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Comparative Evaluation Of Serum Folic Acid, Vitamin B_{12} Levels And Erythrocyte Sedimentation Rate (Esr) In Smokers And Non-Smokers With Chronic Periodontitis: A Clinico-Biochemical Study

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Abstract:
Background: *Periodontitis being an immune-inflammatory disease is an amalgamation of social, behavioral, systemic, environmental, and genetic risk factors. Smoking is one such risk factor that contributes to periodontal destruction. Chronic deficiency of nutrients like Folic acid and Vitamin B_{12} manifests as pathological alterations in the periodontal tissue. It has been reported that Erythrocyte sedimentation rate (ESR) is adversely affected by cigarette smoking. Thus, we have selected this study to compare the serum folic acid, Vitamin B_{12} levels, and ESR in smokers with chronic periodontitis.*
Materials and Methods*: This cross-sectional study comprised **60** subjects divided into four groups: **Group I** – **15** smokers with chronic periodontitis, **Group II – 15** smokers with gingivitis, **Group III- 15** non-smokers with chronic periodontitis, **Group IV-** control group. All subjects were evaluated for clinical parameters which included plaque index (PI), gingival index (GI), probing pocket depth (PPD), and clinical attachment level (CAL). Serum samples were collected for Vitamin B_{12}, Folic acid, and ESR analysis. The results were statistically analyzed.*
Results: *The results revealed that PI, GI, PPD, CAL were significantly higher in Groups I, II, and III while serum folic acid levels were lower in Groups I, II, and III respectively. Serum Vitamin B_{12} levels were higher in Group I as compared to other groups. ESR levels were significantly higher in Group III.*
Conclusion*: This study suggests that among patients with chronic periodontal disease, serum folic acid levels are lower in smokers as compared with non-smokers while Vitamin B_{12} and ESR levels were higher in smokers as compared to nonsmokers.*
Keywords: *ESR, folic acid, periodontitis, smoking, Vitamin B_{12}*

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I. Introduction

Periodontitis is an infectious disease resulting in inflammation of the supporting structures of the teeth culminating in progressive attachment loss and bone loss. It is a multifaceted disease; the demonstration and progression of which are influenced by a plethora of determinants including social, behavioral, systemic, environmental, and genetic. [(1)] Among the environmental factors, cigarette smoking is one of the main and most prevalent risk factors for chronic periodontitis. [(2)] According to the Global Adult Tobacco Survey (GATS) conducted in 2016–17, the overall prevalence of smoking tobacco was 10.38% and smokeless tobacco use was 21.38% in India. The biological conceivability and rate of progression associated with smoking have been hypothesized to be due to interactions among smoking components, and bacterial periodontal pathogens, affecting the innate and immune host responses. [(3)] Nutrition is also one of the modifiable factors affecting the host's immune response and the integrity of the hard and soft tissues of the oral cavity. [(1)] Chronic deficiency of nutrients like Folic acid and Vitamin B_{12} is known to produce pathological alterations in the periodontal tissues. [(4)] Folic acid is vital for the proper maturation of rapidly proliferating epithelial cells. Deficiency of which causes necrosis of gingiva, periodontal ligament, and loss of alveolar bone and causes rapid development and progression of periodontitis. [(5)] Vitamin B_{12} is a water-soluble micronutrient synthesized by microorganisms, deficiency of which is associated with severe diseases, as well as various oral manifestations such as loss of integrity resulting in stomatitis, angular cheilitis, and glossitis indicating a potential role of vitamin B_{12} in oral health. [(6)] Erythrocyte sedimentation rate (ESR) is the measure of the rate at which erythrocytes sediment in anticoagulated whole blood under a given set of conditions which is adversely affected by cigarette smoking. Determination of ESR helps

assess the progress of patients treated for chronic inflammatory disorders. [7] Thus, considering the facts; the purpose of this study was to estimate and compare the serum levels of Vitamin B_{12}, Folic acid, and ESR values in smokers and non-smokers with and without periodontitis.

