

**Original article**

**Comparative evaluation of serum folic acid levels in smokers and non-smokers with chronic periodontitis**

*B Sumona<sup>1</sup>, S Sheetal<sup>2</sup>, M Anil<sup>3</sup>, P Suvarna<sup>4</sup>*

**Abstract**

**Objectives:** Folic acid is a vitamin which belongs to the B-complex group. It is critical to cellular division and new cell production because it is an essential co-factor in DNA synthesis. Repair and maintenance of periodontal tissue generates a high turnover rate of squamous epithelium. Without folic acid, epithelial cells do not divide properly. Cigarette smoking is one of the factors that affect the folic acid levels. The aim of this study was to compare the serum folic acid levels in patients with chronic periodontal disease in relation to the patients' smoking habits. **Materials and Methods:** A total of 60 subjects were included in the study with 30 subjects in each of the following groups, I - patients who have chronic periodontitis and are smokers and II - patients who have chronic periodontitis and are non-smokers. Clinical parameters like gingival index (GI), plaque index (PI), bleeding on probing (BOP), probing depth (PD) and clinical gingival attachment levels (CAL) were recorded for all the patients. Blood was collected and tested in the laboratory for folic acid levels using a fully automated serum analyser. The results were statistically analysed. **Results:** The results suggested that serum folic acid levels of smokers were significantly lower than that of non-smokers ( $p < 0.05$ ). PI, PD and CAL means were significantly higher in Group I (chronic periodontitis and smokers) than Group II (chronic periodontitis and non-smokers). GI and BOP was lower in smokers. **Conclusion:** Among patients with periodontal disease the serum folic acid level is lower in smokers compared with non-smokers.

**Key Words:** Chronic periodontitis, folic acid, smoking.

**Introduction**

Periodontitis is an infectious disease resulting in inflammation of the supporting structures of the teeth, progressive attachment loss and bone loss. Periodontitis is initiated and sustained by bacterial plaque, but host defense mechanisms play an integral role in its pathogenesis.<sup>1</sup> It is a multifactorial disease; manifestation and progression of which is influenced by a wide variety of determinants, including social, behavioral, systemic, environmental and genetic risk factors.<sup>2</sup>

Among the environmental factors, cigarette smoking is undoubtedly one of the main and most prevalent risk factor for chronic periodontitis. It is associated with an increased disease rate in terms of periodontal bone loss,

periodontal attachment loss, as well as periodontal pocket formation.<sup>4</sup> Generally, assessment of risk shows that smoking is associated with 2 to 7 fold increase in risk for having periodontitis and/or periodontal tissue loss compared to nonsmokers. In addition, it exerts a masking effect on gingival symptoms of inflammation, like redness, bleeding on probing.<sup>5-9</sup>

Smoking has a long term chronic effect on many important aspects of the inflammatory and immune responses. Protease release from neutrophils may be an important mechanism in tissue destruction. Tobacco smoke has been found to affect both cell mediated and humoral immunity.<sup>10</sup>

1. \*Dr. Sumona Bhattacharjee, Post Graduate Student,

2. Dr. Sheetal Sanikop, Reader,

Department of Periodontics, KLE VK Institute of Dental sciences, KLE University Belgaum – 590010.

3. Dr. Anil M, Associate Professor, J.N. Medical College, KLE University, Belgaum – 590010.

4. Dr. Suvarna Patil, Professor & Head, Department of Periodontics, KLE VK Institute of Dental sciences, KLE University, Belgaum – 590010.

\***Corresponds to:** Dr. Sumona Bhattacharjee, Post Graduate Student, Department of Periodontics, KLE VK Institute of Dental sciences, KLE University, Belgaum – 590010. **Email:** [sumona1105@gmail.com](mailto:sumona1105@gmail.com).

