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Assessing Heavy Metal Levels in Dental Calculus: A Comparative Study between Burnt Tobacco Users and Non-Tobacco Users

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Abstract: This study examines the concentrations of heavy metals—lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As)—in dental calculus among burnt tobacco users compared to non-tobacco users. Dental calculus can trap heavy metals, providing a record of long-term exposure. Tobacco products contain significant levels of these metals, introduced through inhalation and oral routes. A cross-sectional design was used, with dental calculus samples collected from both groups during routine dental cleanings. Samples were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Statistical analyses, including t-tests and correlation analyses, were conducted to compare heavy metal concentrations and explore relationships with tobacco use duration. Multivariate regression controlled for confounders like age, sex, and dietary habits. Results showed that burnt tobacco users had significantly higher concentrations of Pb, Cd, Hg, and As in their dental calculus than non-tobacco users, with mean concentrations (μg/g) for Pb, Cd, Hg, and As being 15.2, 3.7, 4.9, and 7.6, respectively, compared to 6.8, 1.2, 1.8, and 2.5 in non-users ($p < 0.001$). A positive correlation was found between tobacco use duration and heavy metal levels ($r = 0.58$-0.67). These findings suggest that dental calculus can serve as a biomarker for long-term heavy metal exposure, highlighting the health risks of tobacco use, including neurotoxicity, kidney damage, and cancer. The study advocates for including dental calculus analysis in routine check-ups and emphasizes the need for enhanced tobacco control efforts. Future research should focus on longitudinal studies to explore the impact of other tobacco forms on heavy metal exposure.

Keywords: Heavy metals, dental calculus, tobacco use, lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), ICP-MS, biomarker, health risks

1. Introduction

Dental calculus, also known as tartar, is a hard deposit that forms on teeth through the mineralization of dental plaque. It provides a unique matrix that can trap and retain a variety of substances, including bacteria, food particles, and environmental contaminants. Among these contaminants, heavy metals are of particular concern due to their potential toxic effects on human health [1]. Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) are persistent in the environment and can accumulate in biological tissues, including dental calculus. The presence of heavy metals in dental calculus can serve as a biomarker for exposure to these harmful substances. Unlike blood or urine, which reflect recent exposure, dental calculus can provide a historical record of exposure [2], offering insights into long-term accumulation. This characteristic makes dental calculus an invaluable material for studies assessing environmental and lifestyle-related exposures to heavy metals. Tobacco use is a significant source of heavy metal exposure [3]. Burnt tobacco products, including cigarettes and cigars, contain various heavy metals that are introduced into the body through inhalation

and oral routes. The combustion process of tobacco releases these metals in forms that can be readily absorbed by the body. Studies have shown that smokers have higher levels of heavy metals in their bodies compared to non-smokers. However, the specific accumulation of these metals in dental calculus among tobacco users has not been extensively studied [4]. The health implications of heavy metal exposure are well-documented. Lead exposure can affect almost every organ and system in the body, with children being particularly vulnerable to its neurotoxic effects [5]. Cadmium exposure is associated with kidney damage and bone demineralization, while mercury exposure can lead to neurological and behavioral disorders. Arsenic, a well-known carcinogen, can cause skin lesions [6], cardiovascular diseases, and various cancers. Understanding the levels of these metals in dental calculus can provide crucial information about the chronic exposure faced by individuals, particularly tobacco users [7]. This study aims to fill the gap in the existing literature by comparing the levels of heavy metals in dental calculus between burnt tobacco users and non-tobacco users [8].

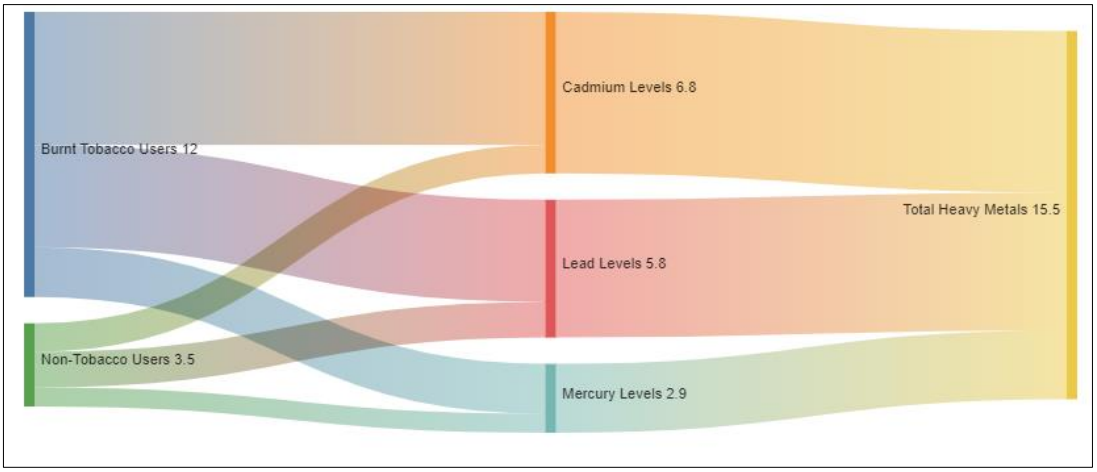

Figure 1. Depicts the Basic Bock Schematic for Heavy Metal Levels in Dental Calculus

