthy volunteers had no site with CAL and less than 30% of sites with a gingival index of  $< 1$ . The study excluded individuals who had systemic diseases that could impact the development or treatment of periodontal disease (such as diabetes or AIDS), pregnant or breastfeeding women, individuals who had taken antibiotics within the past three months, those who had taken any vitamin or health supplements within the past six months, and people who used any form of tobacco. The study was permitted by The Institutional Review Board Ethical Approval on 25/04/2019 communicated via IEC No: 134/A/11/16/Academics/MC/2016/126. Every participant signed a written informed consent before taking part in the study.

### 2.2. Clinical parameters

The following clinical parameters were measured in all the participants: Gingival Index (Loe & Silness, 1963),<sup>22</sup> pocket depth (mm); CAL (mm). The pocket depth and CAL were taken at 6 sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual, and mesiolingual) for all teeth excluding third molars. The pocket depth and CAL measurements were made to the nearest mm using a World Health Organization (WHO) periodontal probe (Hu Friedy, India).

### 2.3. Serum analysis

The patients were instructed to arrive early in the morning on an empty stomach to provide serum samples, which were collected in plain vacutainers for analysis. Subsequently, the samples were sent to the hospital laboratory for assessing the serum concentrations of various vitamins. High-performance liquid chromatography (HPLC) was utilized to determine the levels of *cis*- $\beta$ -carotene,  $\beta$ -cryptoxanthin, and vitamin E, while an enzyme-linked immunosorbent assay (ELISA) was employed to assess the levels of vitamin B12 and folate. The levels of vitamin D were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

### 2.4. Data collection and statistical analysis

Clinical parameters including mean gingival index, mean probing depth, and mean CAL were calculated for each participant followed by means and standard deviation (SD) for each group. Likewise mean and standard deviation for serum levels of micronutrients were also computed. Student's t-test for independent means was utilized for intergroup comparisons. All data was compiled and analyzed using appropriate statistical software and a p-value of  $< 0.05$  was taken as significant.

## 3. Results

### 3.1. Clinical parameters

All the data obtained clinically including gingival index, pocket depth, and CAL are summarized in Table 1. There was no significant disparity between the mean age in both groups ( $p = 0.092$ ). All the other

**Table 1**  
Comparison of Clinical Parameters between Healthy volunteers and Generalized Periodontitis Groups.

	Healthy Volunteers (N = 50)	Generalized Periodontitis (N = 50)	t - value	p-value
Age (years)	30.86 ± 5.81	32.5 ± 6.46	1.334	0.926
Mean GI	0.57 ± 0.29	1.80 ± 0.36	18.814	<0.001 <sup>a</sup>
Mean PPD (mm)	1.51 ± 0.438	4.52 ± 0.904	21.178	<0.001 <sup>a</sup>
Mean CAL (mm)	0	4.69 ± 1.069	31.005	<0.001 <sup>a</sup>

<sup>a</sup> p value <0.05 (significant).

clinical parameters for measurement of periodontal status in the periodontitis group compared to Healthy volunteers were highly significant (p < 0.0001).

### 3.2. Serum vitamin levels

Cis-β-carotene, β-cryptoxanthin, vitamin B 12, folate, vitamin D, and vitamin E were analyzed in both Periodontitis and Healthy Volunteer groups. The results are summarized in Table 2. The mean levels of all the micronutrients, vitamin A precursors cis-β-carotene and β-cryptoxanthin, vitamin B 12, folate, vitamin D & E were lower in the periodontitis group than the healthy volunteers, while the difference was significant only in case of β-cryptoxanthin, vitamin B12 and Vitamin D (p < 0.05) [Figs. 1–5].