Hundreds of different compounds have been identified in tobacco smoke and some occur in concentrations judged to be harmful to health like the alkaloid nicotine, which appears to be responsible for the dependence that characterizes the smoking habit.<sup>10</sup> Organic nitrites, nitrous oxide, cyanates and isocyanates found in cigarette smoke have been shown to interact with folic acid and Vitamin B<sub>12</sub> coenzymes, transforming them into biologically inactive compounds. This could result in low folic acid concentration in smokers.<sup>11</sup>

Folic acid (also known as folate) is an essential vitamin, which is heat sensitive and water-soluble. It belongs to the class of vitamin compounds related to pteroylglutamic acid (PGA), which serve as cofactors in the enzymatic transfer of single carbon units in a variety of metabolic pathways. Folate mediated one-carbon metabolism represents one of the most important biochemical reactions that occur in cells. Folates are necessary for nucleic acid and mitochondrial protein synthesis, amino acid metabolism, and other cellular processes that involve single carbon transfers. It is critical to cellular division and new cell production because it is an essential co-factor in normal DNA synthesis. Folates can serve as carbon donors or acceptors. Hence, folic acid plays an important role in our body functions.<sup>12</sup>

Different metabolic pathways require carbon groups with different levels of oxidation; hence cells contain numerous enzymes that change the oxidation state of carbon groups carried by folates resulting in different metabolically active forms of folate. The predominant form of circulating folate is 5-methyltetrahydrofolic acid (5mTHF). Folates are intensely linked with vitamin B<sub>12</sub> in such a way that vitamin B<sub>12</sub> is necessary to convert 5mTHF into tetrahydrofolate (THF), another metabolically active form of folate. Low serum folate levels reflect the first stage of negative folate balance, and precede tissue depletion.<sup>12</sup>

Folic acid is also important in maintaining the integrity of the periodontium. Deficiency of folic acid is known to cause necrosis of gingiva, periodontal ligament and loss of

alveolar bone.<sup>13</sup> Folic acid deficiency also causes rapid development and progression of periodontitis by altering the defensive mechanisms that include: decreased production of lymphocytes, decreased cytotoxic T-cell activity, decreased phagocytic function of neutrophils.<sup>14</sup> Repair and maintenance of periodontal tissue requires high turnover of squamous epithelium which is impaired in cases of reduced levels of folic acid.<sup>15</sup>

There are many studies on the adverse effects of cigarette smoking on a wide variety of diseases and disturbances but the direct effect of smoking on nutrient concentration is less studied. Hence, there is a need to assess the effect of smoking on folic acid level which plays a role in periodontal disease progression, and this study has been undertaken to assess serum levels of folic acid in smokers and non smokers with chronic periodontitis.

## **Materials and Methods**

### ***Source of Data***

This study was conducted in the Department of Periodontics, KLE V.K. Institute of Dental Sciences and Department of Clinical Biochemistry, Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. An ethical clearance was obtained for the study from the ethical committee, KLE V.K. Institute of Dental Sciences, Belgaum. A total of 60 male patients, 30 smokers and 30 non-smokers in the age range of 40-65 years having chronic periodontitis, reporting to Department of Periodontics, were taken as subjects for the study. A written informed consent was obtained from the subjects at the beginning of the study.

The subjects were divided into 2 groups of 30 each: Group I - patients who have chronic periodontitis and meet the smoking criteria. Group II - patients who have chronic periodontitis and are non-smokers. Inclusion criteria included patients having chronic periodontal disease as evidenced from a pocket depth of 5mm or more and/or clinical gingival attachment loss of 1mm or more in around 30% of the existing natural teeth. Patients

should have been smoking 5 to 10 cigarettes per day for 5 years or more.

Patients who consume tobacco in any form other than smoking cigarettes, have taken a course of anti inflammatory or anti microbial therapy within the past 6 months, patients with a history of use of vitamins or iron supplements within the past 6 months, any systemic diseases or with anaemia, and patients who are alcoholic were excluded from the study.

**Clinical recordings**

Supragingival plaque was scored using the Plaque index (PI) (Silness and Loe 1964). Gingival inflammation was scored using the gingival index (GI) (Loe and Silness 1963). Bleeding on probing (BOP) was measured dichotomously (Ainamo and Bay 1975). Probing Depth (PD) and Clinical Gingival Attachment Level (CAL) were measured at six sites per tooth of all teeth using William’s periodontal probe. The probe was directed parallel to the long axis of the tooth. CAL measurements were made from the cemento-enamel junction to the bottom of the periodontal pocket or sulcus. All clinical data were recorded by one examiner.

**Sample Collection**

2.5 ml of blood was collected from all the patients after a 12 hours fasting period. Venous blood samples were obtained between 8:30 am and 11 am by venepuncture in the antecubital fossa without excessive venous stasis. Blood was taken into vacuum tubes, which was transported to Department of Clinical Biochemistry for analysis. For the determination of serum folic acid fully automated Abbott AxSYM system was used.