II. Material And Methods

The present clinical study was carried out in the Department of Periodontics, Goa Dental College, and Hospital. The study comprised 60 subjects (only males) divided into four groups: **Group I** - 15 smokers with chronic periodontitis, **Group II –** 15 smokers with gingivitis, **Group III-** 15 non-smokers with chronic periodontitis, **Group IV**- Control group.

Study Design: Cross-sectional study

Study Location: This was government dental hospital based study done in Department of Periodontics, Goa Dental College, and Hospital, Bambolim, Goa

Study Duration: October 2021 to January 2022.

Sample size: 60 patients.

Sample size calculation: The sample size was determined using G*Power 3.1.9.7 according to Cohen (1988) at a power of 0.9 and margin of error of 5% (0.05). [8]

Subjects & selection method:

The present clinical study was carried out in the Department of Periodontics, Goa Dental College, and Hospital. The study comprised 60 subjects (only males) divided into four groups: **Group I** - 15 smokers with chronic periodontitis, **Group II –** 15 smokers with gingivitis, **Group III-** 15 non-smokers with chronic periodontitis, **Group IV**- Control group.

Inclusion criteria:

1. Subjects in the age group of 30-65 years
2. Subjects having a minimum of 20 functional teeth excluding the third molars
3. Systemically healthy patients
4. No history of periodontal treatment in the last 3 months
5. Patients smoking 5-10 cigarettes per day for 5 years or more.

Exclusion criteria:

1. Patients who consume tobacco in any form other than smoking cigarettes.
2. Patients who have taken a course of anti-inflammatory or anti-microbial therapy, use of vitamins or iron supplements within the past 6 months.
3. Any systemic diseases like chronic renal failure (e.g., nephritis, nephrosis, etc), malignant diseases (e.g., multiple myeloma, Hodgkin disease, advanced carcinomas, etc), bacterial infections (e.g. Tuberculosis, abdominal infections, acute pelvic inflammatory disease, syphilis, pneumonia, etc), inflammatory diseases (e.g. temporal arteritis, polymyalgia rheumatica, rheumatoid arthritis, rheumatic fever, systemic lupus erythematosus [SLE], etc), necrotic diseases (e.g., acute myocardial infarction, necrotic tumor, gangrene of an extremity, etc), diseases associated with increased proteins (e.g., hyper fibrinogenaemia, macroglobulinemia, etc), and severe anaemias (e.g., iron deficiency or B_{12} deficiency) would be excluded.
4. Alcoholics.

Procedure methodology:

After written informed consent was obtained, a well-designed questionnaire was used to collect the data of the recruited patients retrospectively. The questionnaire included socio-demographic characteristics such as age, gender, nationality, and lifestyle habits like smoking.

All subjects underwent thorough history taking and clinical examination as per clinical examination proforma, followed by evaluation of clinical parameters which included plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL). Serum samples were collected for Vitamin B_{12}, Folic acid and ESR analysis between 9 am and 11 am to minimise the circadian rhythm effects.

Collection of serum samples:

Under aseptic conditions, 5 ml of venous blood was drawn from ante-cubital fossa **(Figure 1)**. 3ml of the blood sample was then transferred into a clean, plain, labeled tube **(Figure 2)**, allowed to clot for 1 hour, and then centrifuged at 3300 rpm for 5 minutes at room temperature **(Figures 3,4)** while 2ml of the blood sample was transferred to a clean, labeled EDTA tube for estimation of Erythrocyte Sedimentation Rate (ESR) using the Westergren method **(Figure 5)**. The clear serum sample was then separated and stored at -20°C till assayed. The serum samples were transported to Thyrocare pathology laboratory for analysis of serum Vitamin B_{12} and folic acid. Estimation of serum Vitamin B_{12} and folic acid was done by ***ADVIA Centaur XP autoanalyzer*** **(Figure 6)**

by chemiluminescence immunoassay. After the collection of blood samples, all patients were provided with supragingival and/or subgingival scaling, and oral hygiene instructions were given.

