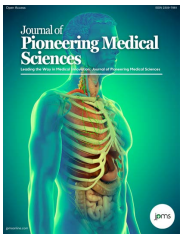
Journal of Pioneering Medical Sciences
Received October 15, 2024 Accepted December 06, 2024 Published January 05, 2025.
Volume 13, Issue 07, Pages 135-140

DOI https://doi.org/10.47310/jpms2024130721



Association of Nutritional Status and Vitamin B12 Levels among Smokers and Non-Smokers

Vishaka Kothari[1], L. Leelavathi[2*] and Selvaraj Jayaraman[3]

[1,2]Department of Public Health Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, 600 077, India

[3]Centre of Molecular Medicine and Diagnostics, Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, 600 077, India

Author Designation: [1]Undergraduate Student, [2]Associate Professor, [3]Professor

Corresponding author: L. Leelavathi (e-mail: leelavathi.sdc@saveetha.com).

©2024 the Author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0

Abstract **Objectives:** Tobacco use remains a leading cause of preventable diseases, adversely impacting numerous physiological processes, including vitamin metabolism. Vitamin B12 deficiency, essential for cellular function and DNA synthesis, is particularly concerning in smokers due to their exposure to harmful tobacco constituents. This research examines the association between smoking habits, nutritional health and salivary vitamin B12 levels, with an emphasis on the broader public health implications of smoking-induced nutritional deficiencies. **Method:** This cross-sectional study involved 240 participants, equally divided into 120 smokers and 120 non-smokers. Salivary samples were collected under standardized conditions and vitamin B12 concentrations were measured using the ELISA technique. A 24-hour dietary recall assessed nutritional adequacy, categorized into four levels: excellent, adequate, barely adequate and not adequate. Statistical analyses were conducted using the Mann-Whitney U test to compare vitamin B12 levels and chi-square tests to evaluate the relationship between smoking and dietary adequacy. **Results:** Smokers exhibited significantly lower salivary vitamin B12 levels compared to non-smokers, with mean ranks of 107.27 and 133.73, respectively (Mann-Whitney U = 8787.5, p = 0.003). Nutritional scores highlighted that smokers had poorer dietary quality, with a higher prevalence of inadequate diets. A significant association between smoking and nutritional adequacy was identified (chi-square value = 12.790, p = 0.005). These findings suggest smoking negatively impacts both vitamin B12 levels and dietary habits. **Conclusion:** This study highlights that smokers experience significantly lower salivary vitamin B12 levels and poorer dietary adequacy compared to non-smokers. The findings emphasize the need for targeted nutritional interventions, dietary education and public health strategies tailored to mitigate smoking-induced nutritional deficiencies. Future research should explore the interaction between smoking, other micronutrients and the potential benefits of dietary supplementation for smokers..

Key Words Tobacco use, vitamin B12 deficiency, nutritional adequacy, oxidative stress, salivary biomarkers, smoking-induced nutritional deficiencies

INTRODUCTION

The widespread use of tobacco in its various forms remains a significant contributor to preventable illness and death worldwide. Tobacco consumption is closely associated with numerous chronic conditions, including cardiovascular disease, chronic obstructive pulmonary disease (COPD) and various cancers [1,2,3]. Cigarette smoke contains a multitude of harmful substances that exacerbate oxidative stress, impairing the body's ability to absorb essential nutrients [4-6].

Nutrients such as vitamin B12 and folate are critical for physiological processes, including DNA synthesis and repair and their deficiencies are commonly linked to hematological and neurological disorders [7-10].

Smoking has been shown to adversely affect vitamin B12 metabolism, leading to increased levels of inactive forms in the body [1]. The toxic constituents of cigarette smoke, such as organic nitrites and nitrous oxide, interfere with the bioavailability and function of vitamin B12, raising significant concerns about the nutritional status of smokers [11]. Barnouin *et al.* [12] reported on Epidemic Neuropathy (EN) in Cuba during the early 1990s, which disproportionately affected smokers. This condition was attributed to a complex interplay of nutritional deficiencies,

particularly reduced levels of carotenoids and riboflavin, emphasizing how smoking exacerbates pre-existing nutritional inadequacies.