**Statistical Analysis**

**Table 2:** Correlation between FA with GI, PI, BOP, PD and CAL in total samples (Group I and Group II) by Karl Pearson’s correlation coefficient method

Variables	Correlation coefficient (r-value)	t-value	p-value
FA with GI	0.5280	4.7345	0.060
FA with PI	0.1755	1.3573	0.1799
FA with PD	-0.1253	-0.9617	0.3402
FA with CAL	-0.4122	-3.4456	*0.0011
FA with BOP	0.0764	0.5838	0.5616

\*p<0.05

Data were expressed as means and standard deviation (SD). The statistical significance differences between groups was tested with Mann-Whitney U-test. Students unpaired ‘t’ test was used to compare clinical parameters between two groups. To find the correlations between serum and clinical parameters in smokers and non-smokers, the Karl-Pearson’s co-efficient of correlation was used. The null hypothesis was rejected at p < 0.05.

**Results**

**Clinical characteristics**

The clinical characteristics of smokers and non-smokers are shown in Table 1 and Graph 1. When the clinical parameters were compared between groups, PI, PD and CAL were higher in smokers compared with non-smokers. Whereas the other parameters like GI, BOP was higher in non-smokers. There was no difference between groups with respect to gender.

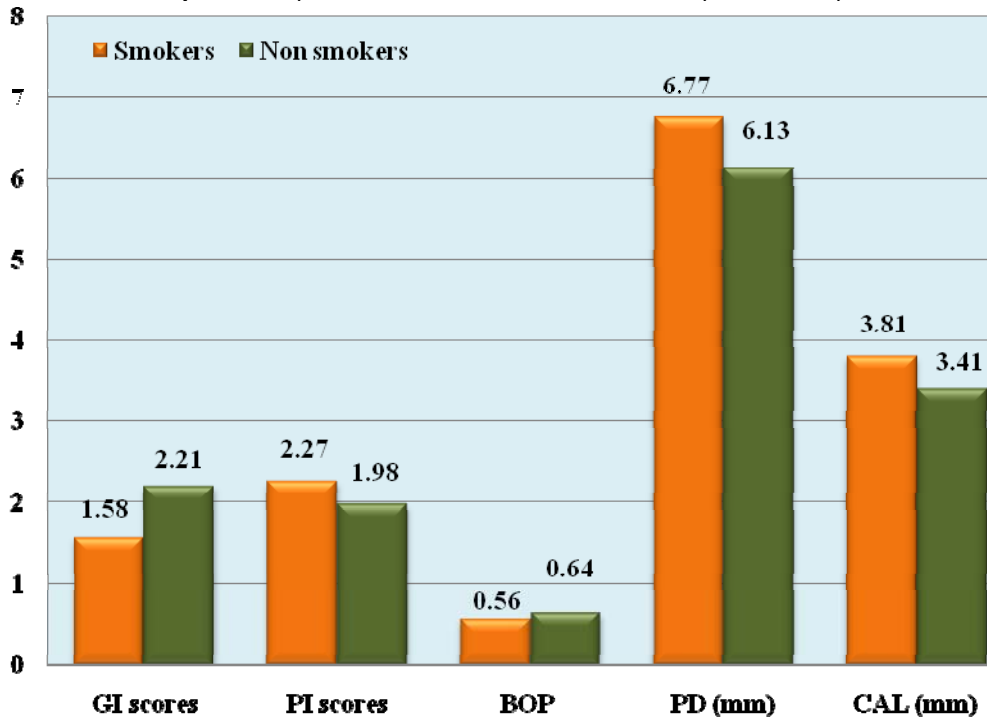
**Table 1:** Comparison of clinical parameters and folic acid levels in Group I and Group II

Parameters	Group I (Mean ± SD)	Group II (Mean ± SD)
P I	2.27 ± 2.40	1.98 ± 2.00
G I	1.58 ± 1.50	2.21 ± 2.40
B O P	0.56 ± 0.55	0.64 ± 0.66
P D	6.77 ± 7.00	6.13 ± 6.00
C A L	3.81 ± 3.79	3.41 ± 3.35
F A	7.20 ± 6.85	9.53 ± 9.70

