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Relationship between smoking and folic acid, vitamin B_{12} and some haematological variables in patients with chronic periodontal disease

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Abstract

Aim: The purpose of this study was to investigate the relationship between cigarette smoking and the serum levels of folic acid, vitamin B_{12} and some haematological variables in patients with periodontal disease.

Patients and methods: The study base consisted of 88 volunteer patients with periodontal disease, including 45 current smokers in the age range 31–68 years and 43 non-smokers in the range 32–66 years. The clinical parameters included plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD) and clinical attachment loss (CAL). Folic acid, vitamin B_{12} and haematological variables were determined from peripheral blood samples.

Results: PI, PD and CAL means were significantly higher in smokers than non-smokers ($p < 0.05$). The serum folic acid concentration of smokers was lower than that of non-smokers ($p < 0.05$), whereas the white blood cell count was higher in smokers than in non-smokers ($p < 0.05$).

Conclusion: The results of this study suggest that among patients with periodontal disease the serum folic acid concentration is lower in smokers compared with non-smokers.

Keywords: chronic periodontitis; folic acid; smoking; vitamin B_{12}

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Periodontal disease is a multifactorial disease, the origins of which remain obscure. However, the manifestation and progression of periodontitis are influenced by a wide variety of determinants including social and behavioural factors, systemic factors and genetic factors (Nunn 2003).

The role of smoking as a risk factor for periodontal disease is well documented (Feldman et al. 1983, Bergstrom & Eliasson 1987a, b, Bergstrom 1989, Genco & Löe 1993, Bergstrom & Preber 1994, Grossi et al. 1994, 1995, Genco 1996, Borrell & Papapanou 2005, Heitz-Mayfield 2005, Palmer et al. 2005, Tonetti & Claffey 2005). Cigarette smoking is a strong predictor of deeper probing depths (PDs) (Bergstrom & Eliasson 1987a, b, Linden & Mullally 1994, Haffajee & Socransky 2001, Bergstrom 2003), greater attachment loss (Grossi et al. 1994, Linden & Mullally 1994, Axelsson et al. 1998, Haffajee & Socransky 2001), more bone loss (Bergstrom & Eliasson 1987a, Grossi et al. 1995, Bergstrom et al. 2000) and fewer teeth (Axelsson et al. 1998, Haffajee & Socransky 2001). Smokers also exhibit more supragingival calculus deposits (Bergstrom 1999). It is widely accepted that smoking impairs various aspects of innate and immune host responses (Kinane & Chestnutt 2000). Numerous functions of oral or peripheral neutrophil are negatively affected by smoking including phagocytosis (MacFarlane et al. 1992), superoxide and hydrogen peroxide generation (Pabst et al. 1995, Ryder et al. 1998a), integrin expression (Ryder et al. 1998b) and protease inhibitor production (Persson et al. 2001). Even exposure to environmental cigarette smoke is associated with increased leucocyte counts, chemotaxis and increased release of reactive oxidants from stimulated neutrophils (Anderson et al. 1991).

Cigarette smoking also affects vitamin B_{12} and folic acid mechanisms. Vitamin B_{12} is a water-soluble, heat-sensitive vitamin of the B-vitamin group (Herbert 1998). It often occurs protein bound as methylcobalamin, hydroxycobalamin and deoxyadenosycobalamin in nutrients (Lee 1993). It is almost only provided through food of animal origin, the largest amounts being found in the liver and kidney and considerable amounts in meat, dairy products and eggs (Herbert 1998).

Folic acid (also known as folate) is also heat-sensitive and water-soluble, closely linked to B_{12} in its metabolism and works with vitamin B_{12} in many functional processes throughout the body, including the periodontium (Haller 1999). It is critical to cellular division and new cell production because it is an essential co-factor in normal DNA synthesis (David & Eaton 2003). Repair and maintenance of periodontal tissue generates a high turnover rate of squamous epithelium. Without folic acid, epithelial cells do not divide properly. It is naturally found in leafy green vegetables, fruits, orange juice, whole grains, and is important in DNA synthesis and repair (Snow 1999).