Vora and Harsh further demonstrated a strong correlation between tobacco consumption and vitamin B12 deficiency, particularly in vegetarian populations. Their findings suggest that tobacco use increases cyanide release, which disrupts vitamin B12 metabolism and leads to lower serum levels [13-15]. Moreover, smokers often follow less nutritious diets characterized by lower intakes of fruits and vegetables, which are key sources of essential micronutrients [16]. These observations underscore the necessity for a comprehensive evaluation of smoking behavior and dietary habits to better understand their combined impact on nutritional health.

Research by Bhattacharjee *et al.* [17] highlighted the protective effects of folic acid and vitamin B12 supplementation against nicotine-induced oxidative stress and apoptosis in pancreatic islets. Their study showed that these vitamins could mitigate nicotine's harmful effects on glucose metabolism and pancreatic cell viability, reinforcing the importance of adequate nutritional status in smokers [18].

Despite extensive evidence linking smoking to nutritional deficiencies, gaps remain in understanding how these deficits manifest in real-world populations, particularly through biomarkers such as salivary vitamin B12. Additionally, the socioeconomic implications of smoking-related nutrient deficiencies and their burden on public health systems warrant further exploration. Addressing these challenges through targeted dietary interventions and public health initiatives could play a pivotal role in mitigating the adverse health outcomes associated with smoking.

The objective of this study is to investigate the relationship between smoking status, nutritional health and salivary vitamin B12 levels. By utilizing saliva as a biomarker and assessing dietary intake patterns, this research aims to provide a comprehensive understanding of the nutritional differences between smokers and non-smokers, with the broader goal of informing dietary recommendations and public health policies.

METHODS

Study Population and Design

This cross-sectional study included 240 participants divided into two equal groups: smokers (n = 120) and non-smokers (n = 120). Participants were recruited based on self-reported smoking habits and informed consent was obtained from each individual prior to data collection. To minimize bias, inclusion criteria were standardized and efforts were made to ensure a diverse participant pool in terms of age, gender and dietary practices. However, the reliance on self-reported smoking behavior may introduce recall bias and no biochemical validation was conducted to confirm smoking status, which is a limitation.

Saliva Collection

Unstimulated saliva samples were collected using sterile, disposable saliva collection containers under standardized conditions. Participants were instructed to abstain from eating, drinking, smoking, or engaging in oral hygiene activities for at least 30 minutes before sample collection to reduce external factors affecting salivary composition. Additionally, efforts were made to control for environmental variables, such as hydration status and time of collection, to minimize variability in salivary composition.

Dietary Analysis

Following saliva collection, participants completed a 24-hour dietary recall. Nutritional intake was analyzed with a focus on key nutrients, including Vitamin B12. A nutrient score was calculated for each participant using validated tools to ensure consistency and reliability. Scores were categorized into four levels: excellent, adequate, barely adequate and not adequate. While the dietary recall method provides valuable insights, its reliance on participant memory introduces potential for bias and future studies could benefit from more objective measures of dietary intake, such as food frequency questionnaires.

ELISA for Vitamin B12 Biomarker

Vitamin B12 concentrations in saliva were measured using an ELISA procedure, specifically the Rat Vitamin B12 ELISA kit, selected for its high sensitivity and specificity. Saliva samples were appropriately diluted and 100 µL of each was added in duplicate to pre-coated wells, following the manufacturer's instructions. After incubation and washing, a conjugate solution was added, followed by the substrate reaction. Absorbance was measured at 450 nm using a microplate reader. Vitamin B12 concentrations were calculated using a standard calibration curve. While saliva is a non-invasive biomarker source, its variability due to environmental or physiological factors could impact reliability; thus, future research might explore complementary biomarkers.

Statistical Analysis

Data analysis was performed using SPSS software. Descriptive statistics, including mean and standard deviation, were calculated for vitamin B12 levels among smokers and non-smokers. Given the non-parametric distribution of the data, the Mann-Whitney U test was employed to compare salivary vitamin B12 levels between the two groups. Nutritional status, based on dietary scores, was analyzed in relation to smoking behavior using the chi-square test. Statistical significance was defined as a p-value of less than 0.05. However, the study did not account for potential confounding factors such as alcohol consumption, stress levels, or co-existing health conditions, which may have influenced the results.

