Original research article

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RELATIONSHIP BETWEEN FOLIC ACID, ESR AND HAEMATOLOGICAL VARIABLES IN PATIENTS WITH CHRONIC PERIODONTAL DISEASE OF SMOKERS AND NON SMOKERS

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Abstract

Aim: Smoking is one of the important factors that can change the folic acid levels. The aim of the present study was to compare the serum folic acid levels, ESR and haematological variables in patients with chronic periodontal disease in relation to the patient's smoking habits. **Materials and methods:** A total of 50 subjects in the age range of 25 to 65 years were included in the study with 25 subjects each in group A – patients of periodontitis who were smokers and group B - patients of periodontitis who were 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 observed for all the patients. Folic acid,ESR and haematological variables were determined from peripheral blood samples. The results were statistically analysed. **Results:** Serum folic acid levels of non-smokers were significantly higher than that of smokers ($p < 0.05$). Plaque index , Probing Depth and Clinical Attachment Level means were significantly lower for Group B (Periodontitis and non-smokers) and higher for Group A (Periodontitis and smokers).ESR was higher whereas BOP was lower in smokers than non smokers ($p < 0.05$). **Conclusion*:*** This study suggests that among patients with chronic periodontal disease, the serum folic acid levels are lower in smokers as compared with non-smokers.

Key words: smoking, folic acid, nutrition, Periodontitis

INTRODUCTION

Periodontitis is an infectious disease that culminates in inflammation within the soft tissues surrounding the teeth causing progressive bone loss. The bone losing characteristic can be identified on a dental X-ray and the formation of pockets and recession. Though common in adults, but can occur at any age. Periodontitis though initiated by bacterial growth into the plaque, but host defence mechanisms play a central role in its pathogenesis.**(1)**

It is a multifactorial disease influenced by a broad variety of determinants like social, behavioural, systemic, environmental and genetic factors.**(2)**

Smoking undoubtedly is one of the most important and prevalent risk factor for chronic periodontitis among all the environmental factor which affect speriodontium via various aspects. It mainly affects innate and immune host responses. Neutrophil functions like phagocytosis, superoxide and hydrogen peroxide generation, integrin expression and protease inhibitor production are negatively affected by smoking. Protease release from neutrophil may cause tissue destruction. Smoking accelerates pocket formation, clinical attachment loss and bone loss. **(3)**

Folic acid is essential for the proper maturation of rapidly proliferating epithelial cells. Folic acid is also known as folate. It is heat sensitive and water-soluble vitamin. It is a member of class of vitamin compounds related to pteroylglutamic acid (PGA).In a number of metabolic pathways, folic acid acts as an essential cofactor in the enzymatic transfer of single carbon units by serving as carbon donor or acceptor. Folate coenzymes function in the single carbon transfer reactions involved in amino acid conversions, methyl-group biogenesis, and nucleoprotein formation. Beingan essential co-factor in normal DNA synthesis, it is also critical to cellular division and new cell production.**(4)** It is due to impairment in dTMP synthesis, which leads to cell cycle hold in the S-phase of fast proliferating cells, predominantly hematopoietic cells.

Folic acid-free diet producesa syndrome, the main sign and symptoms of which are weight loss, lassitude, alopecia, diarrhea, anemia, leukopenia, granulocytopenia, and mucous membrane lesions in the mouth and gastrointestinal tract, which culminates in debilitation,prostration, and death in 59 to 136 days.It also causes bilateral angular cheilosis. Folic acid also reduces the chances of neural tube defects, including spina bifida and facial clefs in cases of pregnancy.

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. 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. Repair and maintenance of periodontal tissue requires high turnover of squamous epithelium which is impaired in cases of reduced levels of folic acid. **(5-10)**

Smoking can be done by various ways. It can be taken in the form of tobacco smoking or either in the form of cigarette smoking. It has been found that Tobacco smoke can affect badly both cell mediated and humoral immunity. Cigarette smoking adversely affects folic acid level and ESR level. Cigarette smoke induces endothelial damage by producing free radicals such as nitric oxide and hydrogen peroxide. This oxidative stress encourages a systemic acute phase reaction thus rising C-reactive protein, fibrinogen, inflammatory cytokines, blood cell count, whole blood viscosity and rouleaux formation. Eventually this leads to rise in ESR values.