## 4. Discussion

Micronutrient malnutrition is the depletion or lack of availability of vitamins and minerals essential for various bodily functions and the maintenance of structural moieties. This deficiency can be provoked by lifestyle factors, intake of drugs (such as antacids, antibiotics, diuretics, laxatives, etc), systemic disorders, malabsorption, and diarrhea among others.<sup>23</sup> The utilization of these micronutrients in the body is influenced by age, gender, physiological state, and disease processes.<sup>24</sup>

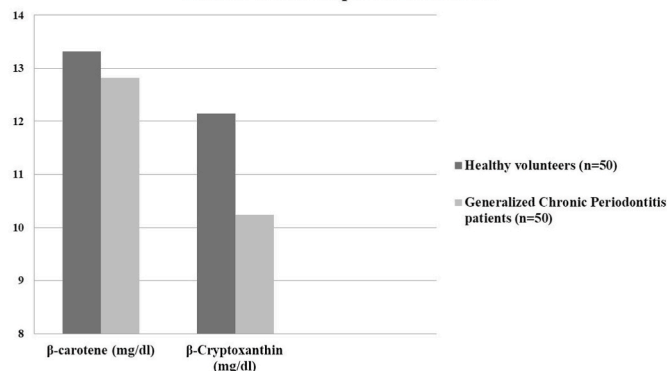
Through the years scientific inquiries to assess the part micronutrients play in the development or advancement of periodontitis have been carried out.<sup>25</sup> Also many micronutrients have been tested for their role in the treatment of periodontal disease as an additional modality apart from clinical management. As the data concerning micronutrients in periodontitis patients in the Indian population is primitive, we undertook this initiative to assess whether micronutrient deficiency could be a modifiable risk factor for the development of chronic periodontitis. The major detection of this study is that serum micronutrient levels especially Vitamin A, Vitamin B 12, and Vitamin D may affect the risk of

**Table 2**  
Comparison of Serum Vitamin levels between Healthy volunteers and Generalized Periodontitis Groups.

	Healthy Volunteers (N = 50)	Generalized Periodontitis (N = 50)	t - value	p-value
β-carotene (mg/dl)	13.31 ± 8.2	12.82 ± 7.09	0.3196	0.7499
β-Cryptoxanthin (mg/dl)	12.14 ± 3.8	10.24 ± 4.35	2.326	<b>0.0221<sup>a</sup></b>
Vitamin B 12 (pg/ml)	279.02 ± 82.21	252.72 ± 61.22	1.81434	<b>0.0363<sup>a</sup></b>
Folate (ng/ml)	13.93 ± 4.17	13.29 ± 2.48	0.93773	0.1753
Vitamin D (nmol/l)	42.99 ± 17.23	34.07 ± 11.78	3.02148	<b>0.0016<sup>a</sup></b>
Vitamin E (mg/dl)	756.1 ± 161.2	732.4 ± 178.0	0.6978	0.4869

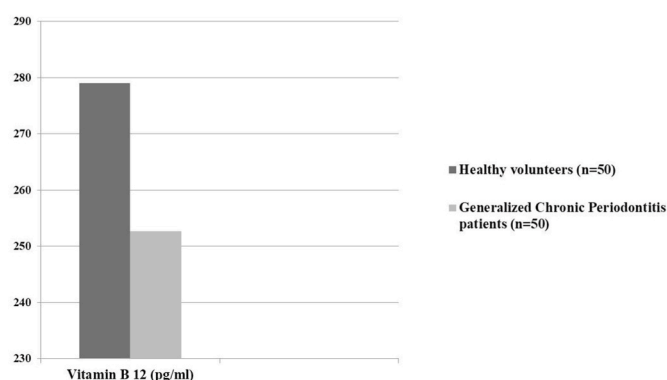
<sup>a</sup> p value <0.05 (significant).

**Serum Vitamin A precursors Levels**



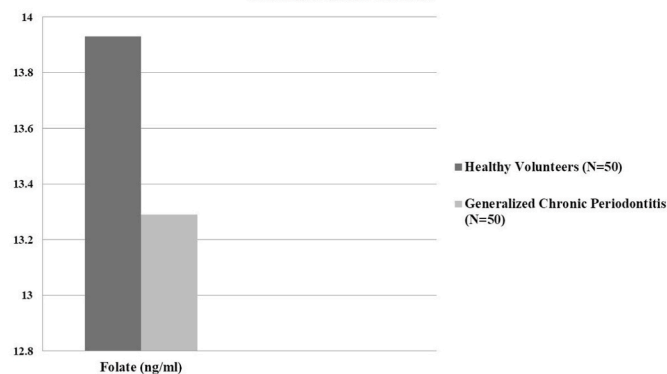
**Fig. 1.** Comparison of serum levels of Vitamin A precursors (Cis-β-carotene and β-cryptoxanthin) in healthy volunteers and periodontitis patients.

