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Comparative Evaluation of Salivary Cotinine and Psychological Nicotine Dependence Among Adolescent Smokeless Tobacco Users

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Abstract **Background:** Adolescent use of smokeless tobacco poses a serious public health concern due to its long-term effects on brain development and increased risk of nicotine dependence. Nicotine exposure during adolescence contributes to greater vulnerability to addiction and long-term behavioral and cognitive consequences. Assessing both biochemical and psychological markers of dependence is essential for developing targeted prevention and cessation strategies. **Objective:** To compare salivary cotinine levels with self-reported psychological nicotine dependence among adolescent smokeless tobacco users and evaluate their readiness to quit. **Methods:** This cross-sectional study was conducted among adolescents aged 13 to 18 years. Nicotine dependence was measured using the Fagerström Test for Nicotine Dependence (FTND) and the Contemplation Ladder. Salivary cotinine was assessed using a non-invasive saliva test kit. Participants were grouped into tobacco users and non-users. Statistical analysis was performed using the Mann-Whitney U test and Kruskal-Wallis test, with significance set at $p<0.05$. **Results:** The mean salivary cotinine level among tobacco users was 124.36 ng/mL, compared to 49.58 ng/mL in non-users. Among the 52 tobacco users, 36.5% showed very high dependence on the FTND scale. On the Contemplation Ladder, 86% had no intention of quitting, while only 0.3% indicated readiness to quit. Despite elevated cotinine levels in users, no statistically significant correlation was found between cotinine concentration and FTND scores ($p = 0.620$). **Conclusion:** Adolescents using smokeless tobacco exhibited higher biochemical markers of nicotine exposure and psychological dependence compared to non-users. However, the absence of a significant correlation between salivary cotinine and self-reported dependence suggests that adolescent nicotine addiction is influenced by multiple factors. These findings highlight the need for comprehensive cessation strategies that integrate both behavioral and biochemical interventions, particularly for youth with low motivation to quit.

Key Words Adolescents, Smokeless tobacco, Salivary cotinine, Nicotine dependence, Fagerström Test, Contemplation Ladder, Public health

INTRODUCTION

Adolescent tobacco use is a significant public health concern, contributing to both immediate and long-term health risks, including increased nicotine dependence and susceptibility to other addictive behaviors [1]. Adolescents are particularly vulnerable to peer influence and experimentation, often initiating tobacco use at an early age. This early initiation increases the likelihood of developing chronic health conditions such as cardiovascular and respiratory diseases later in life [2]. Nicotine's addictive properties have a profound effect on the developing adolescent brain, heightening the risk of long-term dependence when compared to adult users [3].

Salivary cotinine, the primary metabolite of nicotine, has been established as a reliable biomarker for assessing tobacco exposure. Compared to self-reported data, which may be compromised by underreporting or recall bias, cotinine provides an objective measure of nicotine intake [4]. Its ability to detect both active and passive exposure makes it particularly useful for adolescent studies, where secondhand smoke exposure is also prevalent [5]. Moreover, saliva-based cotinine testing is non-invasive, easy

to administer and allows for frequent assessments, making it highly suitable for research in adolescent populations [6,7].

While cotinine effectively quantifies recent nicotine exposure, it does not encompass the behavioral and emotional dimensions of addiction. Psychological dependence involves cravings, tolerance and difficulties with cessation and requires subjective assessment tools to capture these facets [8]. Instruments such as the Fagerström Test for Nicotine Dependence (FTND) are commonly used to evaluate these behavioral indicators. Additionally, the Contemplation Ladder, grounded in the Transtheoretical Model of Behavior Change, helps determine an individual's readiness to quit tobacco use, from no intention to active efforts toward cessation [9].

Nicotine exposure during adolescence poses particular risks to neural development, especially in areas governing reward processing, impulse control and decision-making. This can lead to a greater vulnerability not only to nicotine dependence but also to other addictive behaviors and mental health challenges later in life [10,11]. These risks are compounded by social and environmental influences that encourage early tobacco experimentation and habitual use [12,13]. Research indicates that nicotine's neurotoxic effects during adolescence can impair cognitive development, reduce impulse control and increase the likelihood of anxiety and substance use disorders [14,15]. As a result, adolescent tobacco use presents not only physical health risks but also serious psychological implications that may persist into adulthood [16].