It has been suggested that folic acid deficiency is the most common nutrient deficiency in the world (Krause & Mahan 1984). Gastrointestinal diseases, renal dysfunction, diabetes mellitus, thyroid dysfunction, malignancies, genetics, nutrition, medication, age and gender, chronic inflammatory diseases and alcohol consumption and cigarette smoking are the factors that affect vitamin B_{12} and folic acid (Lökk 2004). Little is known about the influence of deficiency of essential nutrient factors on the periodontium. It may weaken the host resistance of the periodontal tissues as well. The immune suppression, which includes impaired cytokine function as well as diminished acute-phase response to infections, impacts negatively on the natural history of inflammatory periodontal diseases (Enwonwu & Sanders 2001). Low-dose folic acid treatment exerts beneficial effects on patients with hyperhomocysteinaemia by inhibiting pro-inflammatory responses such as chemokine secretion from human monocytes (Wang et al. 2005).

According to some studies, folic acid supplementation produces significant reduction of gingival inflammation as determined by decreased redness, bleeding, tenderness and exudates (da Costa & Rothenberg 1974, Vogel et al. 1976, 1978, Pack & Thomson 1980, Thomson & Pack 1982, Pack 1984). What significance these findings might have on the development and the progression of periodontal disease is currently not known.

Cyanide intake associated with cigarette smoking (Stedman 1968) adversely affects vitamin B_{12} nutritional status (Dastur et al. 1972, Linnell et al. 1986). In addition, many other mechanisms resulting from various direct reactions of smoke components with tetrahydrofolates could result in folate deficiency in tissues affected by cigarette smoke. Among these are reactions of tetrahydrofolates with cyanates to form a biologically inactive derivative (Francis et al. 1977) and the reaction of methyltetrahydrofolates with organic nitrites, leading to decomposition of the coenzymes. Organic nitrites also inactivate methyl cobalamin by cleaving the methyl–cobalamin bond, resulting in the formation of nitrocobalamin (Khaled et al. 1986).

The mechanisms described above and possibly others suggest that it is biologically plausible to expect low folic acid and vitamin B_{12} concentrations in smokers. In support of this, a number of studies have focused on the effect of smoking on the serum levels of folic acid and vitamin B_{12} in adults (Dastur et al. 1972, Witter et al. 1982, Nakazawa et al. 1983, Linnell et al. 1986, Chen et al. 1989, Piyathilake et al. 1992), but no studies have as yet analysed the relationship of smoking with folic acid or with vitamin B_{12} in patients with chronic periodontitis. Therefore, the aim of this study was to investigate serum levels of folic acid, vitamin B_{12}, white blood cell (WBC) count and differential blood count in smokers and non-smokers with chronic periodontal disease.

Material and Methods

Patients

The study population included 88 patients, 45 smokers and 43 non-smokers in the age range of 30–69 years. The patients had chronic periodontal disease as evidenced from a PD of 6 mm or more at 80% of the proximal sites and radiographic bone loss $>50\%$ (Consensus report 1999). All participants were periodontally untreated and had not previously received surgical therapy and were drawn from the waiting list of patients with untreated chronic periodontal disease at the Department of Periodontology at Kirikkale University, Faculty of Dentistry. All subjects had no medical condition that would affect their participation in the study. An extensive medical history was taken both by a written questionnaire and by interview. Exclusion criteria applied were a course of anti-inflammatory or antimicrobial therapy within the previous 3 months, a history of use of vitamin or iron supplementation within the previous 3 months. Pregnant women and individuals who had, apart from periodontitis, any given acute or chronic medical condition, including diabetes, viral, fungal or bacterial infections, or had recent trauma or tooth extractions, were also excluded. None of the patients were alcohol consumers or obese. The purpose and nature of the study, including the types of clinical measurements and sample collection, were explained to all potential participants. After reading and signing the consent form, the subjects were enrolled into the study. The study was approved by the Medical Ethical Committee of Kirikkale University, Faculty of Dentistry.

For all participants, smoking habits were recorded and patients were classified as either current smokers, i.e., regular smokers of 20 cigarettes/day (45 patients), or non-smokers, i.e., individuals who had never smoked tobacco (43 patients). All smokers were cigarette smokers. Patients who had been smokers for 15–20 years were included in the study. The mean $\pm$ SD number of years smoked was 17.5 ± 1.7. The mean $\pm$ SD age of smokers and non-smokers was 45.5 ± 8.5 and 45.9 ± 8.0, respectively. The age difference between groups was not statistically significant ($p > 0.05$). Body mass index (BMI) was also recorded and there was no statistically significant difference between groups ($p > 0.05$).