**Serum Vitamin B 12 levels**



**Fig. 2.** Comparison of serum levels of Vitamin B12 in healthy volunteers and periodontitis patients.

**Serum Folate Levels**



**Fig. 3.** Comparison of serum levels of Folate in healthy volunteers and periodontitis patients.

developing periodontal disease.

Pro-vitamin A carotenoids, such as β-carotene and β-cryptoxanthin, are abundant in plant-based sources, including green leafy vegetables, orange-yellow vegetables, and fruits such as carrots and mangoes. These carotenoids are cleaved into retinol through enzymes and act as a source of vitamin A and prevent its deficiency.<sup>26</sup> Over time, epidemiological research has established an inverse correlation between serum levels of Vitamin A and the incidence of periodontitis.<sup>27</sup> According to Dodington et al., consuming more dietary β-carotene after non-surgical periodontal therapy was associated with a lower percentage of sites with a pocket

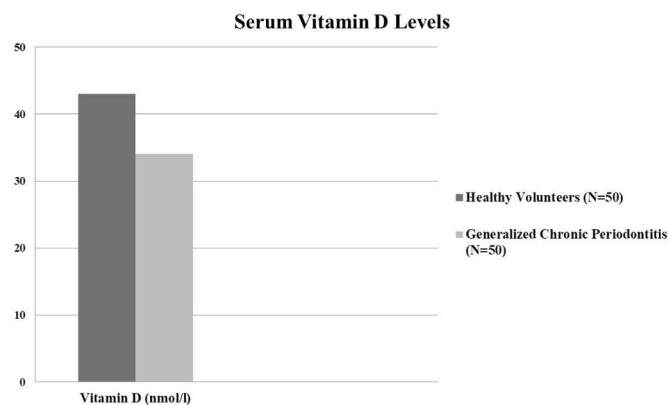


Fig. 4. Comparison of serum levels of Vitamin D in healthy volunteers and periodontitis patients.

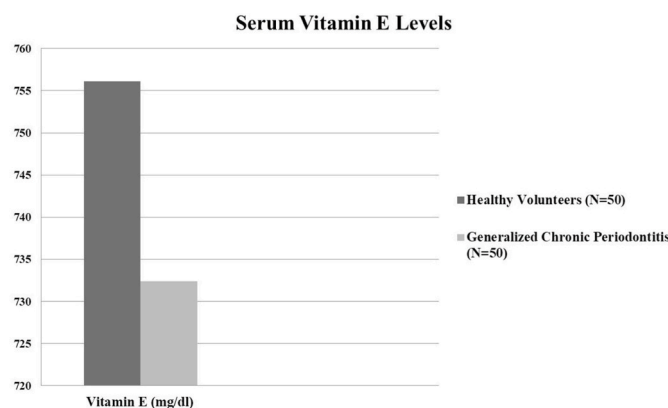


Fig. 5. Comparison of serum levels of Vitamin E in healthy volunteers and periodontitis patients.

depth greater than 3 mm. Also, it resulted in a higher pocket depth reduction in non-smokers compared to smokers.<sup>28</sup> Another study found that beta-carotene and beta-cryptoxanthin were the only antioxidants linked to a higher risk of severe periodontitis with a high threshold. The study concluded that reduced serum levels of several carotenoids, especially beta-cryptoxanthin and beta-carotene, were associated with a higher incidence of periodontitis in a homogeneous group of Western European men aged 60–70.<sup>10</sup> The findings of our study revealed a correlation between carotenoid levels and periodontitis, but only beta-cryptoxanthin showed a statistically significant negative association.

Vitamin B12 and folate belong to the water-soluble vitamin B complex and are involved in multiple processes, including collagen synthesis and blood cell development. Vitamin B12 plays a crucial role in nervous system development, while folic acid is essential for cell division, particularly in DNA synthesis.<sup>11,13</sup> A recent study deduced that reduced serum levels of vitamin B 12 were related to higher periodontal attachment loss and consequently tooth loss.<sup>12</sup> Another study involved 111 participants of varying ages which were divided into four groups based on their health status, periodontitis, smoking habits, and gutkha chewing. Clinical parameters (pocket probing depth, clinical attachment level & gingival index) were assessed, and the levels of vitamin B12 and folate were estimated and analyzed using various statistical methods. The study found that gutkha chewers with chronic periodontitis had higher serum vitamin B12 levels than healthy individuals and those with periodontitis. However, smokers with periodontitis had lower folate levels than healthy individuals.<sup>29</sup> Our study revealed that individuals with chronic periodontitis have significantly lower levels of vitamin B12 compared to healthy individuals. Although the serum levels of folic acid

were lower in periodontitis patients than in healthy individuals, the difference was not statistically significant.