This methodology, while robust, could be further enhanced by incorporating a longitudinal design, subgroup analyses (e.g., vegetarian vs. non-vegetarian dietary habits) and biochemical validation of smoking status. Additionally, future studies could explore the interaction of smoking with other micronutrients, such as folic acid or Vitamin D, to provide a more comprehensive understanding of nutritional deficiencies in smokers.

RESULTS

Dietary Score Distribution

The dietary assessment for the study population (n = 240) revealed varying levels of nutritional adequacy. Overall, 52.08% (n = 125) had an "Adequate" diet score, 24.58% (n = 59) were categorized as "Barely Adequate," 13.33% (n = 32) as "Excellent," and 10.00% (n = 24) as "Not Adequate." (Figure 1).

A comparison of dietary scores between smokers and non-smokers demonstrated a significant disparity in dietary quality. Among non-smokers (n = 120), 10.00% (n = 24) achieved an "Excellent" diet score, compared to only 3.33% (n = 8) of smokers. Conversely, the prevalence of a "Not Adequate" diet score was higher among smokers (6.67%, n = 16) than non-smokers (3.33%, n = 8). This statistically significant difference (chi-square value = 12.790, p = 0.005) underscores the association between smoking and poorer dietary habits (Figure 2).

Additionally, subgroup analysis was not performed but could have revealed further insights into specific dietary patterns, such as vegetarian versus non-vegetarian diets, which might influence nutritional adequacy. Non-smokers exhibited an overall trend toward higher dietary quality, reinforcing the link between smoking status and compromised nutritional behavior.

Salivary Vitamin B12 Levels

Salivary vitamin B12 concentrations were measured using the ELISA method, revealing a significant disparity between smokers and non-smokers.

Smokers (n = 120) exhibited a mean vitamin B12 level of 0.4760 ng/mL with a standard deviation of 0.3718, indicating notable variability in their B12 concentrations. In contrast, non-smokers (n = 120) had a significantly higher mean vitamin B12 level of 0.5900 ng/mL with a much lower standard deviation of 0.0339, reflecting more consistent and elevated levels among non-smokers (Table 1).

The Mann-Whitney U test confirmed these differences, with non-smokers having a higher mean rank (133.73) compared to smokers (107.27). The test yielded U = 8787.5, Z = 2.962 and p = 0.003, indicating statistical significance. (Table 2).

These findings highlight the marked disparity in salivary vitamin B12 levels between smokers and non-smokers. The lower B12 levels observed in smokers may be attributed to the combined effects of inadequate dietary quality, as evidenced by a higher prevalence of "Not Adequate" dietary scores and the physiological impact of smoking, such as impaired nutrient absorption or metabolism. The more stable and elevated vitamin B12 levels among non-smokers further support the negative impact of smoking on B12 status.

