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Evaluation of Folic Acid Level in Serum of Smokers and Non Smokers Patients with Periodontal Disease: A Case-Control Study

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Abstract

Aim of the study: Periodontitis is a complex disease, the reason of which remained unclear. Folic acid belongs to B complex vitamins. It is vital for the formation and division of new cells, as it serves as a crucial cofactor in DNA synthesis. Without the presence of folic acid, epithelial cells fail to divide correctly. Folic acid levels can be influenced by a number of factors, one of which is cigarette smoking. This study aimed to measure serum concentration of folic acid among patients with periodontitis associated to their smoking behaviors.

Material and method: The study included 75 subjects; divided in the following groups: group-I: 25 Smokers with periodontitis, group-II: 25 Non-smokers with periodontitis and group-III: 25 Non-smoker with healthy periodontium (control group). Clinical parameters were recorded including: Plaque Index (PLI), Gingival Index GI, Bleeding on Probing (BOP), Probing Pocket Depth (PPD) and Clinical Attachment Loss (CAL). Blood samples were taken and evaluated in the laboratory for folic acid levels by enzyme-linked immunosorbent assay ELISA technique.

Results: Significantly low concentration of folic acid encountered in serum of smokers with periodontitis as compared to healthy non-smoker controls ($P < 0.001$). Additionally, smokers with periodontitis showed significantly higher PLI, PPD and CAL means than non-smokers with periodontitis.

Conclusion: The results of the present study indicate that folic acid levels were decreased significantly in smokers with periodontitis. Smoking has a negative impact on folic acid level and periodontal health.

Keywords: Periodontitis, Serum, Folic acid, Cigarette Smoking, ELISA.

Introduction

Periodontitis is a common inflammatory disease affecting the tooth-supporting tissues, started by specific pathogens resulting in persistent deterioration of the alveolar bone and periodontal ligament. Periodontal tissue destruction happens by an exaggerated host immune-inflammatory response (Hans et al., 2023) Around 40% and 90% of individuals worldwide affected by periodontitis, resulting in the most common epidemics in the world (Abdul Wahab et al., 2024).

Periodontal health depends on a precise equilibrium among the host, bacteria, environment, and different microorganisms. Periodontal disease results from complex interactions between the host factors and infectious agents. Many risk factors such as environmental, acquired, and genetic could change disease expression and may impact its onset, progression, and treatment response (George et al., 2013).

Tobacco smoking is a widespread habit that has been established as linked to cancer. Also, it may have a role as an environmental factor linked to a higher prevalence and severity of

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periodontal disease, while also adversely affecting nutritional levels. Nutrition is a modified factor that influences the host's immune response as well as integrity of the mouth cavity's soft and hard tissues (Nunn, 2003; Seymour and Heasman, 1992).

The presence of essential nutrients in the host significantly influences periodontal health. Among the human body's most active tissues are the epithelium of the dentogingival junction and the adjoining connective tissues. Periodontium health requires sufficient proteins, mineral salts, lipids, carbs, and vitamins. A chronic deficit in any of these nutrients may cause periodontal tissue pathology (Apatzidou, Riggio and Kinane, 2005; George, 2013).

Folic acid (named as vitamin B9 or folacin), along with folate (the naturally occurring type) are type of vitamin B9, which is water-soluble. It's crucial for biosynthesis of nucleotide and the homocysteine remethylation. Folic acid plays an important role during phases of rapid cellular division and development. Folate and folic acid terms originated from the Latin-word; folium; which means "leaf" (George et al., 2013).