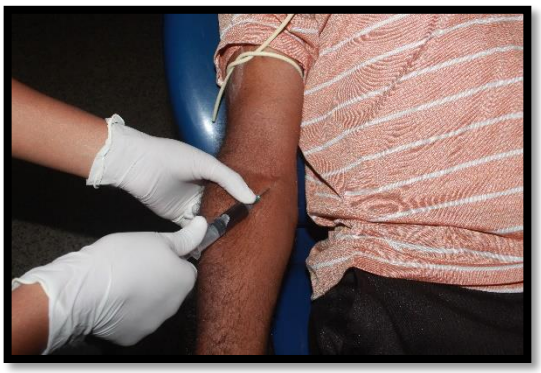

Figure 1: Collection of blood from the antecubital fossa

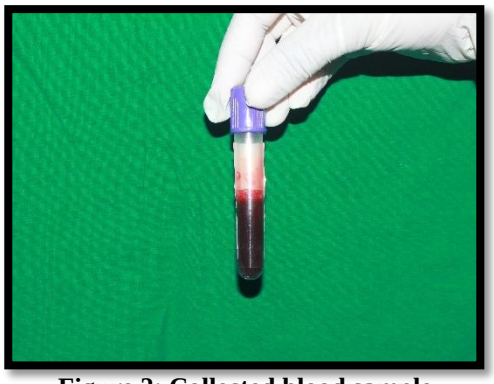

Figure 2: Collected blood sample

Figure 3: Centrifuging machine

Figure 4: Collected serum sample

Figure 5: Estimation of ESR using Westergren's method

Figure 6: Immunoassay analyzer

Statistical analysis

Statistical software namely the R Foundation for Statistical Computing (R version 3.6.2) was used for the analysis of data, and Flourish Studio and Microsoft Office Word have been used to generate graphs and tables. Shapiro Wilk test was conducted to check whether the data followed a normal distribution. For homogenous groups, a parametric test of Analysis of variance, or ANOVA, was used to analyze if the four groups had any difference. Tukey's Post Hoc test was then done to compare each group to the other pair-wise and the *p*-value was given and the mean difference between the two groups was tabulated. The *p*-value was set at 0.05 to be significant,

and a *p*-value less than 0.01 was considered highly significant. For non-homogeneous groups, the non-parametric test of Kruskal Wallis was used to analyze if the four groups had any difference. Pearson's test and Spearman's rho were done to study the correlation of smoking with other variables.

III. Results

Demographics

All groups did not follow normal distribution except for two groups (Group I and Group III) and therefore a non-parametric test of Kruskal Wallis was done. The mean age levels in Group I is 44.4 ± 7.82 years, in Group II is 41.4 ± 9.96 years, Group III is 48.3 ± 9.07 years and Group IV is 48.6 ± 8.41 years.

Periodontal parameters

Below **Table 1** and **Table 2** shows the distribution and inter-group comparison of PI, GI, PD, and CAL across all groups. The mean scores of all the parameters (PI, GI, PD, CAL) were significantly higher among Groups I, II, and III in comparison to Group IV (p <0.001). Likewise, pairwise comparison of PI, GI, PPD, and CAL among four groups by Tukey's Post Hoc test was highly significant (p <0.001). The mean GI score was statistically significant between Group I vs Group IV (p <0.001) while the mean scores of PI, PPD, and CAL showed a statistically significant mean difference (p <0.001) between each pair except Group I vs Group III and Group II vs Group IV

Table 1: Intergroup comparison of PI, GI, PPD, CAL using ANOVA test

		Group I vs Group II	Group I vs Group III	Group I vs Group IV	Group II vs Group III	Group II vs Group IV	Group III vs Group IV
	F (p-value)	MD (p-value)	MD (p-value)	MD (p-value)	MD (p-value)	MD (p-value)	MD (p-value)
CAL	**31.03 (<0.001)**	**1.3 (<0.001)**	0.246 (0.493)	**1.29 (<0.001)**	**-1.052 (<0.001)**	-0.009 (1.00)	**1.04 (<0.001)**
GI	**3.48 (0.022)**	0.368 (0.204)	0.04 (0.996)	**0.497 (0.046)**	-0.32 (0.30)	0.129 (0.898)	0.455 (0.077)
PI	**11.587 (<0.001)**	**0.629 (0.001)**	0.0435 (0.993)	**0.722 (<0.001)**	**-0.586 (0.003)**	0.093 (0.935)	**0.68 (0.001)**
PPD	**20.114 (<0.001)**	**0.976 (<0.001)**	0.113 (0.907)	**0.985 (<0.001)**	**-0.862 (<0.001)**	0.009 (1.0)	**0.872 (<0.001)**