Clinical recordings

Supragingival plaque was scored using the plaque index (PI; Silness & Löe 1964). Gingival inflammation was scored using the gingival index (GI; Löe & Silness 1963). Bleeding on probing (BOP) was measured dichotomously (Ainamo & Bay 1975). PD and clinical attachment level (CAL) were measured at six sites per tooth of all teeth using a conventional periodontal probe (Hu-Friedy, Chicago, IL, USA). 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 (E. O. E.).

Blood analysis

After a 12-h fasting period, venous blood samples were obtained between 08:30 and 11:00 hours by venepuncture in the antecubital fossa without excessive venous stasis. The blood was taken into EDTA-containing vacuum tubes (HEMA, Neuss, Germany) in the Faculty of Medicine, Department of Biochemistry. The laboratory analysis of differential blood count was performed immediately with the Sysmex XT 2000i (Roche, Basel, Switzerland).

Folic acid, vitamin B_{12}, haemoglobin, WBC count and differential blood count were calculated according to standardized and automated procedures

Statistical analysis

Data were expressed as means and SD. The statistical significance of differences between groups was tested with the Mann–Whitney *U*-Test. Simple pairwise correlation coefficients were calculated according to the product–moment correlation method of Pearson. The null hypothesis was rejected at $p<0.05$.

Multiple linear regression analysis was performed with folic acid as the dependent variable. Logistic regression was used to estimate the relative risk expressed as odds ratio (OR) and 95% confidence interval (CI). Folic acid was the dependent variable, dichotomized (<7.0 ng/ml $=1$, $n=22$, otherwise $=0$, $n=66$). Age was stratified according to (1) 30–45 years ($n=47$), and (2) 46–71 years ($n=41$); vitamin B_{12} according to (1) <290 pg/ml ($n=29$), (2) 291–390 pg/ml ($n=30$) and (3) >390 pg/ml ($n=29$); and haemoglobin according to (1) <13.5 g/dl ($n=28$), (2) 13.5–15.4 g/dl ($n=30$) and (3) >15.4 g/dl ($n=30$).

Results

Clinical characteristics

The clinical characteristics of smokers and non-smokers are shown in Table 1. When the clinical parameters were compared between groups, PI, PD and CAL were significantly higher in smokers compared with non-smokers ($p<0.05$). There were no significant differences between smokers and non-smokers in the mean values of GI and BOP ($p>0.05$). The number of teeth present was comparable in study groups, the mean $\pm$ SD for smokers and non-smokers being 24.6 ± 4.2 and 24.5 ± 3.6, respectively. There was no difference between groups with respect to gender.

Blood analysis

The mean values of serum parameters are shown in Table 2. The levels of folic acid and haemoglobin were lower, whereas WBC counts and the percentage of neutrophils were higher in smokers compared with non-smokers ($p<0.05$). The levels of vitamin B_{12} and the percentages of lymphocytes, monocytes, eosinophils and basophils did not differ between groups ($p>0.05$).

Correlations

Correlations between serum and clinical parameters are shown in Tables 3 and 4 for smokers and non-smokers, respectively. In smokers, there was a positive correlation between GI and folic acid ($p<0.01$). In addition, there were positive correlations between monocytes in serum and PI, PD and CAL ($p<0.05$). There were no significant correlations between serum parameters and clinical parameters in non-smokers ($p>0.05$).

The relation between folic acid as the dependent variable and HGB, WBC, vitamin B_{12}, neutrophils, lymphocytes, monocytes, eosinophils, basophils, PI, GI, BOP, PD, CAL, smoking, age and gender as predictors was analysed by means of multiple linear regression. Vitamin B_{12}, GI and smoking were the only significant predictors. The variables explained 27% of the variance in the dependent variable [R^2 (adjusted) $=0.27$, $F(16, 71)=3.0$, $p<0.001$] (Table 5).

An analysis of covariance was run with folic acid as the dependent variable and smoking as the independent factor and vitamin B_{12} and GI as covariates. The results suggested that a highly significant association between smoking and folic acid remained when controlling for vitamin B_{12} and GI [ANCOVA $F(1, 84)=17.1$, $p<0.001$; Scheffé's test $p<0.0001$].