The practicality and reliability of salivary cotinine have made it a preferred tool in adolescent research for measuring recent nicotine exposure [17]. Its non-invasive nature supports repeated sampling, a valuable attribute in longitudinal or school-based studies. However, despite its strengths in quantifying nicotine intake, cotinine alone cannot capture the full scope of dependence, which also includes behavioral, social and psychological components. Instruments like the FTND help quantify these subjective aspects, yet studies frequently report a weak correlation between biochemical markers like cotinine and self-reported dependence, underscoring the complexity of nicotine addiction [18].

Smokeless tobacco, often mistakenly perceived as a safer alternative to smoking, has seen increasing use among adolescents. This trend has been associated with rising rates of dependency and health complications, including oral cancers [19,20]. Salivary cotinine continues to serve as a reliable indicator for quantifying tobacco exposure in this demographic [21,22]. However, the psychological drivers of continued use must also be evaluated to inform effective intervention strategies.

This study aims to compare salivary cotinine levels with self-reported psychological nicotine dependence among adolescent users of smokeless tobacco. By addressing both biochemical and behavioral dimensions of nicotine use, this study seeks to deepen understanding of adolescent tobacco addiction and contribute to the development of targeted prevention and cessation efforts tailored to this vulnerable population.

METHODS

A cross-sectional study was conducted among adolescents aged 12 to 19 years, recruited from government schools in Chennai, Tamil Nadu. The study population included 150 participants, with a minimum of six months of smokeless tobacco use and no significant medical illness. Adolescents with existing oral conditions such as xerostomia, oral infections, or other systemic illnesses were excluded to eliminate confounding factors.

Nicotine dependence was assessed using two validated tools: the Fagerström Test for Nicotine Dependence (FTND) and the Contemplation Ladder [23]. The FTND consists of a standardized set of questions measuring behavioral patterns associated with nicotine use. Responses are scored to categorize dependence levels as follows: very low (0-2), low (3-4), moderate (5-6), high (7-8) and very high (9-10). This scoring system aids in identifying individuals with varying levels of nicotine addiction, thereby guiding the need for tailored intervention strategies.

The Contemplation Ladder was employed to evaluate psychological readiness to quit tobacco. This tool, grounded in the Transtheoretical Model of Behavior Change, assesses motivational stages from pre-contemplation to maintenance. Participants responded to questions exploring their awareness of health consequences, perceived benefits of quitting and social or psychological influences such as peer pressure and familial tobacco use. This dual-assessment approach helped capture both physiological dependence and motivational readiness.

Salivary cotinine levels were measured using a standardized oral fluid collection kit. Participants were instructed to abstain from food, drink, or tobacco products for at least 10 minutes prior to sample collection. Saliva was collected using a sponge-based collector placed under the tongue until saturated. The collected fluid was transferred to a testing cassette and results were interpreted within 10 minutes following manufacturer instructions. Cotinine levels were used as a biochemical marker to quantify recent nicotine exposure.

Ethical approval was obtained from the Institutional Review Board of Saveetha Institute of Medical and Technical Sciences. Written informed consent was obtained from all participants and assent from parents or guardians, in accordance with ethical standards for research involving minors.

Statistical analysis was performed using the Mann-Whitney U test for comparing two independent groups and the Kruskal-Wallis test for multiple group comparisons. A significance level of $p<0.05$ was considered statistically significant. All analyses were conducted using IBM SPSS Statistics.

RESULTS

A total of 150 adolescents participated in the study. Among them, 98 adolescents were not exposed to smokeless tobacco, while 52 were identified as current users of smokeless tobacco products.

Fagerström Test for Nicotine Dependence (FTND) revealed key behavioral patterns among the 52 adolescent tobacco users. Of these, 28 participants (53%) reported using tobacco within 30 minutes of waking, while 24 (46%) delayed use beyond 30 minutes. When ill, 32 participants (61%) continued to use tobacco, while 20 (38%) refrained. Regarding usage frequency, 32 participants (61%) used smokeless tobacco more than twice but fewer than four times per week, while 20 (38%) reported using it less frequently.