Table 2: Inter-group comparison for PI, GI, PPD, CAL using Tukey's Post Hoc test

PI	Mean	Standard Deviation	ANOVA	
			F	p-value
Group I	1.58	0.348	11.587	<0.001***
Group II	0.954	0.347		
Group III	1.54	0.654		
Group IV	0.861	0.278		
GI	Mean	Standard Deviation	ANOVA	
			F	p-value
Group I	1.1	0.382	3.48	0.022*
Group II	0.728	0.311		
Group III	1.05	0.831		
Group IV	0.595	0.307		
PD	Mean	Standard Deviation	ANOVA	
			F	p-value
Group I	3.31	0.372	20.114	<0.001***
Group II	2.33	0.227		
Group III	3.2	0.742		
Group IV	2.32	0.338		
CAL	Mean	Standard Deviation	ANOVA	
			F	p-value
Group I	3.52	0.39	31.103	<0.001***
Group II	2.22	0.342		
Group III	3.28	0.742		
Group IV	2.23	0.285		

Hematological parameters

The below **Table 3** and **Table 4** show the distribution and inter-group comparison of Vitamin B_{12}, Folic acid, and ESR across all groups. When the hematological parameters were compared between groups, Folic acid levels were lower in smokers compared with nonsmokers, though not statistically significant, while serum Vitamin B12 levels were found to be higher in smokers as compared to non-smokers, statistically insignificant. However, ESR levels were found to be higher in non-smokers as compared to smokers (p <0.001). Upon pairwise comparison of Vit B_{12}, Folic Acid, and ESR among four groups using Tukey's test showed no statistically significant mean difference between each group. The mean ESR score, however, was statistically significant between Group III vs Group IV and Group II vs Group III (p <0.001).

VIT B_{12}	Mean	Standard Deviation	One-way ANOVA test	
			F	p-value
Group I	502	212	1.59	0.201
Group II	419	179		
Group III	357	137		
Group IV	448	214		
FOLIC ACID	Mean	Standard Deviation	One-way ANOVA test	
			F	p-value
Group I	6.03	3.69	0.67	0.575
Group II	4.49	2.8		
Group III	5.66	3.96		
Group IV	6.5	5.4		
ESR	Mean	Standard Deviation	One-way ANOVA test	
			F	p-value
Group I	19.2	13.7	10.52	**<0.001*** **
Group II	10.1	4.63		
Group III	31.3	16.9		
Group IV	12.5	4.64		

Table 3: Intergroup comparison of Vit B_{12}, Folic Acid, and ESR using ANOVA test

		Group I vs Group II	Group I vs Group III	Group I vs Group IV	Group II vs Group III	Group II vs Group IV	Group III vs Group IV
	F (p-value)	MD (p-value)	MD (p-value)	MD (p-value)	MD (p-value)	MD (p-value)	MD (p-value)
Vitamin B12	1.59 (0.201)	83 (0.625)	114.8 (0.164)	54.1 (0.86)	61.8 (0.805)	-28.9 (0.975)	-90.7 (0.555)
Folates	0.86 (0.471)	1.54 (0.729)	0.37 (0.995)	-0.47 (0.989)	-1.17 (0.859)	-2.01 (0.533)	-0.84 (0.942)
ESR	**8.33 (<0.001)**	9.07 (0.14)	**-12.13 (0.025)**	6.73 (0.374)	**-21.2 (<0.001)**	-2.33 (0.943)	**18.87 (<0.001)**

Table 4: Inter-group comparison for Vit B_{12}, Folic Acid, and ESR for using Tukey's Post Hoc test

Correlation of smoking with periodontal and hematological parameters

A negative correlation was found between smoking and ESR (-0.26) (p 0.042), and age (-0.31) (p 0.017) though not statistically significant while a weak positive correlation was noted between smoking and serum Vitamin B_{12} levels (0.15) (p 0.243) **Table 5**.