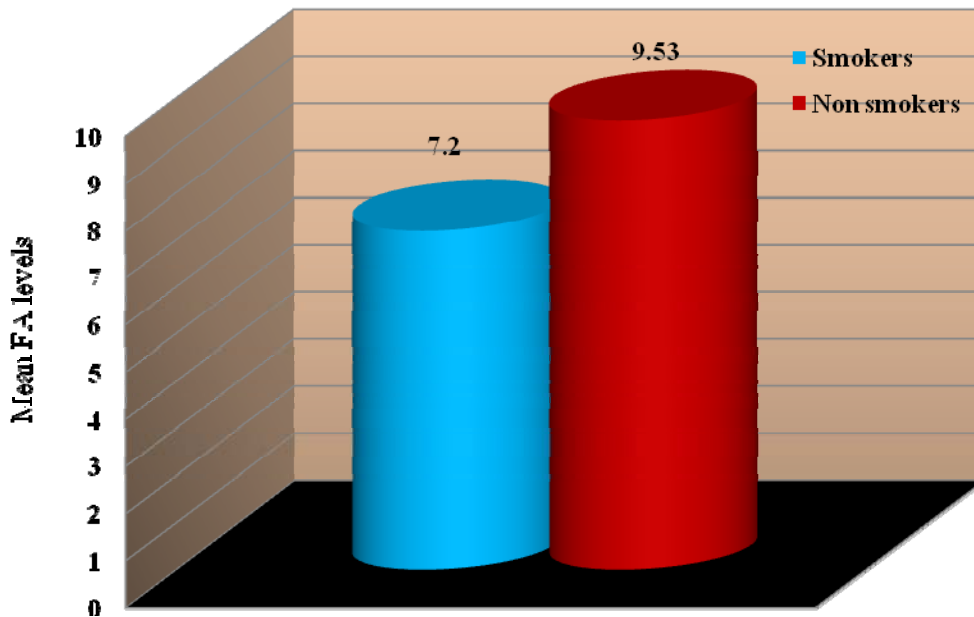
**Blood analysis**

The mean value of serum folic acid is shown in Table 2 and Graph 2. Level of serum folic acid was lower in smokers compared to nonsmokers.

**Graph 1: Comparison of Clinical Parameters in Group I and Group II**



**Graph 2: Comparison of Mean Serum Folic Acid Levels in Group I and Group II**



**Discussion**

Periodontitis is a multifactorial disease with microbial dental plaque as the initiator of periodontal disease. However, the manifestation and progression of periodontitis is influenced by a wide variety of determinants

and factors including subject characteristics, social, behavioral, systemic and genetic factors. Recent research has suggested a possible association between oral health and nutritional status in older adults.<sup>2, 16, 17</sup> Nutrition has a strong influence on the integrity of the periodontium and its deficient state can

modify the expression of the primary etiologic factor.<sup>18</sup> Periodontium is amongst the most dynamic tissues in the body, the maintenance of which is dependent upon an adequate supply of nutrients.<sup>18</sup>

Folic acid, an essential vitamin which belongs to the vitamin B group, is generally known as a hemocytopoietic vitamin and is also a factor for the growth of animals. Although the terms folic acid and folate are often used interchangeably, correctly *folic acid* refers to the oxidized compound, pteroyl monoglutamate, and the various tetrahydrofolate derivatives are collectively known as folates.<sup>19</sup> Folic acid deficiency is the most common nutrient deficiency in the world<sup>2</sup> and is associated with increased oxidative stress, endothelial dysfunction, genomic instability, defective DNA repair, and apoptosis. It has been shown to be related to a number of human diseases, including periodontal disease.<sup>16</sup> However, little is known about the influence of folic acid on patients with chronic periodontitis in relation to their smoking status. Clinical research has shown that folic acid supplementation results in a significant reduction in gingival inflammation as determined by gingival redness, bleeding tendency, tenderness, and presence of exudates.<sup>20</sup>

Cigarette smoking is a major risk factor in the incidence and progression of periodontal disease. Organic nitrites, nitrous oxide, cyanates and isocyanates found in cigarette smoke have been shown to interact with folic acid and Vitamin B<sub>12</sub> coenzymes, transforming them into biologically inactive compounds. Various direct reactions of smoke components with tetrahydrofolates could result in folic acid deficiency in smokers. Among these are reactions of tetrahydrofolates with cyanates to form a biologically inactive derivative and the reaction of methyltetrahydrofolates with organic nitrites, leading to decomposition of the co-enzymes. Thus, it is biologically plausible to expect low folic acid concentration in smokers.<sup>11</sup>

The present study was undertaken to estimate and compare serum folic acid levels in smokers and non smokers with chronic periodontitis. In addition the clinical parameters like (GI), (PI), (BOP), (PD) and clinical attachment level (CAL) were compared between the two groups.