Cigarette smoking is among those factors that negatively impact folic acid levels (Erdemir and Bergstrom, 2006). Cigarette smoke contains nitric oxide, superoxide and various species of reactive oxygen. Smoking's negative consequences may arise from oxidative damage to endothelial cells (Enwonwu and Sanders, 2001). In inflammatory reactions; the production of significant amounts of nitric oxide and superoxide, their interaction results in reactive nitrogen species generation, including the peroxynitrite anion, known as a toxic byproduct of nitric oxide together with superoxide. Peroxynitrite-induced tissue injury due to exaggerated local immune response and inflammatory cells migration. The bioactive form of folic acid (5-Methyl Tetrahydro-folic acid) has a demonstrated ability to decrease superoxide generation, thereby manifesting an antioxidant capacity. Conversely, an oxidant and a free radical, which induce damage to DNA and membrane, found in elevated levels in tobacco smoke and could potentially contribute to the reduction of folic acid levels. Cigarette smoke comprises organic the nitrite nitrous oxide (NO), cyanates, and isocyanates which react with co-enzymes of B12 vitamins and folic acid to transform them into molecules that are not biologically active. In addition to the negative impacts of smoking on immunity and interaction between host and bacteria, it may contribute to its damaging effect on periodontal health ((Enwonwu and Sanders, 2001)). Moreover, smoking adversely influences folic acid mechanisms of action. The insufficient amount of this vitamin could hinder the formation and evolution of the periodontal tissues, as well as the beginning and advancement of periodontal diseases (George et al., 2013). Thus; the purpose of this study was to assess and juxtapose serum the amount of folic in smokers and non-smokers, with and without periodontitis, and to investigate result of folic acid levels on periodontal health status.

Subjects, Material and Methods:

Study design: Case-control study.

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Sample Size: The sample size was calculated with G*Power 3.1.9.7 at a power of 0.9 and margin of error of 5% (0.05) (Faul et al., 2009). The calculated sample size for periodontitis group is 50, while it will be 25 for healthy controls. So, the total sample size would be 75 (25 control, 50 smokers and non-smokers with periodontitis).

Subject Population:

The study included seventy-five individuals with age range of 30- 55 years old; consisting of fifty participants with generalized stage II periodontitis divided into two groups each group consist of twenty-five patients with stage II periodontitis smokers and non-smokers; and twenty-five periodontally healthy non-smokers volunteers. The subjects involved were possessed at least 20 natural teeth, showed the desire with the ability for signed a consent form and systemically healthy without any diseases or conditions that affect the periodontium. Smokers' patients, who smoked for five years or more, between 5 and 10 cigarettes\day.

The criteria of generalized stage II periodontitis were defined as the presence of more than 30% of sites with pocket depths less than 5 mm and/or 30% of sites with clinical attachment loss > 4 mm. In healthy individuals, less than 30% of sites had a gingival index less than 1, and no CAL (Tonetti, 2018). The participants with systemic diseases that might affect the development or treatment of periodontal disease, those who had received antibiotics in the last three months, and individuals who used any vitamins or nutrition supplements in the past six months were excluded. The ethical committee of the College of Dentistry approved this study under the reference number of REC143 and study number MUOSU-202112. Every participant signed a written informed consent form before being enrolled in the study.

Clinical Parameters

Clinical parameters PLI, GI, PPD and CAL were measured for all the participants. The recordings were done at 6 sites per tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual, mid-lingual and mesio-lingual) for all teeth except third molars. The records were taken using William probe.

Serum Analysis

Samples of blood were obtained from fasting participants, as recent food consumption can significantly elevate serum folate levels (Robert and Schifferle, 2009). Under aseptic conditions; 5 milliliters of intravenous blood were obtained from all participants; the blood sample was then placed to plain tube to clot for 1 hour, and then at room temperature the sample centrifuged at 3300 rpm for 5 minutes. The clear serum sample was then separated and stored at -20°C until analysis. Then the samples were sent to the laboratory to assess serum levels of folic acid, utilizing ELISA technique.

Data Collection and Statistical Analysis

The mean and standard deviation (SD) of plaque Index, gingival index, PPD and CAL were calculated. Also, serum levels of folic acid's mean and standard deviation were calculated. All study groups follow a normal

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distribution therefore a parametric test of one-way ANOVA; Least significant difference test (LSD) and T-test was conducted for intergroup comparisons. Data was collected and analyzed via IBM® SPSS® version 27 with a P-value of <0.05 considered as significant.

Results:

According to the demographic results, the mean age of Group I was 41.72 ± 7.34 years, Group II was 39.76 ± 7.06 years and Group III was 38 ± 7.29 years respectively. The clinical periodontal parameters (PI, GI, PPD and CAL) were higher in group I followed by group II and III respectively.

Folic acid values demonstrated least levels in smokers with periodontitis (group I) as compared to non-smokers with periodontitis (group II) 4.004 ng\ml, 9.572 ng\ml respectively; while the controls (group III) showed highest level of folic acid (16.8 ng\ml) (Table I).