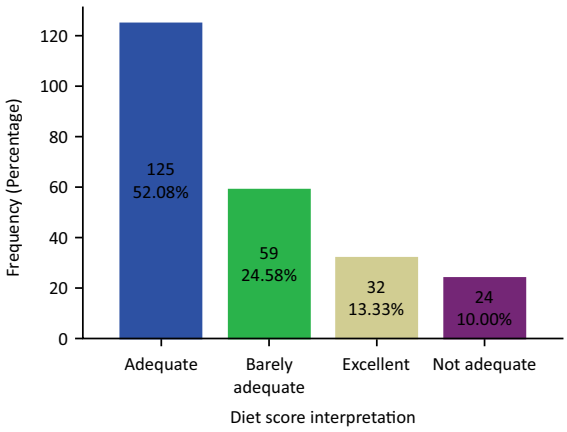


Figure 1: Pie chart depicting study participant's nutritional status

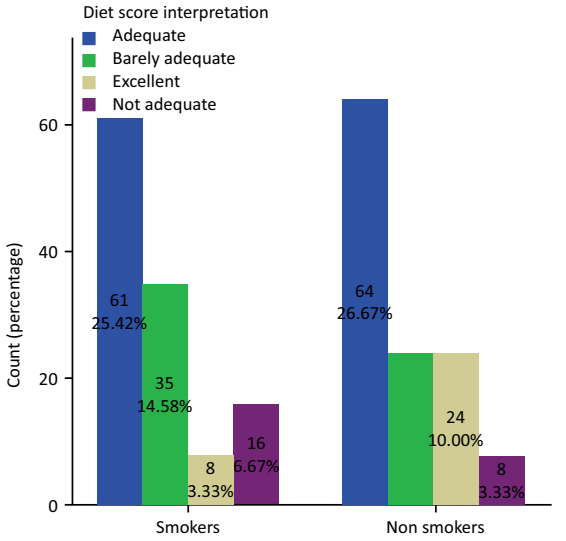


Figure 2: Barchart showing nutritional status among smokers and non smokers

Table 1: Vitamin B12 values among smokers and non smokers

	Vitamin B12 levels (pg/mL)			
	N	Mean	Standard Deviation	Standard Error Mean
Smokers	120	0.4760	0.371796	0.03394
Non smokers	120	0.5900	0.033940	2.43838

Table 2: Results of the Mann-Whitney U Test Comparing Vitamin B12 Levels Between Smokers and Non-Smokers

	Vitamin B12 levels				
	N	Mean Rank	U	Z	P
Smokers	120	107.27	8787.5	2.962	0.003
Non smokers	120	133.73			

While these results provide critical insights, they do not account for confounding factors such as alcohol consumption, stress, or co-existing medical conditions that might influence vitamin B12 levels. Future studies should explore these variables to provide a more comprehensive understanding of the observed disparities. Additionally, longitudinal data could elucidate the dose-dependent effects of smoking on vitamin B12 metabolism and the potential recovery of B12 levels following smoking cessation.

DISCUSSION

The findings of this study confirm that non-smokers exhibit significantly higher salivary vitamin B12 levels compared to smokers, which aligns with prior research on smoking's detrimental effects on vitamin metabolism. For instance, Tungtrongchitr *et al.* [1] reported lower serum vitamin B12 levels in smokers due to tobacco constituents interfering with vitamin metabolism. Similarly, Vardavas *et al.* [19] observed that smokers have lower dietary vitamin intake, exacerbating the impact of smoking on vitamin B12 levels.

Barnouin *et al.* [12] emphasized the role of oxidative stress induced by smoking in exacerbating nutritional deficits, particularly of B vitamins and carotenoids. This deficiency hampers the body's ability to detoxify harmful byproducts like cyanide, a significant contributor to conditions such as Epidemic Neuropathy [1,20]. These findings underscore the interplay between smoking, oxidative stress and nutrient deficiencies.

Dietary patterns, especially in vegetarian populations, significantly influence health outcomes in smokers. Smokers already face elevated risks from toxic exposures and the increased prevalence of vitamin B12 deficiency among vegetarians further compounds these risks [12,13]. Ensuring adequate dietary intake or supplementation in these groups is essential to mitigate tobacco-related health hazards. Additionally, cultural background, local food availability and socio-economic factors play a vital role in dietary choices, directly impacting nutrient levels [1,21].

Vitamin B12 and folic acid supplementation have shown promise in counteracting nicotine-induced oxidative stress and apoptosis in pancreatic cells, as demonstrated by Bhattacharjee *et al.* [17] and Pflipsen *et al.* [18]. These findings highlight the therapeutic potential of nutritional interventions in reducing the harmful effects of smoking. Public health initiatives should incorporate these strategies to address smoking-induced nutritional deficiencies.

Analysis of dietary scores revealed significant differences between smokers and non-smokers. A higher proportion of non-smokers attained "excellent" dietary scores, while smokers were more likely to fall into the "not adequate" category. This trend aligns with prior research, emphasizing the importance of healthy dietary patterns in mitigating nutrient deficiencies [9,23]. Variations in dietary scores partially explain the disparities in vitamin B12 levels between smokers and non-smokers.