	Periodontitis	**Gingivitis**	**Smoking**
	r (p-value)	r (p-value)	r (p-value)
Age	0.076 (0.567)	**-0.273 (0.035)**	**-0.31 (0.017)**
Smoking	0 (1.0)	**0.577 (<0.001)**	
Vitamin B12	-0.01 (0.939)	-0.038 (0.772)	**0.15 (0.243)**
Folates	0.044 (0.740)	-0.170 (0.193)	-0.10 (0.434)
ESR	**0.509 (<0.001)**	**-0.343 (0.007)**	**-0.26 (0.042)**
CAL	**0.782 (<0.001)**	**-0.455 (<0.001)**	0.08 (0.548)
GI	**0.386 (<0.001)**	-0.153 (0.242)	0.08 (0.545)

PI	**0.615 (<0.001)**	**-0.305 (0.018)**	0.06 (0.627)
PPD	**0.718 (<0.001)**	**-0.410 (0.001)**	0.05 (0.718)

Table 5: Correlation of Smoking with periodontal and hematological variables

IV. Discussion

The global rise in the number of people with addiction to smoking and the associated mortality and morbidity with it has made smoking a grave public health hazard.[9] But the exact mechanism of how smoking increases the severity of periodontitis is unfathomable. Whether smoking causes a local effect on the periodontium or the systemic effects of smoking that causes the periodontal disease is abstruse. The present cross-sectional study was carried out to assess the effect of smoking on periodontal and hematological parameters.

The age group of 30-65 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 A et al (2014), the prevalence of smoking was higher among the higher age adults, those living in the rural areas, the uneducated, and the poor as compared to the younger population, those living in the urban areas, the educated, and the rich. [10] Also, most of the epidemiological studies have shown that both the severity and prevalence of chronic periodontitis are higher at this age.

Female patients were excluded from this study as females are known to have higher levels of ESR as compared to men. Also, it would have been difficult to recruit females who admit that they smoke. [11]

Alcoholics were also omitted because chronic alcohol consumption leads to deficiency of Vitamin B_{12} and folic acid due to their dietary inadequacy, intestinal malabsorption, decreased hepatic uptake, and increased body excretion, mainly via urine. Also, alcohol consumption was negatively associated with the ESR, that is, regular drinkers of low, moderate, and high quantities of alcohol registered a lower ESR than abstainers/occasional drinkers. [12,13]

The plaque index (Silness & Loe 1964) was recorded to assess the oral hygiene status of patients. On intergroup comparison, there was a statistically highly significant difference between all groups (p <0.001) except group I and group III (p =0.993), group II, and group IV (p =0.935). The results of the present study indicate that plaque accumulation was minimal in the healthy control group, but similar between smokers with periodontitis group, smokers with gingivitis group, and chronic periodontitis group. The oral hygiene status as depicted by plaque scores was almost similar in both the smokers and non-smokers groups even though smokers had slightly higher scores and this finding agrees with previous studies by Haffajee A. D (2001)[14], Calsina G (2002)[15]. Contradicting these studies, Torrungruang et al (2005)[16] has shown significantly higher plaque levels in smokers.

The mean gingival index of patients in group I (smokers with periodontitis group) was 1.10 ± 0.382, in group II (smokers with gingivitis group) was 0.728 ± 0.311, in group III (non-smokers with periodontitis group) was 1.05 ± 0.831 and in group IV (healthy control group) was 0.595 ± 0.307. The difference between the mean gingival index in all groups was statistically significant (p <0.002). On intergroup comparison, there was a statistically significant difference between Group I and Group IV (p=0.046). The results of the present study indicate that the gingival index was higher in the smokers' group when compared to the non-smokers group and healthy controls group. These results agree with previous studies by A Johanssen et al (2005)[17], and Arowojolu et al (2013)[18]. Contrary to the results obtained in our study, studies done by R J Bastiaan (1978)[19], Feldman et al (1983)[20], and Naderi et al (2015)[21] have shown lower levels of the gingival index. This could be attributable to tobacco smoke products which are known to interfere with the vascular inflammatory response.