For intergroup comparisons there were highly significant differences (P<0.0001) both between and within study groups for serum folic acid level, PLI, GI, PPD and CAL (Table II).

Upon intergroup comparison of three study groups, significant differences encountered in mean of serum folic acid between group I and II; group I and III; group II and III (p<0.0001) suggesting its level being lower in smokers. Likewise, highly significant differences in mean of PLI and GI was found between (group I and group III) and between (group II and group III) (P<0.0001). While and nonsignificant differences between group I and II regrading PLI and GI (P>0.05) as shown in (Table-III).

According to Pearson's rank correlation (r) of clinical parameters and levels of Folic Acid in study groups (Table- IV); the result reflect an inverse significant association were demonstrated between serum folic acid and PLI in group II; and between folic acid and GI, PPD, CAL in group I and II (P<0.05).

Table 1: Mean and Standard Deviation of Age, Periodontal Parameters, and Serum Folic Acid of the Study Groups

Parameters	Group I	Group II	Group III
	Mean ± SD	Mean ± SD	Mean ± SD
Age	**41.72 ± 7.346**	**39.76 ± 7.061**	**38 ± 7.292**
PLI	2.376 ± 0.32	2.13 ± 0.41	0.30 ± 0.16
GI	2.12 ± 0.67	2.56 ± 0.287	0.37 ± 0.16
PPD	5.27 ± 0.65	4.28 ± 0.38	-
CAL	7.28 ± 0.85	4.45 ± 0.57	-
FolicAcid (ng\ml)	4.004 ± 1.14	9.572 ± 1.48	16.8 ± 1.08

SD: Standard Deviation; PLI: Plaque Index; GI: Gingival Index; PPD: Probing Pocket Depth; CAL: Clinical Attachment Loss; ng\ml: nanogram\milliliters.

Table 2: ANOVA Test for Serum Folic Acid and Clinical Periodontal Parameters Among Study Groups

Index	ANOVA Test	SS	df	MS	F- test	P-value	Significance

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Folic Acid	Between Groups	2058.20	2	1029.10	662.74	<0.00001	*HS
	Within Groups	111.8	72	1.55			
	Total	2170.00	74				
PLI	Between Groups	64.24	2	32.12	323.26	<0.0001	*HS
	Within Groups	7.15	72	0.09			
	Total	71.40	74				
GI	Between Groups	66.84	2	33.42	175.645	<0.0001	*HS
	Within Groups	13.70	72	0.19			
	Total	80.548	74				
PPD	T- test	6.4791	<0.0001				*HS
CAL	T- test	13.6765	<0.0001				*HS

HS: highly Significant; Sum of Squares: SS; DF:Degree of Freedom;MS: Mean Square and statistical expressions: F and P; PLI: Plaque Index; GI: Gingival Index; PPD: Probing Pocket Depth; CAL: Clinical Attachment Loss.

Table 3: Least Significant Difference Test (LSD) to Compare the Means of Serum Folic Acid Level and Clinical Periodontal Parameters Among Study Groups

Parameters	**Groups**	**MD**	**SE**	**P- value**	**Significance**
Folic Acid	GI- GII	5.57	22.34	0.000	HS
	GI- GIII	12.80	51.34	0.000	HS
	GII- GIII	7.23	29.00	0.000	HS
PLI	GI- GII	0.25	3.90	0.0198	S
	GI- G III	2.07	32.91	0.000	*HS
	G II- GIII	1.83	29.01	0.000	*HS
GI	GI- GII	0.44	5.00	0.0020	S
	GI- GIII	1.75	20.04	0.000	*HS
	GII- GIII	2.18	25.04	0.000	*HS

S: Significant, NS: Non-significant, * HS: Highly Significant; PLI: Plaque Index; GI: Gingival Index; PPD: Probing Pocket Depth; CAL: Clinical Attachment Loss; MD: Mean Difference, SE: Standard Error, P: P-value.