MATERIALS AND METHODS

Source of Data

This study was conducted in the Department of Periodontology and Implantology, Mahatma

Gandhi Dental College and Department of Clinical Biochemistry, Mahatma Gandhi medical college and Hospital, Jaipur (Rajasthan).Ethical clearance was obtained from institutional ethical committee. For all participants, smoking habits were recorded and patients were classified as 25 cigarette smokers and 25non-smokers. Patients who had been smokers for 10–20 years were included in the study.

The study population included 50 patients in the age range of 25-65 years; Group 1 comprised of 25 smokers with chronic periodontitis and group 2 of 25 non-smokers with chronic periodontitis. The patients had chronic periodontal disease as evidenced from "more than 30% of the teeth were affected (with) probing depths more than 4mm and (the) amount of clinical attachment loss was consistent with the presence of mineralized plaque".

Patient who took a course of anti-inflammatory or antimicrobial therapy within the previous 3 months, or a history of use of vitamin or iron supplementation or folic acid within the previous 3 months, pregnant women and obese patients and patients who had recent trauma or tooth extractions, were excluded. Alcoholic patients were totally excluded. Clinical measurements and sample collection were explained to all potential participants. After reading and signing the consent form, the subjects were included into 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 Attachment Level (CAL) were measured at six different sites of affected tooth using William's periodontal probe that was directed parallel to the long axis of the tooth. CAL measurements were made from the cement enamel junction to the bottom of the periodontal pocket or sulcus. All clinical data were recorded by a single examiner.

Sample Collection

2.5 ml of blood was collected from all the patients after a 12 hours fasting period. Collection of venous blood samples was done between 8:30 am and 11 am by venepuncture in the antecubital fossa. Blood was collected into vacuum tubes and transported to Department of Clinical Biochemistry for analysis. Fully automated Abbott AxSYM system was used for determination of serum folic acid.

Statistical Analysis

Data were expressed as mean and standard deviation (SD). The statistical difference between groups was tested with independent t-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, 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. When the clinical

parameters were compared between groups, PI, PD,BOP 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 ($p>0.05$). 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 ESR higher in smokers compared with non-smokers ($p<0.05$). The percentages of neutrophils, lymphocytes, monocytes, eosinophils and basophils did not differ between groups ($p>0.05$).

Correlations:

Correlations between serum and clinical parameters are shown in Tables 4 and 5 for smokers and non-smokers, respectively.

In non smokers, there was a positive correlation between haemoglobin and PI ($p<0.05$). In smokers, there was a positive correlation between haemoglobin and PI ($p<0.05$) as well as WBC and PD ($p<0.05$).In addition, there were positive correlations between ESR and BOP ($p<0.05$).

DISCUSSION

Periodontitis is common dental disorder, which is caused by many factors with microbial dental plaque as the initiator of periodontal disease. However, the manifestation and progression of periodontitis is influenced by a broad variety of determinants like social, behavioural, systemic, environmental and genetic factors. Recent researches are suggestive of a possible association between oral health and nutritional status in older adults.**(2-11-12)**

Nutrition strongly influences the integrity of the periodontium and its deficient state can modify the primary etiology. As periodontium is amongst the most dynamic tissues in the body, so its maintenance depends upon an adequate nutrients supply. **(13)** Folic acid is essential vitamin of vitamin B group and is commonly known as a hemocytopoietic vitamin. It 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, pteroylmonoglutamate, and the various tetrahydrofolate derivatives are collectively known as folates.**(14)** Folic acid deficiency is the most common nutrient deficiency in the world.**(2)** 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. However, little is known about the influence of folic acid on patients with chronic periodontitis in relation to their smoking status. Researches have shown a significant reduction in gingival inflammation following folic acid supplementation as determined by gingival redness, bleeding tendency, tenderness, and presence of exudates.**(15)**