ELISA analysis confirmed that smokers had significantly lower salivary vitamin B12 levels than non-smokers. These findings align with evidence linking smoking to systemic inflammation and impaired nutrient metabolism [24]. Elevated inflammatory markers such as IL-6 and STAT-3 in smokers may contribute to their lower nutrient status, further substantiating the study's results.

Smoking's negative impact on oral health is also significant. Conditions such as periodontal disease and oral lesions in smokers are well-documented [25]. These conditions can indirectly affect nutrient absorption and dietary choices, compounding the observed deficiencies. Tobacco's effect on the oral mucosa and systemic health reflects broader disruptions in nutrient levels, including vitamin B12 [26].

Despite awareness of smoking's risks to respiratory and cardiovascular health, there is a significant gap in knowledge about its specific effects on nutrient levels and oral health [27,28]. Targeted health education programs are critical for bridging this gap and promoting healthier lifestyle choices, particularly among at-risk populations.

The role of other nutrients, such as vitamin D, in mitigating smoking-induced inflammation should also be considered. Recent research highlights the benefits of vitamin D3 in modulating immune responses and reducing inflammation [29]. Future research could explore the synergistic effects of multiple nutrients to address the broader deficiencies observed in smokers.

In summary, this study highlights the negative impact of smoking on nutritional status, particularly vitamin B12 levels. Understanding the intricate link between smoking and nutrient deficiencies is critical for addressing the broader health consequences of tobacco use. Future research should address the noted limitations and focus on exploring smoking's interaction with other micronutrients, dietary habits and potential recovery following smoking cessation.

Limitations

While this study provides valuable insights, several limitations must be acknowledged. First, the cross-sectional design does not establish causality between smoking and vitamin B12 levels. Second, reliance on self-reported smoking habits introduces potential recall bias, as biochemical validation of smoking status was not conducted. Third, the dietary recall method is subject to memory bias and may not fully capture habitual dietary patterns. Additionally, the study did not account for confounding factors such as alcohol consumption, stress and co-existing health conditions that could influence nutrient levels. Finally, while saliva was chosen as a non-invasive biomarker source, its variability due to environmental and physiological factors may affect reliability. Future studies should incorporate longitudinal designs, larger and more diverse sample sizes and objective dietary assessments to address these limitations.

CONCLUSION

This study demonstrates that smokers have significantly lower salivary vitamin B12 levels and poorer dietary quality compared to non-smokers. Non-smokers were more likely to achieve higher dietary adequacy scores, while smokers predominantly fell into the "not adequate" dietary category. These findings underscore the adverse impact of smoking on both nutritional status and dietary habits, highlighting an urgent need for targeted nutritional interventions for smokers. To address these deficiencies, dietary education, vitamin supplementation and public health strategies should be prioritized for smokers. Health professionals should integrate nutrition-focused counseling into smoking cessation programs to enhance their effectiveness.

Future research should explore the recovery of nutrient levels post-smoking cessation, the interaction of smoking with other micronutrients and the long-term benefits of combined nutritional and behavioral interventions. Addressing the interplay between smoking, nutrition and systemic health is vital for developing comprehensive approaches to reducing the health burden associated with tobacco use.

Acknowledgments

The authors thank all study participants, the research team and funding sources for their invaluable support.

Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Considerations

The study was conducted with ethical approval from the relevant institutional review board. Participant confidentiality was strictly maintained and informed consent was obtained prior to data collection.

REFERENCES

[1] Tungtrongchitr, Rungsunn, *et al.* "Relationship of tobacco smoking with serum vitamin b_{12}, folic acid and haematological indices in healthy adults." *Public Health Nutrition,* vol. 6, no. 7, October 2003, pp. 675-681. http://dx.doi.org/10.1079/phn2003483

[2] Doll, R, *et al.* "Mortality in relation to smoking: 40 years' observations on male british doctors." *BMJ,* vol. 309, no. 6959, October 1994, pp. 901-911. http://dx.doi.org/10.1136/bmj.309.6959.901.