Blood samples were collected from fasting individuals because recent food intake may appreciably increase the serum folate concentration.<sup>19</sup>

Our study revealed that the mean concentration of folic acid in Group I (smokers with chronic periodontitis) was 7.20 ng/mL and in Group II (non smokers with chronic periodontitis) was 9.53 ng/mL ( $p < 0.05$ ). From this we can infer that serum folic acid levels are significantly lower in smokers with chronic periodontitis compared to nonsmokers with comparable periodontal destruction. This was in agreement with the study done by Erdemir and Bergstrom.<sup>4, 11</sup> The low folic acid levels could be attributed to the interactions of cyanates and isocyanates with folic acid making it biologically inactive and hence decreasing its level. However, it could also be a result of low dietary intake, impaired absorption or metabolism.

In the present study the clinical parameters were assessed to determine the periodontal status and to compare the periodontal destruction in both the groups. It was observed that GI and BOP in smokers is 1.58 and 0.56 respectively compared to non-smokers who had a value of 2.21 and 0.64 respectively. The development of gingival redness was lower in smokers, suggesting a suppression of the normal inflammatory response to plaque. The reduced gingival bleeding as a result of smoking must be considered detrimental because it may lead to an inaccurate assessment of periodontal status and fail to alert the patient the presence of the disease. It may also indicate a diminution of the defense capabilities of the gingival tissues.<sup>21</sup> This effect is due to the potential vasoconstrictive effect of nicotine. Nicotine from cigarette smoke stimulates the sympathetic ganglia to produce

neurotransmitters including catecholamines. These affect the alpha receptors on blood vessels which in turn causes vasoconstriction. The vasoconstriction of peripheral blood vessels caused by smoking can also affect the periodontal tissue and can lead to less overt signs of gingival inflammation such as redness, bleeding and exudation.<sup>22</sup> The results of our study are in agreement with various other studies<sup>23-24</sup> which showed that smokers experienced less gingival bleeding than non-smokers.

Our study illustrates that the PI in smokers is 2.27 and non-smokers is 1.98, indicating that smokers have a statistically higher value. The higher plaque levels could be because of less favourable oral hygiene behaviour in smokers. Smokers have significantly more plaque than non-smokers and there is a trend towards increased plaque deposits with increasing cigarette consumption<sup>25</sup> also the tooth brushing efficiency of smokers is much less and the calcium concentration in the dental plaque of smokers is found to be significantly higher than in non-smokers.<sup>26-28</sup> Our study corroborates well with the studies done by Preber et al, Bergstrom et al, Feldman et al. and Macgregor I et al.<sup>23, 27, 29-30</sup>

The assessment of periodontal damage is a mandatory component in a periodontal examination and for this the PD and CAL were measured using a William's graduated periodontal probe in both the groups. PD and CAL values for smokers (6.77 and 3.81) were significantly higher than for non-smokers (6.13 and 3.41). Adequate tissue levels of FA are essential for maturation of epithelia with high mitotic activities. Therefore, deficiency of this vitamin, may adversely affect the sulcular epithelium predisposing the gingiva to inflammation from local factors.<sup>31</sup> Moreover, the maturation of the junctional epithelium, which has a rapid turnover rate, is of prime importance in the prevention and control of periodontal disease. Since folic acid deficiency has been associated with abnormalities in rapidly proliferating epithelial cells it is conceivable that the junctional epithelium would also be affected.<sup>32</sup> Folic acid also has an

impact on the immune system by altering the defensive mechanisms which include decreased production of lymphocytes, cytotoxic T-cell activity and phagocytic function of neutrophils.<sup>14</sup> Thus in presence of cigarette smoking which is a risk factor for periodontal disease, deficiency of folic acid may further aggravate the destruction. The results of this study are in accordance with other studies which reported higher probing depths and attachment loss in smokers.<sup>27, 29, 33-35</sup>

When folic acid was correlated with the clinical parameters in both the groups, a strong negative correlation was found with CAL, indicating that higher folic acid levels resulted in lesser loss of attachment in chronic periodontitis patients irrespective of their smoking status. From this we can infer that a higher folic acid level may result in reduced periodontal destruction.