In terms of usage behavior, 31 adolescents (59%) habitually retained the tobacco dip, while 9 (17%) occasionally swallowed it and 12 (23%) had never swallowed it. When asked about dip retention duration, 28 participants (53%) kept the dip for 20-30 minutes and 24 (46%) kept it for 10-19 minutes. The daily duration of use, defined as time from first to last dip, was over 14.5 hours for 30 adolescents (57%) and under 14 hours for 12 (23%). Regarding quantity, 30 participants (57%) reported using 1-9 dips per day and 22 (42%) used 10-15 dips per day. Cravings were reported by 28 participants (53%) if more than two hours had passed without use, while 24 (46%) did not report strong cravings.

Based on the FTND scoring system, 19 adolescents (36%) were classified as having very high dependence, 21 (40%) had medium dependence, 11 (21%) had high dependence and 1 participant (1%) had very low dependence (Figure 1, 2).

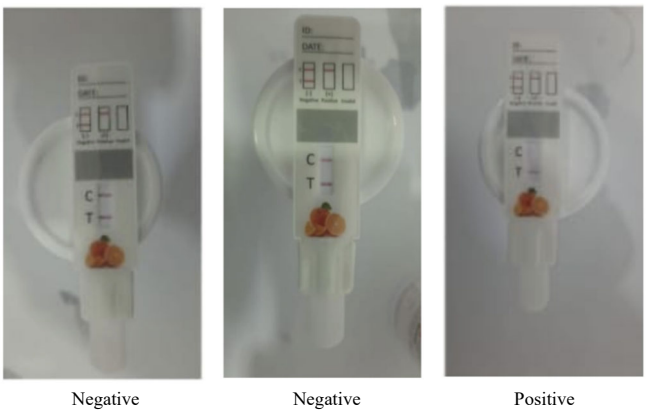

Figure 1: Cotinine assessment test

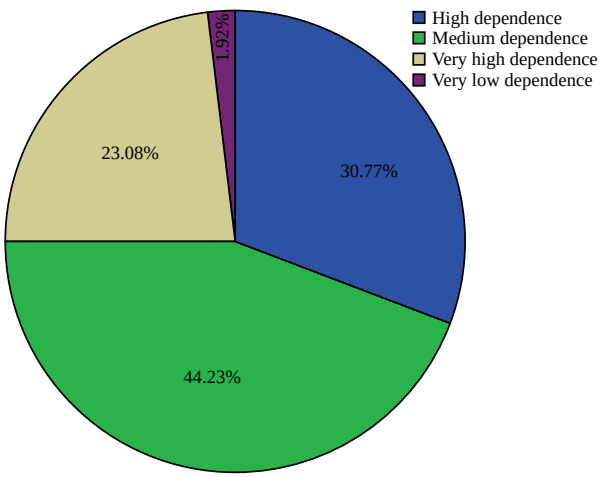

Figure 2: Percentage distribution of smokeless tobacco users based on Fagestrom dependence test

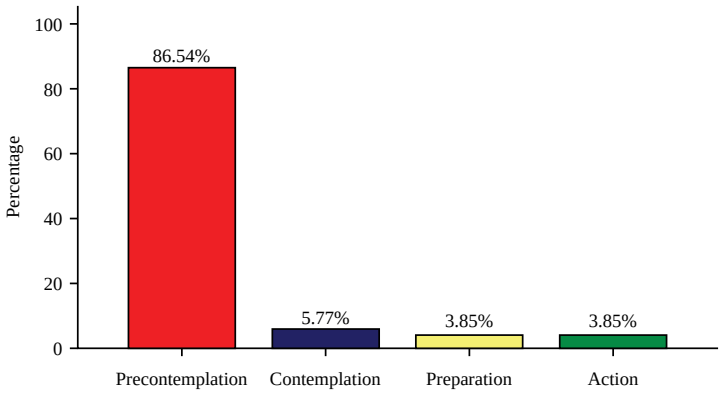

Figure 3: The percentage distribution of smokeless tobacco users based on contemplation ladder scale

Table 1: Descriptive statistics of salivary cotinine levels

	Salivary cotinine (ng/mL)			
	N	Mean	Standard deviation	Standard error Mean
Adolescents not consuming tobacco	92	13.12	2.950	0.298
Adolescents consuming smokeless tobacco	58	28.94	5.011	0.695