Smoking undoubtedly is one of the most important and prevalent risk factor for chronic periodontitis among all the environmental factors which affects periodontium via various aspects. It mainly affects innate and immune host responses. Neutrophil functions like phagocytosis, superoxide and hydrogen peroxide generation, integrin expression and protease

inhibitor production are negatively affected by smoking. Organic nitrites, nitrous oxide, cyanates and isocyanates found in cigarette smoke have been shown to interact with folic acid 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. **(16)**

The present study was undertaken to estimate and compare serum folic acid and ESR levels in smokers and non smokers with chronic periodontitis. Also, the clinical parameters like (GI), (PI), (BOP), (PD) and clinical attachment level (CAL) were compared between the two study groups. Blood samples were collected from fasting individuals because recent food intake may appreciably increase the serum folate concentration.**(17)**

The mean concentration of folic acid in Group I (smokers with chronic periodontitis) and Group II (non smokers with chronic periodontitis) was 10.52ng/mL and 15.48ng/mL ($p < 0.05$) respectively. From this we concluded that serum folic acid levels were significantly lower in smokers with chronic periodontitis compared to non-smokers. This was in tune with the study done by Erdemir and Bergstrom. **(17)** 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.

ESR was 29.60ng/mL in smokers and 18.00 ng/mL in non smokers($p < 0.05$).From this we concluded that ESR levels were significantly higher in smokers with chronic periodontitis compared to non-smokers. The mechanisms described above and possibly others suggest that it is biologically plausible to expect low folic acid and ESR level in smokers. In support of this, a number of studies have focused on the effect of smoking on the serum levels of folic acid, but no studies have as yet analysed the relationship of smoking with folic acid or with ESR in patients with chronic periodontitis. Therefore, the aim of this study was to investigate serum levels of folic acid, ESR white blood cell (WBC) count and differential blood count in smokers and non-smokers with chronic periodontal disease.

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 BOP in smokers is 0.378 ± 0.363 (.072) and in non-smokers was 0.621 ± 0.240 (.048). 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 defence capabilities of the gingival tissues. This effect is due to the potential vasoconstrictive effect of nicotine. Nicotine from cigarette smoke stimulates the sympathetic ganglia to produce neurotransmitters including catecholamines which act on the blood vessels

via alpha receptors causing vasoconstriction. The smoking induced vasoconstriction of peripheral blood vessels can also affect the periodontal tissue leading to less overt signs of gingival inflammation such as redness, bleeding and exudation.**(18)**The results of our study are in agreement with various other studies.**(19,20)** which showed that smokers experienced less gingival bleeding than non smokers.

Our study illustrates PI in smokers to be 1.84 $\pm$ 0.374 (.075) and non-smokers be 1.04 $\pm$ 0.200 (.040), a statistically higher value in smokers. The higher plaque levels could be because of less favourable oral hygiene in smokers. Smokers have significantly more plaque than non-smokers and there is a trend towards increased plaque deposits with increasing cigarette consumption.**(25)** Also the tooth brushing efficiency of smokers is much less so the calcium concentration in the dental plaque of smokers is significantly higher than in non-smokers.**(21,22,23)** Our study corroborates well with the studies done by Preber et al, Bergstrom et al, Feldman et al and Macgregor I et al. **(24,25)**

The assessment of periodontal damage is a mandatory component in a periodontal examination, so PD and CAL were measured using a William's graduated periodontal probe in both the groups. PD and CAL values for smokers (3.68 and 4.56) were significantly higher than for non-smokers (2.96 and 3.04). 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. **(26).** Moreover, the maturation of the junctional epithelium, which has a quick turnover rate, is of central 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.**(27)**

Folic acid also affects the immune system by altering the defensive mechanisms which include decreased lymphocyte production, cytotoxic T-cell activity and phagocytic function of neutrophils.**(28)** 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.**(29,30,31)**

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. **(32)**

CONCLUSION

Based on the findings of the present study, 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 serum ESR level of smokers was significantly higher compared to non smokers. The clinical parameters like PI, CAL and PD were higher and BOP was lower in smokers, whereas GI was higher in non-smokers.