The periodontal status of the patients was assessed by the commonly used clinical parameter i.e., probing depth. The mean probing pocket depth of patients in group I (smokers with periodontitis group) was 3.31 ± 0.372, in group II (smokers with gingivitis group) was 2.33 ± 0.227, in group III (non-smokers with periodontitis group) was 3.20 ± 0.742 and in group IV (healthy control group) was 2.32 ± 0.338. The difference between the mean probing pocket depth in all groups was statistically significant (p <0.001). On intergroup comparison, there was a statistically significant difference between all groups (p <0.001) except between group I and group III (p =0.907) and group II and group IV (p=1.00). The results of our study agree with a study done by Ragghianti et al. (2004)[22], and Velidandla et al (2019)[23].

Concerning CAL, the difference between the mean clinical attachment level in all groups was statistically significant (p <0.001). On intergroup comparison, there was a statistically significant difference between all groups (p <0.001) except between group I and group III (p =0.493) and group II and group IV (p=1.00). This highlights that the clinical attachment level was more in the smokers as compared to the non-smoker's group. Clinical attachment levels can be regarded as a result of an inflammatory burden from the past into the present, in contrast to probing pocket depth level which reflects the ongoing pathophysiological status of periodontitis and thus, the findings from our study may be attributed to the change in the sub-gingival plaque composition, the virulence of subgingival bacteria and change in the host response which increase the destruction of periodontium and bone resorption. The use of nicotine in tobacco can cause damage to the collagen tissues, by increasing the production of collagenase, suppressing the growth of gingival fibroblast, and the production of collagen and

fibronectin, ultimately deteriorating the periodontal tissues. The results obtained in our study agreed with studies by G. Calsina et al (2002)[15], Susin C et al (2004)[24], K. Torrungruang (2005)[16], Rudziński R et al (2011)[25].

For the estimation of serum Folic acid and Vitamin B_{12}, serum samples were collected between 9 am and 11 am to minimize the circadian rhythm effects. Also, serum folate levels increase with the intake of food. Therefore, the use of fasting determinations has been recommended. The mean serum Vitamin B_{12} level of patients in all groups was statistically insignificant (p=0.201). On intergroup comparison, no statistically significant mean difference in serum Vitamin B_{12} levels between each group was observed. Smoking may modify the appetite and consequently affect nutrient intake and serum micronutrients. The effect of smoking on Vitamin B_{12} status has been considered in several studies and research has proposed that organic nitrites, nitrous oxide, cyanides, and isocyanides of cigarette smoke interfere with Vitamin B_{12} metabolism, and convert it to inactive forms. Erdemir EO et al (2006)[26], concluded that among patients with periodontal disease, the serum folic acid concentration was lower in smokers compared with non-smokers. However, the results of our study found an increase in the levels of serum Vitamin B_{12} levels which is following a study done by Tungtrongchitr R et al (2003)[27], in which serum Vitamin B_{12} levels were significantly higher in smokers than non-smokers. Also, it was observed that Vitamin B_{12} levels were found to be lower in patients with chronic periodontitis as compared to healthy controls. These results were in tandem with studies done by Yu et al (2007)[28], and Zong et al (2016)[29]. The above-mentioned authors found an inverse association between serum Vitamin B_{12} status and changes in mean probing depth (PD) and clinical attachment level (CAL). The mean serum folic acid levels in all groups were statistically insignificant (p=0.545). On intergroup comparison, no statistically significant mean difference in serum folic acid levels between each group was observed. However, it was observed that smokers showed a decline in the levels of serum folic acid as compared to non-smokers, although these results were not statistically significant. These results are in congruence with studies done by Tungtrongchitr R et al (2003)[27], Erdemir EO et al (2006)[26], Sumona B et al (2011)[5], Agarwal et al (2014)[30].