Table 4: Spearman's Rank Correlation (r) of Serum Folic Acid and Clinical Periodontal Parameters Among Study Groups

Groups and Parameters		**Group I**	**Group II**	**Group III**
		Folic Acid		
PLI	r	0.209	-0.389	-0.068
	P	0.316	0.054*	0.7467
GI	r	-0.938	-0.609	-0.058
	P	<0.0001**	0.0012**	0.7830
PPD	r	-0.671	-0.382	-
	P	0.0002 *	0.053*	-
CAL	r	-0.821	-0.50	-
	P	<0.0001**	0.0109*	-

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r: Spearman's Rank Correlatio; * Significant; ** Highly Significant; PLI: Plaque Index; GI: Gingival Index; PPD: Probing Pocket Depth; CAL: Clinical Attachment Loss.

Discussion:

Periodontal disease is a multifactorial condition characterized by complex etiology and wide phenotypic variation. Alongside the primary factor of bacterial plaque, inherited and social conditions, as well with the individual's behaviors, play crucial supporting roles. The increasing prevalence of smoking addiction and its related mortality as well as mortality renders smoking a serious public health risk (Omare et al., 2022). However, the exact mechanism through which smoking affects is still unknown. It is unclear if smoking induces systemic effects on the periodontium or local effects that promote periodontal disease (14. Rahul et al., 2024; Alwan, Al Karawi and Abdul Wahab, 2024).

The age group of 30-55 years was selected for the present study because patients in this age range are most likely to indulge in deleterious habits like cigarette smoking. According to a study done by Singh *et al* (2014), The prevalence of smoking was greater among older persons, individuals residing in rural areas, the uneducated, and the impoverished, in contrast to the younger population and those living in metropolitan areas (Singh et al., 2014). Also, the majority of epidemiological research found that both the severity and prevalence of chronic periodontitis are higher at this age; female patients were excluded from this study as recruiting women who acknowledge that they smoke would ultimately be challenging.

The periodontal parameters of the study groups were recorded to assess the oral hygiene status. On intergroup comparison, there was a statistically significant and highly significant difference between all groups ($p < 0.001$). The results of the present study indicated that plaque accumulation was minimal in the healthy control group (0.30 ± 0.16), followed by non-smokers with periodontitis group (2.13 ± 0.41), then smokers with periodontitis group (2.376 ± 0.32). The oral hygiene status indicated by plaque levels correlates with prior studies by Torrungruang *et al* in 2005; who shown significantly higher plaque levels in smokers (Torrungruang et al., 2005). While contradicting to findings of other previous studies (Rahul et al., 2024; Haffajee and Socransky, 2001; Calsina et al., 2002).

The difference between the mean gingival index in all study groups was statistically highly significant ($p < 0.001$). On intergroup comparison, a statistical significant difference was observed between Group I and Group II ($P = 0.002$). The results of this study indicate that the GI was greater in the non-smokers' group in contrast to both the smokers and the healthy controls groups. These results agree with previous studies by Jalayer *et al* (2015) (Jalayer et al., 2015) who have shown lower levels of the gingival index. This could be due to tobacco products, which impact vascular inflammation. Contrary to these results, the findings of researchs performed by Johanssen et al in 2005 (Johannsen et al., 2005), and Arowojolu *et al* in 2013 (Arowojolu et al., 2013).

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Highly significant differences were also observed in the mean of PPD,and CAL scores between smokers and non-smokers with periodontitis. The mean of PPD in (smokers with periodontitis group) was 5.27 ± 0.65 and in (non smokers with periodontitis group) was 4.28 ± 0.38. The difference between the mean of PPD was statistically highly significant ($p < 0.001$) as the smokers exhibit a higher scores of PPD compared to non-smokers group. The results of our study agree with a study done by Rahul et al, 2024; Ragghianti et al., 2004; and Velidandla et al; 2019 (Rahul et al., 2024; Ragghianti et al., 2004; Velidandla, 2019).