Logistic regression analysis was run to estimate the relative risk for a low folic acid level as defined by the 25th percentile of the distribution (<7.0 ng/ml). In univariate analysis, smoking was associated with a 9.7-fold elevated risk for a low folic acid level (OR = 9.7, 95% CI 2.6–36.9, $p<0.001$). Further low vitamin B_{12} levels increased the risk for a low folic acid level by 2.7 times/level. None of the other variables were associated with an increased risk. When smoking and vitamin B_{12} were run together in a bivariate model, the relative risk for a low folic acid level was 12.7-fold elevated in smokers compared with non-smokers (OR = 12.7 95% CI 3.1–52.7, $p<0.001$, Table 6). Including, additionally, HGB, BOP,

Table 1. Clinical parameters of smokers and non-smokers (mean $\pm$ SD)

Parameters	Smokers ($n=45$)	Non-smokers ($n=43$)
PI	2.06 ± 0.33*	1.79 ± 0.27
GI	1.82 ± 0.31	1.92 ± 0.16
BOP	0.56 ± 0.40	0.59 ± 0.34
PD	5.04 ± 0.61*	4.75 ± 0.56
CAL	4.84 ± 0.51*	4.41 ± 0.54

PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, clinical attachment loss.
*$p<0.05$ according to the Mann–Whitney *U*-test.

Table 2. Serum parameters in smokers and non-smokers (mean $\pm$ SD)

Parameters	Smokers ($n=45$)	Non-smokers ($n=43$)
Folic acid (ng/ml)	8.01 ± 2.79*	10.8 ± 2.87
Vitamin B_{12} (pg/ml)	363.8 ± 150.3	363.4 ± 121.3
HGB (g/dl)	13.4 ± 2.19*	14.9 ± 1.58
WBC (10^3/μl)	7.72 ± 2.10*	6.69 ± 1.76
Neutrophils (%)	61.0 ± 7.61*	55.4 ± 10.8
Lymphocytes (%)	33.6 ± 7.62	33.7 ± 9.32
Monocytes (%)	7.58 ± 1.55	7.53 ± 2.52
Eosinophils (%)	2.29 ± 0.99	2.30 ± 1.72
Basophils (%)	0.34 ± 0.15	0.36 ± 0.19

HGB, haemoglobin; WBC, white blood cell.
*$p<0.05$ according to the Mann–Whitney *U*-test.

Table 3. Correlations between serum and clinical parameters in smokers ($n = 45$)

Parameters	PI	GI	BOP	PD	CAL
Folic acid (ng/ml)	− 0.051	0.388**	0.067	0.235	0.222
Vitamin B_{12} (pg/ml)	0.230	− 0.033	0.269	0.151	0.057
HGB (g/dl)	0.036	− 0.175	− 0.132	− 0.062	− 0.215
WBC (10^3/μl)	0.029	− 0.172	0.087	− 0.033	− 0.207
Neutrophils (%)	− 0.060	0.026	0.031	0.192	0.147
Lymphocytes (%)	− 0.176	0.010	− 0.159	0.251	− 0.104
Monocytes (%)	0.340*	0.150	0.289	0.344*	0.325*
Eosinophils (%)	− 0.075	− 0.038	− 0.059	0.024	− 0.019
Basophils (%)	0.107	− 0.169	0.103	0.022	0.024

Pearson correlation coefficients
*$p < 0.05$,
**$p < 0.01$ level
HGB, haemoglobin; WBC, white blood cell.

Table 4. Correlations between serum and clinical parameters in non-smokers ($n = 43$)

Parameters	PI	GI	BOP	PD	CAL
Folic acid (ng/ml)	0.002	− 0.033	− 0.055	− 0.105	0.048
Vitamin B_{12} (pg/ml)	− 0.029	− 0.073	− 0.291	− 0.022	0.029
HGB (g/dl)	0.036	− 0.175	− 0.132	− 0.062	− 0.215
WBC (10^3/μl)	− 0.195	− 0.173	0.055	0.009	0.044
Neutrophils (%)	0.145	− 0.140	0.152	0.199	0.024
Lymphocytes (%)	− 0.177	0.098	− 0.113	− 0.220	− 0.030
Monocytes (%)	− 0.108	0.108	− 0.165	0.179	0.234
Eosinophils (%)	0.144	0.198	− 0.121	− 0.109	− 0.017
Basophils (%)	0.069	0.255	0.050	0.142	0.085

Pearson's correlation coefficients
HGB, haemoglobin; WBC, white blood cell; PI, plaque index; GI, gingival index; PD, pocket depth; CAL, clinical attachment loss.