Table 2: Maan Whitney U-test results

		Salivary cotinine levels			
	N	Mean rank	U	Z	P
Adolescents not consuming tobacco	92	124.36	5088.50	10.064	0.000
Adolescents consuming smokeless tobacco	58	49.58			

Table 3: Kruskal Wallis analysis between nicotine dependence and salivary cotinine

	Salivary cotinine				
Dependence level	n	Mean rank	df	χ^2	p-value
Very low	1	27.50	3	1.779	0.620
Medium	23	23.39			
High	16	29.38			
Very high	12	28.54			

Assessment using the Contemplation Ladder revealed that 45 participants (86%) had no intention to quit, while 4 (8%) were ready to quit and 3 (6%) were considering quitting but were not yet ready (Figure 3). This highlights a strong resistance or lack of motivation toward cessation among most users.

Salivary cotinine levels were significantly higher among adolescents who used smokeless tobacco compared to those who did not. The Mann-Whitney U test indicated that users had a mean cotinine level of 124.36 ng/mL, while non-users had a mean level of 49.58 ng/mL, suggesting more than a two-fold increase in cotinine concentration among users. The difference was statistically significant ($U = 5088.50$, $Z = 10.064$, $p<0.001$) (Table 1, 2).

However, when comparing salivary cotinine levels across different nicotine dependence categories (as per FTND), no statistically significant association was found ($p = 0.620$) (Table 3). This suggests that while cotinine levels objectively measure nicotine exposure, they may not directly correspond to self-reported psychological dependence levels in adolescents.

DISCUSSION

This study offers a comprehensive view of nicotine dependence among adolescents by simultaneously evaluating biochemical exposure through salivary cotinine and psychological dependence using validated self-report tools. Unlike many tobacco-related studies that primarily focus on smoking, this research specifically investigates smokeless tobacco (Coollip) use among adolescents-a growing concern with distinct patterns of use and associated health risks. By targeting this often-overlooked population and examining both physiological and behavioral dimensions of nicotine addiction, the study contributes meaningfully to public health literature and early intervention research.

Salivary cotinine emerged as a reliable biomarker for detecting recent nicotine exposure among adolescent tobacco users. The results showed significantly elevated cotinine levels in smokeless tobacco users compared to non-users, confirming its utility for objective exposure assessment. However, in contrast to findings from some previous studies, this study did not observe a statistically significant correlation between salivary cotinine levels and scores on the Fagerström Test for Nicotine Dependence ($p = 0.620$). This discrepancy highlights the complex and multifactorial nature of nicotine addiction in adolescents, where psychological dependence may not always mirror biochemical markers.

Cotinine, a stable and long-lasting metabolite of nicotine, is widely recognized for its effectiveness in estimating tobacco exposure across biological fluids such as saliva, plasma and urine [24,25]. Its half-life of 16-20 hours, substantially longer than nicotine's, allows for detection of exposure even days after last use [26,27]. Following nicotine intake, especially through oral routes like smokeless tobacco, the substance undergoes hepatic metabolism-primarily via the cytochrome P450 2A6 enzyme-resulting in cotinine production [28,29]. The pharmacokinetics of cotinine remain consistent across studies, making it a preferred indicator in population-level assessments of tobacco use.

Previous research supports the value of cotinine as a predictor of dependence. For instance, Foulds *et al.* [30] observed a strong association between elevated cotinine levels and psychological dependence in adolescents. Similarly, Lanza *et al.* [31] linked higher cotinine concentrations with increased cravings and withdrawal symptoms. However, as this study indicates, psychosocial variables may play an equally significant role. Kwan *et al.* [32] emphasized that environmental and social influences-such as peer pressure and exposure to tobacco marketing-can shape both behavior and biochemical exposure, often independent of internal dependence mechanisms. Moreover, Cheng *et al.* [33] found that adolescents with lower initial cotinine levels responded more positively to early cessation support, underscoring the importance of early intervention.