[3] Selhub, Jacob. "Vitamin status and intake as primary determinants of homocysteinemia in an elderly population." *JAMA: The Journal of the American Medical Association,* vol. 270, no. 22, December 1993,. http://dx.doi.org/10.1001/jama.1993.03510220049033.

[4] Erdemir, Ebru Olgun and Jan Bergstrom. "Relationship between smoking and folic acid, vitamin b_{12} and some haematological variables in patients with chronic periodontal disease." *Journal of Clinical Periodontology,* vol. 33, no. 12, November 2006, pp. 878-884. http://dx.doi.org/10.1111/j.1600-051x.2006.01003.x.

[5] Axelsson, P., *et al.* "Relationship between smoking and dental status in 35 , 50 , 65 and 75 year old individuals." *Journal of Clinical Periodontology,* vol. 25, no. 4, April 1998, pp. 297-305. http://dx.doi.org/10.1111/j.1600-051x.1998.tb02444.x.

[6] Chen, Andrew T. L., *et al.* "Increased chromosome fragility as a consequence of blood folate levels, smoking status and coffee consumption." *Environmental and Molecular Mutagenesis,* vol. 13, no. 4, January 1989, pp. 319-324. http://dx.doi.org/10.1002/em.2850130407.

[7] Shekoohi, N. *et al.* Smoking discriminately changes the serum active and non-active forms of vitamin B_{12}. *Acta Medica Iranica,* vol. 55, no. 6, 2017, pp. 389-394. https://pubmed.ncbi.nlm.nih.gov/28843240/.

[8] Mcphillips, Janice B, *et al.* "Dietary differences in smokers and nonsmokers from two southeastern new england communities." *Journal of the American Dietetic Association,* vol. 94, no. 3, March 1994, pp. 287-292. http://dx.doi.org/10.1016/0002-8223(94)90370-0

[9] Piyathilake, CJ, *et al.* "Local and systemic effects of cigarette smoking on folate and vitamin b_{-12}." *The American Journal of Clinical Nutrition,* vol. 60, no. 4, October 1994, pp. 559-566. http://dx.doi.org/10.1093/ajcn/60.4.559.

[10] Khaled, M.Abu, *et al.* "Inactivation of B12 and folate coenzymes by butyl nitrite as observed by NMR: implications on one-carbon transfer mechanism." *Biochemical and Biophysical Research Communications,* vol. 135, no. 1, February 1986, pp. 201-207. http://dx.doi.org/10.1016/0006-291x(86)90963-0.

[11] Gariballa, Salah and Sarah Forster. "Associations between smoking, nutritional status and clinical outcome following acute illness." *Proceedings of the Nutrition Society,* vol. 67, no. 8, May 2008,. http://dx.doi.org/10.1017/s0029665108000906.

[12] Barnouin, Jacques, *et al.* "Vitamin and nutritional status in cuban smokers and nonsmokers in the context of an emerging epidemic neuropathy." *International Journal for Vitamin and Nutrition Research,* vol. 70, no. 3, May 2000, pp. 126-138. http://dx.doi.org/10.1024/0300-9831.70.3.126.

[13] Vora, Jyoti H. and Harsh N. Oza. Study of serum vitamin B12 level in population consuming tobacco in various forms as compared to non-tobacco users. *National Journal of Community Medicine*, vol. 8, no. 7, 2017, pp. 353-355. https://njcmindia.com/index.php/file/article/view/1063.

[14] Saini, T., *et al.* Study of Vitamin B12, folic acid and related Hematological indices in tobacco users. Proceedings, 2014, pp..

[15] Mishra, Gauravi A, *et al.* "An overview of the tobacco problem in India." *Indian Journal of Medical and Paediatric Oncology,* vol. 33, no. 3, July 2012, pp. 139-145. http://dx.doi.org/10.4103/0971-5851.103139.

[16] Bergström, J. and S. Ellasson. "Noxious effect of cigarette smoking on periodontal health." *Journal of Periodontal Research,* vol. 22, no. 6, November 1987, pp. 513-517. http://dx.doi.org/10.1111/j.1600-0765.1987.tb02364.x.