Concerning CAL, the difference between the mean of CAL in smokers and non-smokers was statistically highly significant ($p < 0.001$). The smokers group with periodontitis record a higher mean of CAL (7.28 ± 0.85); while the non-smokers recorded (4.45 ± 0.57). This highlights that the clinical attachment level was more in the smokers as compared to the non-smoker's group. CAL levels are a consequence of an inflammatory burden from the past into the present, while probing pocket depth levels reflect the ongoing pathophysiological status of periodontitis. Our study's findings may be related to modifications in subgingival plaque structure, and subgingival bacteria virulence and host response, which increase periodontium deterioration and bone resorption. Tobacco's nicotine can harm collagen tissues by increasing the production of collagenase, inhibiting the growth of gingival fibroblasts and decreasing the synthesis of collagen and fibronectin which ultimately compromises periodontal health. The results of our study agreed with Rahul et al, 2024; Torrungruang, 2005, Calsina et al, 2002, Susin et al,2004; Rudziński and Banach, 2011 studies (Rahul et al., 2024; Torrungruang et al., 2005; Calsina et al., 2002; Susin et al., 2004; Rudziński et al., 2011).

Recent research suggests that nutritional habits and dental health have potential link in older persons (Esaki et al., 2010; Vogel, 1977; Ainamo and Bay, 1975). Nutrition greatly impacts the health of the periodontium, and its deficiency can alter the presentation of the main etiological factors. Periodontium, one of the body's most dynamic tissues, with its sustainability reliant on a sufficient nutrients supply (Newman et al., 2010).

As folic acid, considered as a key vitamin within the B vitamin group, is recognized as a hemo-cytopoietic vitamin and plays a role in growth (Robert et al., 2009). Folic acid deficiency represents the most prevalent nutrient deficiency globally and it correlates with elevated oxidative stress, malfunctioning endothelial cells, genomic disorder, weakened DNA repair, and apoptosis. It is has been demonstrated to be associated with various diseases of humans, including periodontal diseases. However, very little has been discovered about how smoking affects folic acid level in chronic periodontitis patients. Clinical studies suggest that folic acid supplementation reduces gingival inflammation, characterized by redness, bleeding, tenderness and exudates (Neiva et al., 2003).

According to this study the folic acid mean in smokers with periodontitis was 4.004 ± 1.14 ng/ml, while non-smokers were 9.572 ± 1.48 ng/ml and controls were 16.8 ± 1.08 ng/ml.

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Intergroup comparisons indicated highly significant differences; concentrations of serum folic acid were notably less in smokers with chronic periodontitis group versus nonsmokers with similar periodontal destruction group and controls with healthy periodontium group. This aligns with the research conducted by Erdemir and Bergstrom (Erdemir et al., 2006; Erdemir and Bergstrom, 2007), Sumona et al (Sumona et al., 2011) Yu et al. (Yu et al., 2007).

Low folic acid levels may result from the reaction between isocyanates and cyanates together with folic acid, turning it inactive biologically and consequently reducing its levels. However, it may also result from insufficient intake of nutrients, poor absorption or metabolic dysfunction.

The correlation between the clinical parameters and folic acid was a strong negative correlation was found with PLI in non-smokers group and negative significant correlation with GI, PPD and CAL in both smokers and non-smokers with periodontitis, these inverse correlations signifying more folic acid levels were associated with reduced loss of attachment in patients with chronic periodontitis, regardless of smoking status. It can be inferred that increased folic acid levels may lead to decreased destruction of periodontium. Consequently, cigarette smoking habit, a known risk factor for periodontal diseases, could magnify the destruction resulting from folic acid deficiency. These results were in accordance with findings of Sumona et al, 2011 (Erdemir et al., 2007).

While smoking cessation is the preferred goal, it is not always achievable; thus, any strategy aimed at minimizing the harmful effects of smoking is valuable. Smokers with inadequate folic acid levels may benefit from enhanced dietary intake or supplementation of folic acid. A longitudinal study might explain the exact period of the deficient state and disease initiate, and providing stronger proof. Thus, longitudinal and interventionist studies in more epidemiological population will be required to determine the link between periodontal disease, folic acid, and smoking.

Conclusion:

The current research suggests that one significant environmental risk factor associated with further periodontal destruction is cigarette smoking. The escalated progression and excessive deterioration of periodontal support in later life largely influenced by excessive smoking in youth. Also, smoking can have negatively impacted hematological parameters like serum folic acid levels.

These findings highlight the necessity for preventive strategies targeting both younger and older individuals increasing folic acid intake by diet or folic acid supplementation may beneficial to dental health.

Supplementary Material

None.

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Data Availability Statement

Data are available from the authors upon reasonable request.

Conflict of interest

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The authors reported that they have no conflicts of interest.

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