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Ethical approval: The study was approved by the institutional ethics committee

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Table 1. Clinical parameters of smokers and non-smokers (mean + SD) Std. Error Mean

Parameters	Smokers(n=25)	Non-smokers(n=25)
PI	1.84±.374(.075)	1.04±.200(.040)
GI	1.88±.440(.088)	1.72±.458(.092)
BOP	.378±.363(.072)	.621±.240(.048)
PD	3.68±.748(.150)	2.96±.841(.168)
CAL	4.56±.870(.174)	3.04±.790(.158)

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

Table 2. Serum parameters in smokers and non-smokers (mean + SD) Std. Error Mean.

Parameters	**Smokers (n=25)**	**Non-smokers (n=25)**
Folic acid (ng/ml)	10.52±1.939 (.388)	15.48±3.630 (.726)
HGB (g/dl)	14.72±1.768 (.354)	13.80±1.658 (.332)
RBC	4.56±.6506 (.130)	4.50±.7237 (.144)
WBC (10^3/ml)	9.64±4.443 (.889)	8.40±2.533 (.507)
Neutrophils(%)	61.92±12.33 (2.46)	59.32±13.74 (2.75)
Lymphocytes(%)	31.52±12.72 (2.54)	33.12±12.37 (2.47)
Monocytes (%)	3.24±2.634 (.527)	2.88±2.743 (.549)
Eosinophils (%)	3.12 ±2.315 (.463)	4.76±3.491 (.698)
Basophils (%)	0.04±.2000 (.040)	0.08±.2770 (.055)
ESR	29.60±15.27 (3.05)	18.00±8.416 (1.68)

HGB, haemoglobin;RBC, red blood cell; WBC, white blood cell;ESR,erythrocytes sedimentation rate. *p<0.05 according to the Mann–Whitney U-test

Table 3. Logistic analysis of all the variables with t-test,p value and confidence interval lower and upper limit.

Parameters	t-value	p-value	95% Confidence Interval of the Difference (upper)	95% Confidence Interval of the Difference (lower)
PI	9.428	.000	.972	.628
BOP	-2.786	.008	-.0669219	-.4188621
GI	1.260	.214	.415	-.095
CAL	6.470	.000	1.993	1.047
PPD	3.199	.002	1.173	.267
S. FA	-6.026	.000	-3.292	-6.628
Hb	1.898	.064	1.895	-.055
WBC	1.212	.231	3.297	-.817
N	.704	.485	10.024	-4.824
L	-.451	.654	5.537	-8.737
M	.473	.638	1.889	-1.169
E	-1.957	.056	.045	-3.325
B	-.586	.561	.097	-.177
ESR	3.327	.002	18.663	4.537
RBC	.284	.778	.44655	-.33615

Table 4. Correlations between serum and clinical parameters in non-smokers (n=25)

Parameter	**PI**	**BOP**	**GI**	**CAL**	**PD**
Folic acid (ng/ml)	0.135	0.134	-0.157	0.303	0.327
RBC	0.0296	0.160	-0.043	-.007	-.036
HGB (g/dl)	0.443*	-0.048	-0.060	0.176	0.105
WBC (103/ml)	-0.223	0.178	0.186	-0.004	0.038
Neutrophils (%)	0.182	-0.050	0.039	0.112	0.073
Lymphocytes (%)	-0.133	0.012	-0.002	-0.112	-0.090
Monocytes (%)	-0.198	-0.258	0.153	-0.177	-0.151
Eosinophils (%)	-0.078	0.355	-0.271	0.101	0.153
Basophils(%)	-0.300	0.003	0.014	-0.138	-0.128
ESR	0.265	0.028	-0.230	0.291	0.301

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

Table 5. Correlations between serum and clinical parameters in smokers (n=25)