Several reports have associated higher serum levels of vitamin D with the reduced risk of tooth loss as a consequence of periodontitis.<sup>17,30</sup> One of the studies found that there is a significant inverse relationship between the predicted 25-hydroxyvitamin D score and the incidence of tooth loss. The higher the 25-hydroxyvitamin D score, the lower the risk of tooth loss. In addition, UV-B exposure was also associated with a lower risk of tooth loss. The study suggests that there is an association between predictors of vitamin D and a lower incidence of tooth loss and periodontitis.<sup>30</sup> Data from our research depicted deficient levels of Vitamin D in both the comparison groups, even though the levels were significantly lower in the periodontitis group. These findings are consistent with similar studies that have been conducted previously.<sup>31,32</sup> One of these studies investigated the link between Plasma 25-hydroxyvitamin D concentrations and periodontal disease in postmenopausal women. It found that women with adequate vitamin D status had 33% lower odds (95% CI = 5%–53%) of periodontal disease and 42% lower odds (95% CI = 21%–58%) of having  $\geq 50\%$  of gingival sites that bled, compared to women with deficient/inadequate vitamin D status.<sup>31</sup> Vitamin D has a significant role in bone metabolism and its serum levels have a direct relation to bone mineral density. Vitamin D deficiency negatively impacts the absorption of calcium along with phosphorus from the intestines, which are the building blocks for bone formation. It is also responsible for an enhanced epithelial innate immune function along with antimicrobial peptide secretion. It also negatively influences NF $\kappa$ B activation in addition to cytokine secretion by monocytes and macrophages.<sup>16</sup> This might have an additional impact on the way vitamin D influences periodontal disease.

Vitamin E is an antioxidant that helps to obstruct the production of reactive oxygen species through inter-communication with cell membranes and adipose tissues.<sup>18</sup> We found no significant difference in the serum levels of Vitamin E in the compared groups. The literature concerning vitamin E and its relationship with periodontitis is rather limited. A study was conducted using data from the NHANES with 4708 participants to explore the correlation between periodontal status and serum tocopherol levels. The study measured serum tocopherols through HPLC and adjusted values with total cholesterol (TC). The results revealed that increased serum  $\alpha$ -tocopherol:TC quartiles had an inverse correlation with mean clinical attachment loss, mean probing pocket depth, and total periodontitis.<sup>33</sup> A supplementation of Vitamin E seems to exhibit improved healing of periodontal tissues.<sup>34</sup> Moreover, an augmented uptake of  $\alpha$ -tocopherol in diet along with other antioxidants and omega-3 fatty acids diminished the periodontal pocket depth in periodontitis patients, although the encouraging results were limited only to non-smokers.<sup>28</sup>

The notion of the effect of nutrition on periodontal tissues has been the concern of various investigations. The results of which may be applied by enhanced nutritional uptake and supplementation for improved periodontal health. An imperative postulation that appears from our study is a diminished probability of any single nutrient being efficient in the deterrence or management of inflammation and alveolar bone loss in periodontitis. Nevertheless, an aimed approach applying a blend of these bioactive nutritional agents possibly will provide a supplementary perspective for managing periodontitis. There are a few drawbacks to our study. As is the case concerning every cross-sectional epidemiologic study, our data cannot define a causal relationship between the effect of vitamin deficiency and periodontal disease outcome. However, our findings relating to the potential impact of vitamin deficiency on periodontal disease advocate that the nutrient components could be evaluated in more prospective studies, to delineate causal linkages in protection from periodontal disease.

## 5. Conclusion

The findings of our study suggest that serum vitamin levels especially

Vitamin A, Vitamin B 12, and Vitamin D may be modifiable risk factors for periodontal disease. Imparting an optimized combination of micro-nutrients in the daily serving of food in combination with adequate oral hygiene practices may present an opportunity to prevent periodontitis.

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