Cotinine levels measured in saliva, urine and hair offer insights into recent and long-term exposure, including environmental tobacco smoke (ETS) [34]. Among these, saliva testing stands out for its non-invasive nature, making it particularly suitable for adolescent research. When paired with psychological tools like the FTND and Contemplation Ladder, cotinine testing provides a multidimensional profile of nicotine use. This combined approach aligns with public health recommendations to adopt integrated screening tools in adolescent cessation programs [35,36].

Importantly, the study revealed a troubling trend: despite high levels of nicotine exposure and psychological dependence, a vast majority (86%) of participants expressed no intention to quit. This lack of readiness poses a critical barrier to intervention and reflects broader psychosocial dynamics at play. As noted by Kwan *et al.* [32] and others, factors such as family tobacco use, cultural acceptance, misinformation about smokeless tobacco and peer normalization contribute to ongoing use. The data reinforce the urgency of developing youth-centered cessation programs that address both motivation and support systems.

Given these findings, comprehensive public health strategies should be prioritized. These include awareness campaigns in schools, motivational interviewing in clinical settings, family-based counseling and ongoing biochemical monitoring. Tailored interventions that consider both behavioral readiness and objective exposure are essential to reduce initiation, support cessation and prevent relapse among adolescents [37].

In conclusion, while salivary cotinine confirms elevated nicotine exposure among adolescent smokeless tobacco users, its lack of correlation with psychological dependence measures underscores the need for dual-focused strategies. Addressing both the biochemical and psychosocial dimensions of addiction will be critical in breaking the cycle of early tobacco initiation and long-term dependence.

CONCLUSION

This study highlights the importance of assessing both biochemical and psychological dimensions of nicotine dependence in adolescents. Salivary cotinine levels were significantly higher in smokeless tobacco users compared to non-users, confirming its effectiveness as an objective marker for recent nicotine exposure. However, the absence of a statistically significant correlation between cotinine levels and self-reported dependence scores underscores the complex nature of nicotine addiction in this age group. Psychological dependence may be influenced by behavioral, social and environmental factors that are not directly reflected in biochemical markers alone. These findings support the need for comprehensive tobacco cessation strategies that integrate both biochemical screening and behavioral assessment to better identify at-risk adolescents and provide tailored interventions.

Ethical Consideration

The study was conducted in accordance with ethical standards and approved by the Institutional Review Board of Saveetha Institute of Medical and Technical Sciences. Written informed consent was obtained from all participants, along with assent from parents or legal guardians, ensuring compliance with ethical guidelines for research involving minors.

Conflict of Interest

The authors declare no conflict of interest related to this study.

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REFERENCES

[1] Balasubramaniam, Arthi, and Meignana Indiran Arumugham. "Salivary oxidative stress level among tobacco chewers and smokers." *Journal of Advanced Pharmaceutical Technology & Research,* vol. 13, no. 1, November 2022, pp. S21-S25. http://dx.doi.org/10.4103/japtr.japtr_116_22.

[2] Lidón-Moyano, Cristina *et al.* "Third-hand exposure at homes: Assessment using salivary cotinine." *Environmental research,* vol. 196, May 2021. https://pubmed.ncbi.nlm.nih.gov/33129855/.

[3] Patel, Vrushali D. *et al.* "Comparative assessment of salivary cotinine level and psychological dependence among tobacco users." *Dental research journal,* vol. 14, no. 2, March 2017, pp. 125-130. https://pubmed.ncbi.nlm.nih.gov/28584536/.

[4] Takai, Noriyasu *et al.* "Gender-specific differences in salivary biomarker responses to acute psychological stress." *Annals of the New York Academy of Sciences,* vol. 1098, March 2007, pp. 510-515. https://pubmed.ncbi.nlm.nih.gov/17435161/.

[5] Singh, Satvinder *et al.* "Assessment of Salivary Catalase, a-Amylase, and Cotinine Levels in Chronic Smokers: A Comparative Study." *The journal of contemporary dental practice,* vol. 19, no. 3, March 2018, pp. 253-256. https://pubmed.ncbi.nlm.nih.gov/29603693/.

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

[7] Dhavan, Poonam *et al.* "Using Salivary Cotinine to Validate Self-Reports of Tobacco Use by Indian Youth Living in Low-Income Neighborhoods." *Asian Pacific journal of cancer prevention,* vol. 12, no. 10, March 2012, pp. 2551-2554. https://pmc.ncbi.nlm.nih.gov/articles/PMC3310162/.