Parameter	PI	BOP	GI	CAL	PD
Folicacid (ng/ml)	-.026	.228	.192	.121	.099
RBC	.050	.029	-.277	.354	.293
HGB (g/dl)	-.428*	.338	.044	.310	.267
WBC (103/ml)	-.143	-.228	-.181	-.193	-.415*
Neutrophils (%)	.054	.037	.092	-.309	-.296
Lymphocytes (%)	-.100	-.047	-.105	.326	.248
Monocytes (%)	.179	.140	.190	-.090	.073
Eosinophils (%)	.040	-.099	-.124	-0.060	0.092
Basophils(%)	.102	.022	.232	0.041	0.151
ESR	0.119	-0.500*	-0.220	0.031	-0.209

Pearson's correlation coefficients

HGB, haemoglobin; WBC, white blood cell; PI, plaque index; GI, gingival index; PD, pocket depth; CAL, clinical attachment loss;ESR,erythrocytes sedimentation rate.

The relation between folic acid as the dependent variable and HGB, WBC, neutrophils, lymphocyte s,monocytes, eosinophils, basophils, ESR, PI, GI, BOP, PD, CAL, smoking as predictors was analysed by means of multiple linear regression.

PI, lymphocytes and basophils were the only significant predictors. The variables explained 31% of the variance in the dependent variable[R^2 (adjusted) =0.312]

Table 6. Multiple regression analysis with folic acid as the dependent variable and including all variables as predictors

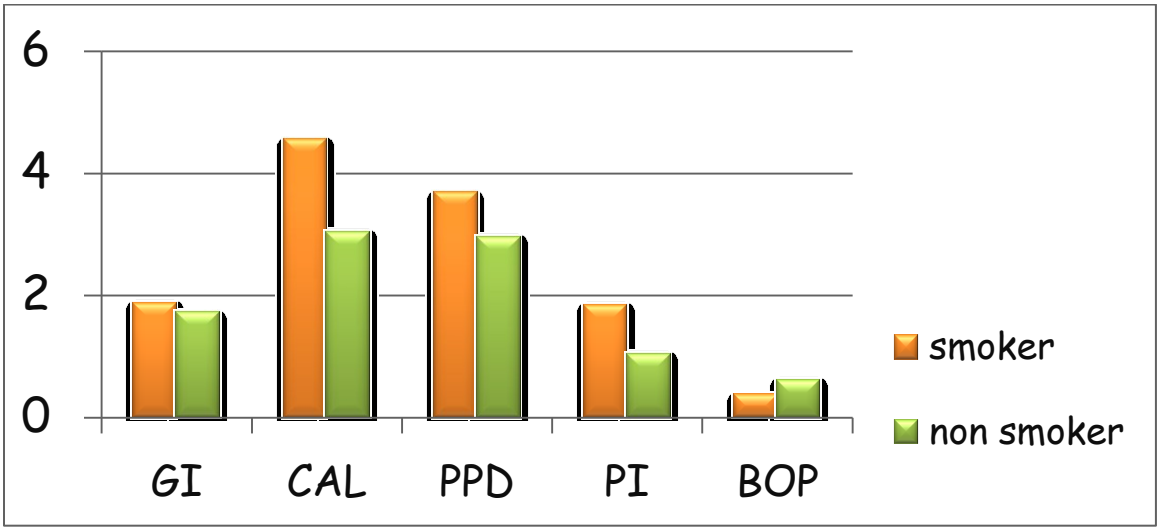
n=50	Std. Error	p value
(CONSTANT)	6.954	<0.05*
PI	1.175	0.0078*
BOP	0.017	0.2353
GI	1.315	0.3301
CAL	0.943	0.4080
PPD	1.133	0.6655
HB	0.357	0.4526
RBC	0.894	0.2752
WBC	0.153	0.0751
L	0.049	0.0196*
M	0.293	0.6897
E	0.177	0.2252
B	4.431	0.0422*
ESR	0.045	0.5194

R^2 adjusted=(0.321)

*($p<0.05$) according to multiple regression analysis.

HGB, haemoglobin; WBC, white blood cell; PI,plaque index; GI, gingival index; PD, pocketdepth; CAL, clinical attachment loss.

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



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