Table 5. Multiple regression analysis with folic acid as the dependent variable and including all variables as predictors

$n = 88$	Coefficient	SE	*p*-value
Smoking	− 2.233	0.756	0.004*
Vitamin B_{12}	2.187	0.856	0.012*
HGB	0.156	0.216	0.473
WBC	− 0.251	0.186	0.182
Neutrophils	0.174	0.151	0.252
Lymphocytes	0.160	0.162	0.325
Monocytes	0.029	0.167	0.863
Eosinophils	1.017	1.013	0.319
Basophils	0.155	2.504	0.951
PI	− 6.251	3.268	0.060
GI	8.377	3.603	0.023*
PD	− 0.569	0.869	0.514
CAL	0.751	0.892	0.402
Age	0.072	0.041	0.083
Gender	− 0.785	0.839	0.352

R^2 adjusted = 0.27
*$p < 0.05$ according to multiple regression analysis.
HGB, haemoglobin; WBC, white blood cell; PI, plaque index; GI, gingival index; PD, pocket depth; CAL, clinical attachment loss.

Table 6. Logistic regression analysis with folic acid as the dependent variable and smoking and vitamin B_{12} as independent variables

$n = 88$	OR	95% CI	*p*-value
Smoking			
No	1.0		
Yes	12.7	3.0; 52.7	0.0004
Vitamin B_{12}			
High	1.0		
Moderate	3.3	1.5; 7.1	
Low	10.7	2.3; 50.2	0.002

OR, odds ratio; CI, confidence interval.

CAL and age in a multivariate model, the smoking-associated risk for a low folic acid level was 14.7-fold elevated (OR = 14.7, 95% CI 3.0–73.4, $p < 0.001$).

Discussion

There are many studies on the adverse effects of cigarette smoking on a variety of diseases and disturbances, but the direct effect of smoking on nutrient concentrations is less well studied (Preston 1991). In the present study, the serum levels of folic acid, vitamin B_{12} and some haematological factors were explored in patients with chronic periodontal disease in relation to the patients' smoking habits. The results suggested that folic acid and haemoglobin levels were decreased, whereas WBC count and the proportion of neutrophils were increased in smoking patients compared with non-smoking patients. The most salient finding was that the folic acid level of smokers was lowered by 26% in comparison with that of non-smokers. The result is in line with several previous studies indicating lowered serum folic acid concentrations in smokers (Piyathilake et al. 1994, Mansoor et al. 1997, O'Callaghan et al. 2002, van Wersch et al. 2002, Ozerol et al. 2004).

Although none of the smokers exceeded the lower limit of the reference range for serum folic acid (3.0 ng/ml, Snow 1999), all patients with levels below 5.0 ng/ml and 86% of patients with levels below 6.0 ng/ml were smokers. Adjusting for the influences of vitamin B_{12} and other haematological factors as well as periodontal inflammatory characteristics in multivariate analysis, the relative risk to belong to the 25% with the lowest serum folic acid levels (< 7.0 ng/ml) was more than 10-fold elevated among smokers in this patient group. The clinical relevance of this finding remains uncertain, although folic acid is known to be of importance in DNA synthesis, host resistance and repair. In particular, it may modulate homocysteine metabolism. An inverse relationship between low folate levels and high homocysteine levels in smokers is associated with increased risk of cardiovascular disease (O'Callaghan et al. 2002). Thus, folic acid may be a marker of hyperhomocysteine. What impact hyperhomocysteine may have on periodontal disease progression is, however, currently not known. On the other hand, a lowered folic acid concentration may simply reflect a reduced vitamin intake in smokers (O'Callaghan et al. 2002).

There are several causes of inadequate serum levels of vitamin B_{12} and folic acid: the binding proteins of the vitamins may be falsely high or low, the distribution to the cell may be disturbed and enzymatic defects may demand higher vitamin levels and serum levels do not mirror tissue levels. Serum levels may be considered specific, however non-sensitive (Lökk 2004). The levels of folic acid in serum reflect recent intake, whereas red blood cell levels reflect long-term intake and tend to be more stable (Snow 1999). It is therefore not possible to conclude anything about

the relation between folic acid and periodontal disease.