The results of our study have shown that among patients with chronic periodontitis, smokers displayed significantly reduced levels of folic acid. This corroborates well with the observations of other studies.<sup>4, 11</sup> However, there could be several causes for the same as enumerated above.

Although cessation of smoking is the ideal objective, it is not always attainable and therefore any strategy to prevent the detrimental effects of smoking is desirable. For smokers with a deficient folic acid status, improved dietary intake of folic acid or its supplements may prove beneficial. Stronger evidence would be provided by a longitudinal design, which will clarify the timing between the deficiency state and the onset of the disease. Thus, further longitudinal and interventional studies need to be conducted on larger epidemiological groups to delineate the relationship between chronic periodontitis, folic acid and smoking.

### **Conclusion**

Based on the findings of the present study the following conclusions can be made: the serum folic acid level of Group I (smokers with

chronic periodontitis) was significantly lower compared to Group II (non-smokers with chronic periodontitis). The clinical parameters

like GI, BOP was higher in Group II, whereas the other parameters PI, PD and CAL were higher in Group I.

## References

1. Jan Lindhe. Consensus report: Chronic periodontitis. *J Periodontol* vol 4 no 1; 38: 1999. doi:10.1902/annals.1999.4.1.38.
2. M Esaki, M Morita, R Akhter, K Akino, O Honda. Relationship between folic acid intake and gingival health in non-smoking adults in Japan. *Oral Diseases* Volume 16, Issue 1, pages 96–101, January 2010 doi:10.1111/j.1601-0825.2009.01619.x. PMID:19732352.
3. Giovanni E. Salvi, Herenia P. Lawrence, Steven Offenbacher & James D. Beck. Influence of risk factors on the pathogenesis of periodontitis. *Periodontology* 2000, Vol. 14, 1997, 173-201. doi:10.1111/j.1600-0757.1997.tb00197.x. PMID:9567971.
4. Erdemir EO, Bergstrom J. Relationship between smoking and folic acid, vitamin B12 and some haematological variables in patients with chronic periodontal disease. *J Clin Periodontol* 2006; 33: 878–884. doi:10.1111/j.1600-051X.2006.01003.x. PMID:17092240.
5. Gelskey SC, Young TK, Singer DL. Factors associated with adult periodontitis in a dental teaching clinic population. *Community Dent Oral Epidemiol* 1998; 26: 226–232. doi:10.1111/j.1600-0528.1998.tb01955.x. PMID:9758422.
6. Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE, Norderyd OM, Genco RJ. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *J Periodontol* 1994; 65: 260–267. doi:10.1902/jop.1994.65.3.260. PMID:8164120.
7. Grossi SG, Genco RJ, Machtei EE, Ho AW, Koch G, Dunford R, Zambon JJ, Hausmann E. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *J Periodontol* 1995; 66: 23–29. doi:10.1902/jop.1995.66.1.23. PMID:7891246.
8. Tomar SL, Asma S. Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. *J Periodontol* 2000; 71: 743–751. doi:10.1902/jop.2000.71.5.743. PMID:10872955.
9. Linden GJ, Mullally BH. Cigarette smoking and periodontal destruction in young adults. *J Periodontol* 1994; 65: 718–723. PMID:7608851.
10. Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanism of action of environmental factors – tobacco smoking. *J Clin Periodontol* 2005; 32 (Suppl. 6): 180-195. doi:10.1111/j.1600-051X.2005.00786.x. PMID:16128837.
11. Erdemir EO, Bergstrom J. Effect of smoking on folic acid and vitamin B12 after nonsurgical periodontal intervention. *J Clin Periodontol* 2007; 34: 1074–1081. doi:10.1111/j.1600-051X.2007.01154.x. PMID:17953692.
12. [www.Abbott.com](http://www.Abbott.com).
13. Tungtrongchitr R, Pongpaew P, Soonthornruengyot M, Viroonudomphol D, Vidhuvai N, Tungtrongchitr A, et al. Relationship of tobacco smoking with serum vitamin B12, folic acid and haematological indices in healthy adults. *Public Health Nutrition* 2003, Oct; 6(7) 675-681. doi:10.1079/PHN2003483. PMID:14552668.
14. Linda D. Boyd, Theresa E. Madden. Nutrition, infection and periodontal disease. *The Dent Clin North America* 2003, Vol 47: No 2; 337-354. doi:10.1016/S0011-8532(02)00103-9.
15. Kinane DF. Causation and pathogenesis of periodontal disease. *Periodontol* 2000 2001; 25:8–20. doi:10.1034/j.1600-0757.2001.22250102.x. PMID:11155179.
16. Vogel R and M Deasy. Folic acid and experimentally produced gingivitis. *IADR abstracts* 1977.
17. Ainamo J and Bay I 1975. Problems and proposals for recording gingivitis and plaque. *International Dental Journal* 25, 229-235. PMID:1058834.
18. Newman MG, Takei HH and Carranza FA. *Clinical Periodontology* 10th edition; Saunders.
19. Robert E. Schifferle. Periodontal disease and nutrition: separating the evidence from current fads. *Periodontology* 2000, Vol. 50, 2009, 78–89. doi:10.1111/j.1600-0757.2008.00297.x. PMID:19388955.