[8] Pérez-Martín, Hipólito *et al.* "Changes in the salivary cotinine cut-offs to discriminate smokers and non-smokers before and after Spanish smoke-free legislation." *Cancer epidemiology,* vol. 80, October 2022. https://pubmed.ncbi.nlm.nih.gov/35878525/.

[9] Miskimins, Kelsey *et al.* "Comparative Analysis of Alternate Measures of Readiness to Quit Smoking: Stages of Change and the Contemplation Ladder." *Substance abuse and rehabilitation,* vol. 7, no. 14, December 2023, pp. 167-171. https://pubmed.ncbi.nlm.nih.gov/38089114/.

[10] Eswaramoorthy, Rajalakshmanan *et al.*, "Inflammation in smokers and non-smokers during implant surgery among Indians." *Bioinformation,* vol. 19, no. 4, April 2023, pp. 510-513. https://pubmed.ncbi.nlm.nih.gov/37822839/.

[11] Park, Sang-Hee. "Smoking and adolescent health." *Korean journal of pediatrics,* vol. 54, no. 10, October 2011, pp. 401-404. https://pubmed.ncbi.nlm.nih.gov/22232621/.

[12] Dawes, Kelsey *et al.* "Saliva DNA Methylation Detects Nascent Smoking in Adolescents." *Journal of child and adolescent psychopharmacology,* vol. 29, no. 7, August 2019, pp. 535-544. https://pubmed.ncbi.nlm.nih.gov/31180231/.

[13] Figueiredo, Valeska Carvalho *et al.* "Determinants of salivary cotinine level: a population-based study in Brazil." *Journal of public health,* vol. 41, no. 6, December 2007, pp. 954-962. https://pubmed.ncbi.nlm.nih.gov/17992352/.

[14] Sri, S. Soumya, *et al.* "Immunostimulating influence of herbal biomedicines on nonspecific immunity in grouper Epinephelus tauvina juvenile against Vibrio harveyi infection.." *Journal of Pioneering Medical Sciences*, vol. 13, no. 7, December 2024, pp. 116-120. https://www.semanticscholar.org/paper/Assessment-of-Mental-Health-Status-and-its-with-in-Sri-Leelavathi/ec84b8909c5d2ad3dcaf51255c9e0abca115cdb2.

[15] National Center for Chronic Disease Prevention and Health Promotion (US). *Preventing tobacco use among youth and young adults: A report of the Surgeon General.* Centers for Disease Control and Prevention (US), Office on Smoking and Health. Atlanta (GA), 2012.

[16] Fu, Marcela *et al.* "Salivary cotinine concentrations in daily smokers in Barcelona, Spain: a cross-sectional study." *BMC Public Health,* vol. 9, September 2009. https://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-9-320.

[17] Morris, Matthew C. *et al.* "Salivary stress biomarkers of recent nicotine use and dependence." *The American journal of drug and alcohol abuse,* vol. 42, no. 6, November 2016, pp. 640-648. https://pubmed.ncbi.nlm.nih.gov/27463324/.

[18] Han, Yineng *et al.* "Salivary Exosomes: Emerging Roles in Systemic Disease." *International journal of biological sciences,* vol. 14, no. 6, April 2018, pp. 633-643. https://pubmed.ncbi.nlm.nih.gov/29904278/.

[19] Warren, Charles W. *et al.* "Global youth tobacco surveillance, 2000-2007." *Morbidity and mortality weekly report,* vol. 57, no. 1, January 2008, pp. 1-28. https://pubmed.ncbi.nlm.nih.gov/18219269/.

[20] Gupta, Prakash C. *et al.* "Smokeless tobacco: a major public health problem in the SEA region: a review." *Indian journal of public health,* vol. 55, no. 3, September 2011, pp. 199-209. https://pubmed.ncbi.nlm.nih.gov/22089688/.

[21] Benowitz, Neal L. *et al.* "Nicotine chemistry, metabolism, kinetics and biomarkers." *Handbook of experimental pharmacology,* vol. 192, September 2011, pp. 29-60. https://pubmed.ncbi.nlm.nih.gov/19184645/.