Although positively correlated with folic acid in multiple regression analysis, vitamin B_{12} did not significantly differ between smoking groups. This observation is in line with most previous studies (Piyathilake et al. 1994, O'Callaghan et al. 2002, van Wersch et al. 2002, Ozerol et al. 2004).

As expected, we observed that the WBC and neutrophil counts of smokers were greater than those of non-smokers. It is well known that smoking is associated with increased WBC and neutrophil counts (Corre et al. 1971, Bridges et al. 1985). Fredriksson et al. (1998) reported significantly higher leucocyte counts in 17 treated periodontitis patients compared with 17 age- and sex-matched healthy controls. No information was given regarding the smoking habits of the subjects investigated. In another study by the same group, significantly higher leucocyte counts were reported in non-smoking periodontitis patients compared with non-smoking healthy controls. The difference between smoking periodontitis patients and smoking controls was not statistically significant (Fredriksson et al. 1999).

Interestingly, a positive correlation was observed between GI and folic acid concentration in smokers but not in non-smokers, suggesting that both GI and folic acid were dependent on smoking. This was confirmed by means of analysis of covariance with GI as a covariate. In addition, GI was significantly lower in smokers than non-smokers when PI and PD were controlled for (data not shown). These observations agree with several previous reports suggesting a depressed clinical inflammatory response in smoker patients (Preber & Bergstrom 1985, Bergstrom & Preber 1986, Bergstrom 1989, 1990, Axelsson et al. 1998, Lie et al. 1998, Bergstrom & Bostrom 2001, Dietrich et al. 2004). The literature on smoking and clinical parameters of inflammation, however, contains somewhat conflicting results. Some studies have failed to find a difference between smokers and non-smokers or have found higher values in smokers (Haber et al. 1993, van der Weijden et al. 2001).

In the present study, PD and CAL values were higher in smokers than non-smokers. This observation, suggesting that among patients of comparable age smokers suffer more severe disease than non-smoker, is in agreement with several previous studies, which report higher PD (Feldman et al. 1983, Bergstrom & Eliasson 1987a, Stoltenberg et al. 1993, Linden & Mullally 1994, Zambon et al. 1996, Bergstrom et al. 2000, Machuca et al. 2000, van der Weijden et al. 2001) and CAL values (Grossi et al. 1995, Zambon et al. 1996, Axelsson et al. 1998, Albandar et al. 2000, Machuca et al. 2000, van der Weijden et al. 2001) in smokers than non-smokers.

The BMI measures were also collected due to the well-recognized effect of adiposity on systemic host response (Tanaka et al. 1993, Stallone 1994). Nishida et al. (2005) suggested that the immunological disorders or inflammation might be the reason why obese smokers tend to exhibit escalating poor periodontal status relative to non-obese and non-smoking individuals. Because of this, obese patients were excluded from the study and also the difference between the groups was not significant.

A limitation of this study is its cross-sectional design, in which outcomes and risk factors are ascertained concurrently. Stronger evidence would be provided by a longitudinal design, which will clarify the timing between the deficiency state and the onset of the disease. In the present study, no control for disease status was used and hence no conclusion can be drawn with regard to the possible importance of folic acid for disease occurrence. However, bearing in mind that smokers are more susceptible to the disease, the observations presented might give some clue as to why smoking is rendering the individual more susceptible.

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 insufficient intake and/or a deficient folic acid status, improved dietary intake or a folic acid supplement is advisable.

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Clinical Relevance

A number of studies have focused on the effect of smoking on the serum levels of folic acid and vitamin B_{12} in adults, but no studies have as yet analysed the relationship of smoking with folic acid or vitamin B_{12} in patients with chronic periodontal disease in relation to the patients' smoking habits. The results suggested that folic acid and haemoglobin levels were decreased, whereas WBC count and the proportion of neutrophils were increased in smokers compared with non-smokers. The most salient finding was that the folic acid level of smokers was lowered by 26% in comparison with that of non-smokers. For smokers with insufficient intake and/or a deficient folic acid status, improved dietary intake or a folic acid supplementation is advisable.