20. Neiva RF, Steigenga J, Al-Shammari KF, Wang H-L. Effects of specific nutrients on periodontal disease onset, progression and treatment. *J Clin Periodontol* 2003; 30: 579–589. doi:10.1034/j.1600-051X.2003.00354.x. PMID:12834494.
21. David A. Bender. *Nutritional Biochemistry of the Vitamins*. 2nd edi.2003.
22. Burtis CA, Ashwood ER. *Biochemical aspects of Hematology*. Tietz Textbook of Clinical Chemistry. Second edition. WB Saunders, 1994: 2056.
23. Bergström J, Floderus-Myrhed B. Co-twin control study of the relationship between smoking and some periodontal disease factors. *Community Dent Oral Epidemiol* 1983; 11: 113–116. doi:10.1111/j.1600-0528.1983.tb01367.x. PMID:6573237.
24. Hanioka T, Tanaka M, Ojima M, Takaya K, Matsumori Y, Shizukuishi S. Oxygen sufficiency in the gingiva of smokers and non-smokers with periodontal disease. *J Periodontol* 2000; 71: 1846–1851. doi:10.1902/jop.2000.71.12.1846. PMID:11156041.
25. Bergström J, Eliasson S, Dock J. Exposure to tobacco smoking and periodontal health. *J Clin Periodontol* 2000; 27: 61–68. doi:10.1034/j.1600-051x.2000.027001061.x.
26. Christen AG. The clinical effects of tobacco on oral tissue. *J Am Dent Assoc* 1970; 81: 1378-1382. PMID:5273603.
27. Ana Pejic, Radmila Obradovic, Ljiljana Kesic. *Smoking and Periodontal Disease: A Review*. *Medicine and Biology* Vol 14, No 2, 2007; 53-59.
28. Feldman RS, Bravacos JS, Rose CL. Association between smoking different tobacco products and periodontal disease indexes. *J Periodontol* 1983; 54: 481-487. PMID:6578319.
29. Philip M. Preshaw, Brenda Lauffart, Elena Zak, Marjorie K. Jeffcoat. *Progression and Treatment of Chronic Adult Periodontitis*. *J Periodontol* 1999; 70:1209-1220.
30. Amarasena N, Ekanayaka ANI, Herath L, Miyazaki H. tobacco use and oral hygiene as risk indicators for periodontitis. *Comm Dent Oral Epid* 2002; 30; 115-123. doi:10.1034/j.1600-0528.2002.300205.x. PMID:12000352.
31. Hyman JJ, Reid BC; Epidemiologic risk factors for periodontal attachment loss among adults in the United States. *J Clin Periodontol* 2003; 30: 230–237. doi:10.1034/j.1600-051X.2003.00157.x. PMID:12631181.
32. Vogel R and Richard A Fink. The effect of folic acid on gingival health. *J Periodontol*. 11: 667-668. 1976.
33. Krall EA, Garvey AJ, Garcia RI. Alveolar bone loss and tooth loss in male cigar and pipe smokers. *J Am Dent Assoc* 1999; 130: 57–64. PMID:9919032.
34. Jan Bergstrom, Soren Eliasson, Jan Dock. A 10-year prospective study of tobacco smoking and periodontal health. *J Periodontol* 2000;71: 1338-1347. doi:10.1902/jop.2000.71.8.1338. PMID:10972650.
35. Macgregor I, Edgar W, Greenwood A. Effects of cigarette smoking on the rate of plaque formation. *J Clin Perio* 1985; 12; 35-41. doi:10.1111/j.1600-051X.1985.tb01351.x. PMID: 3855869.