[17] Bhattacharjee, Ankita, *et al.* "Synergistic protective effect of folic acid and vitamin b_{12}Against nicotine-induced oxidative stress and apoptosis in pancreatic islets of the rat." *Pharmaceutical Biology,* vol. 54, no. 3, May 2015, pp. 433-444. http://dx.doi.org/10.3109/13880209.2015.1043561.

[18] Pflipsen, M. C., *et al.* "The prevalence of vitamin b12 deficiency in patients with type 2 diabetes: A cross-sectional study." *The Journal of the American Board of Family Medicine,* vol. 22, no. 5, September 2009, pp. 528-534. http://dx.doi.org/10.3122/jabfm.2009.05.090044.

[19] Vardavas, Constantine I, *et al.* "Smoking status in relation to serum folate and dietary vitamin intake." *Tobacco Induced Diseases,* vol. 4, no. 1, December 2007,. http://dx.doi.org/10.1186/1617-9625-4-8.

[20] Ortega, R M, *et al.* "Influence of smoking on folate intake and blood folate concentrations in a group of elderly spanish men.." *Journal of the American College of Nutrition,* vol. 13, no. 1, February 1994, pp. 68-72. http://dx.doi.org/10.1080/07315724.1994.10718374.

[21] Daniel, M. and M. D. Cargo. "Association between smoking, insulin resistance and β cell function in a north western first nation." *Diabetic Medicine,* vol. 21, no. 2, January 2004, pp. 188-193. http://dx.doi.org/10.1046/j.1464-5491.2003.01064.x.

[22] Mukherjee, Sandip, *et al.* "Synergistic effect of folic acid and vitamin b12 in ameliorating arsenic-induced oxidative damage in pancreatic tissue of rat." *The Journal of Nutritional Biochemistry,* vol. 17, no. 5, May 2006, pp. 319-327. http://dx.doi.org/10.1016/j.jnutbio.2005.08.003.

[23] Francis, Twinkle, *et al.* "Awareness of vitamin and mineral supplements among college students." *Journal of Advanced Pharmaceutical Technology and Research,* vol. 13, no. 1, November 2022, pp. S320-S324. http://dx.doi.org/10.4103/japtr.japtr_418_22.

[24] Ep, Raguladhithya *et al.* "Inflammation in smokers and non-smokers during implant surgery among Indians." *Bioinformation*, vol. 19, no. 4, April 2023, pp. 510-513.

[25] Ahmed, Naveed, *et al.* 2021. Smoking a dangerous addiction: A systematic review on an underrated risk factor for oral diseases. *International Journal of Environmental Research and Public Health*, Vol. 18, No. 21,. 10.3390/ijerph182111003. http://dx.doi.org/10.3390/ijerph182111003.

[26] Ramalingam, Karthikeyan, *et al.* 2023. Assessment of oral lesions with tobacco usage: A cross-sectional clinicopathological study in Sri Ganganagar, Rajasthan, India. *Cureus*, Vol. 15, No. 1,. 10.7759/cureus.33428. http://dx.doi.org/10.7759/cureus.33428.

[27] Nivethitha, R. and L. Leelavathi. 2022. Awareness on ill effects of tobacco usage among tobacco users. *Journal of Advanced Pharmaceutical Technology and Research*, Vol. 13, No. 1,. 10.4103/japtr.japtr_147_22 pp: S217-S222. http://dx.doi.org/10.4103/japtr.japtr_147_22.

[28] Antony, J.Vini Mary, *et al.* 2021. Particle size, penetration rate and effects of smoke and smokeless tobacco products-an *in vitro* analysis. *Heliyon*, Vol. 7, No. 3,. 10.1016/j.heliyon.2021.e06455. http://dx.doi.org/10.1016/j.heliyon.2021.e06455

[29] Sagar, Sandra, *et al.* "Role of 1, 25 dihydroxycholecalciferol on the rate of orthodontic tooth movement- a systematic review." *International Journal of Orthodontic Rehabilitation,* vol. 14, no. 4, January 2024, pp. 19-32. http://dx.doi.org/10.56501/intjorthodrehabil.v14i4.877.1