[22] Etzel, R.A. "A review of the use of saliva cotinine as a marker of tobacco smoke exposure." *Preventive medicine,* vol. 19, no. 2, March 1990, pp. 190-197. https://pubmed.ncbi.nlm.nih.gov/2193308/.

[23] Biener, L. and D. B. Abrams. "The Contemplation Ladder: validation of a measure of readiness to consider smoking cessation." *Health psychology,* vol. 10, no. 5, 1991, pp. 360-365. https://pubmed.ncbi.nlm.nih.gov/1935872/.

[24] Norderhaug, Inger Natvig. *Health Effects and Dependency Associated with Snuff Consumption [Internet].* Knowledge Centre for the Health Services at The Norwegian Institute of Public Health (NIPH), Report from Norwegian Knowledge Centre for the Health Services (NOKC) No. 06-2005. 2005, https://pubmed.ncbi.nlm.nih.gov/29319988/.

[25] Rubinstein, Mark L. *et al.* "Cotinine levels in relation to smoking behavior and addiction in young adolescent smokers." *Nicotine & tobacco research,* vol. 9, no. 1, January 2007, pp. 129-135. https://pubmed.ncbi.nlm.nih.gov/17365743/.

[26] Mulcahy, M. *et al.* "Secondhand smoke exposure and risk following the Irish smoking ban: an assessment of salivary cotinine concentrations in hotel workers and air nicotine levels in bars." *Tobacco control,* vol. 14, no. 6, December 2005, pp. 384-388. https://pubmed.ncbi.nlm.nih.gov/16319361/.

[27] Chaffee, Benjamin W. *et al.* "Exposure to a Tobacco-Specific Carcinogen Among Adolescent Smokeless Tobacco Users in Rural California, United States." *Nicotine & tobacco research,* vol. 22, no. 10, October 2020, pp. 1764-1771. https://pubmed.ncbi.nlm.nih.gov/31504879/.

[28] Riis, Jenna L. *et al.* "Salivary cytokines in healthy adolescent girls: Intercorrelations, stability, and associations with serum cytokines, age, and pubertal stage." *Developmental psychobiology,* vol. 56, no. 4, May 2014, pp. 797-811. https://pubmed.ncbi.nlm.nih.gov/23868603/.

[29] Jenkins, R. A. and R. W. Counts "Personal exposure to environmental tobacco smoke: salivary cotinine, airborne nicotine, and nonsmoker misclassification." *Journal of exposure analysis and environmental epidemiology,* vol. 9, no. 4, August 1999, pp. 352-363. https://pubmed.ncbi.nlm.nih.gov/10489160/.

[30] Baskerville, Neill Bruce *et al.* "Effect of a digital social media campaign on young adult smoking cessation." *Nicotine & Tobacco Research,* vol. 18, no. 3, June 2015, pp. 351-360. https://academic.oup.com/ntr/article-abstract/18/3/351/2583993.

[31] Lanza, S.T. *et al.* "Cotinine levels and psychological symptoms in adolescents using smokeless tobacco." *Addictive Behaviors*, vol. 90, 2019, pp. 155-162.

[32] Kwan, P. *et al.* "Social and environmental influences on tobacco dependence in adolescents: The role of peer influence and marketing." *Journal of Adolescent Health*, vol. 68, no. 3, 2021, pp. 543-550.

[33] Cheng, J. *et al.* "Early intervention effects on salivary cotinine levels and psychological outcomes in adolescents: A randomized controlled trial." *Tobacco Control*, vol. 29, no. 4, 2020, pp. 445-451.

[34] Ahmed, Naveed *et al.* "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, October 2021. http://dx.doi.org/10.3390/ijerph182111003.

[35] Nivethitha, R. and L. Leelavathi. "Awareness on ill effects of tobacco usage among tobacco users." *Journal of advanced pharmaceutical technology and research,* vol. 13, no. Suppl 1, November 2022, pp. S217-S222. https://pubmed.ncbi.nlm.nih.gov/36643121/.

[36] Haley, N.J. and D. Hoffmann. "Analysis for nicotine and cotinine in hair to determine cigarette smoker status." *Clinical chemistry,* vol. 31, no. 10, October 1985, pp. 1598-1600. https://pubmed.ncbi.nlm.nih.gov/4042325/.

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