Deficiency in folic acid levels could be a result of low dietary intake, impaired absorption, or metabolism. Chemical components of tobacco smoke have also been shown to interact with folic acid coenzymes, transforming them into biologically inactive compounds. It was also observed that serum folic acid levels were found to be lower in patients with chronic periodontitis as compared to healthy controls. Similar findings were observed in a study done by Yu et al (2007)[28].

ESR is a nonspecific reaction that is a measure of the present severity of pathological processes, the values of which are increased in all acute and inflammatory conditions. All blood samples were collected in EDTA and assayed within 4 hours of venipuncture, and the blood was mixed carefully before mechanical aspiration, according to the International Council for Standardization in Haematology (ICSH) recommendation. Undiluted blood specimens anticoagulated with EDTA were used for analysis by the Westergren method. Thompson et al (1964) in a study confirmed the view that Westergren's method is superior to Wintrobe's method of ESR measurement. Thus, Westergren's method was employed for the estimation of ESR in this study. The mean ESR values of patient's value in all groups were statistically highly significant with $p<0.001$. On intergroup comparison, no statistically significant mean difference in the ESR values between each group was found. However, group III patients (non-smokers with periodontitis) group showed significantly higher values of ESR as compared to the other groups. The results of our study are in conjunction with studies done by Hutter JW et al (2001)[31], Prakash et al (2012)[32], Latha S et al (2015)[33], Siddhesh ST et al (2016)[34]. The above authors found a significant increase in the levels of ESR in chronic periodontitis patients when compared to healthy controls. Increased levels of ESR are commensurate with progressive inflammatory disease. Cigarette smoke can cause damage to the endothelium by producing reactive oxygen species such as nitric oxide and hydrogen peroxide. Systemic acute phase reaction promoted by this oxidative stress will subsequently increase the inflammatory cytokines such as C-reactive protein, fibrinogen, blood cell count, blood viscosity, and rouleaux formation. These could lead to a rise in ESR values. The results of our study showed elevated levels of ESR in smokers as compared to healthy controls which is in conjunction with studies done by Gitte et al (2018)[35], Alende-Castro V et al (2019)[13], Sultana et al (2019)[7], Thriveni R et al (2020)[36].

V. Limitations

The limitations of the present study were a cross-sectional study design; hence a causal relationship could not be elucidated. The sample size was small. Additionally, controlled studies involving larger sample sizes may provide more conceptual evidence. The gingival condition was assessed by bleeding on probing (sulcus bleeding) only. Additional indicators of inflammation such as ILs, CRP, TNF-α, and MMPs could be explored in conjunction with smoking habits for a better understanding of causal relationships. Only a single sample at a particular time was collected from the subjects. Due to circadian rhythms, additional samples could have been collected at different intervals for better accuracy. The role of nutrition was not taken into consideration. Also, a lack of serum Vitamin B12 and folic acid values standardization could result in different reference values from

one laboratory to another. Lastly, no intervention was carried out to assess the serum Vitamin B12, folic acid levels, and ESR values before and after treatment.

VI. Conclusion

To conclude, the present study suggests that cigarette smoking is a major environmental risk factor associated with accelerated periodontal destruction. The rapid progression and excessive deterioration of periodontal support in later life depend to a greater extent upon excessive smoking in youth. Also, smoking can have deleterious effects on hematological parameters such as serum folic acid, vitamin $B_{12,}$ and ESR levels. However, in this study serum vitamin B_{12} levels were found to be higher in patients with smoking habits, though not statistically significant. The serum ESR levels of smokers were significantly higher as compared to non-smokers. These findings highlight the need for preventive strategies aimed at younger and older individuals, many of whom take up smoking as a habit, early in life. Dental public health efforts, therefore, need to include and emphasize the role of smoking and not only oral hygiene in primary preventive efforts. For smokers with a deficient folic acid status, improved dietary intake of folic acid or its supplements may prove beneficial.

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