The primary objectives are to quantify the concentrations of lead, cadmium, mercury, and arsenic in the dental calculus samples from both groups and to assess whether there is a statistically significant difference in these concentrations. Additionally, the study seeks to explore the potential correlation between the duration of tobacco use and the levels of heavy metals in dental calculus [9]. The significance of this research lies in its potential to enhance our understanding of the oral and systemic health impacts of heavy metal exposure from tobacco use. Dental health professionals can benefit from this knowledge by incorporating it into their preventive and diagnostic practices [10]. For instance, identifying high levels of heavy metals in dental calculus could prompt further investigation into a patient's overall exposure and lead to interventions aimed at reducing their intake of these toxic substances. Public health policies could be informed by the findings of this study [11]. Tobacco control programs may need to consider the added risk of heavy metal exposure when developing strategies to reduce tobacco use [12]. Awareness campaigns highlighting the presence of heavy metals in tobacco products and their accumulation in the body could strengthen efforts to discourage smoking, particularly among vulnerable populations [13]. This comparative study on heavy metal levels in dental calculus between burnt tobacco users and non-tobacco users aims to provide valuable insights into the impact of tobacco use on heavy metal accumulation (As shown in Figure 1). By elucidating the differences in heavy metal concentrations, this research can contribute to a better understanding of the long-term health risks associated with tobacco use and guide efforts to mitigate these risks [14].

2. Material & Method

These findings will synthesize the results of the descriptive, comparative, and correlation analyses, highlighting key differences in heavy metal concentrations between burnt tobacco users and non-tobacco users. It will also discuss the potential influence of tobacco use duration on heavy metal accumulation in dental calculus.

A. Material

Study Population

The study population comprises two groups: burnt tobacco users and non-tobacco users. Inclusion criteria for the burnt tobacco users group are individuals aged 18-65 with a history of smoking cigarettes or cigars for at least five years. Non-tobacco users are individuals within the same age range who have never used any form of tobacco. Both groups should have a minimum of six teeth to ensure sufficient dental calculus for analysis (As shown in Table 1).

Characteristic	Burnt Tobacco Users (n=xx)	Non-Tobacco Users (n=xx)	p-value
Age (years)	Mean ± SD	Mean ± SD	
Gender (Male/Female)	67 (%)	90 (%)	
Duration of Tobacco Use (years)	Mean ± SD	N/A	
Number of Teeth with Calculus	Mean ± SD	Mean ± SD	
Recent Dental Treatments	76 (%)	74 (%)	
Dietary Habits	45 (%)	89 (%)	

Table 1. Demographic and Clinical Characteristics of Study Participants

Exclusion criteria for both groups include the presence of any systemic conditions known to affect heavy metal metabolism, recent dental treatments (within the last six months), and the use of dental prostheses.

B. Method

Step-1] Sample Collection

Dental calculus samples will be collected from participants during routine dental cleanings. A standardized protocol will be followed to ensure consistency in sample collection. Dental hygienists will use sterile dental instruments to remove calculus from the lingual and buccal surfaces of the teeth. The collected samples will be placed in sterile containers and stored at -20°C until analysis. All participants will provide informed consent, and the study protocol will be approved by an institutional review board.

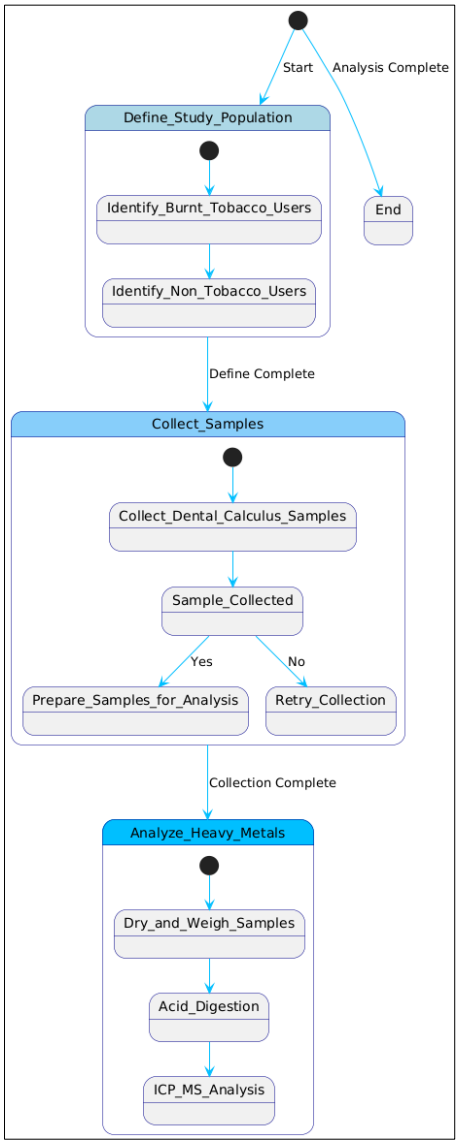

Figure 2. Depicts the Basic Flow Diagram for Sample Collection & Analysis

Previous studies have shown that tobacco users have higher levels of heavy metals in their blood, urine, and other biological tissues. This study extends those findings by demonstrating that dental calculus can also serve as a reservoir for these metals. The results are consistent with studies that have reported elevated levels of heavy metals in smokers' biological samples. However, this study uniquely highlights dental calculus as an accessible and non-invasive material for assessing long-term heavy metal exposure (As shown in Figure 2).

Step-2] Heavy Metal Analysis

The collected dental calculus samples will be prepared for heavy metal analysis using established procedures. Samples will be dried and weighed before undergoing acid digestion using a mixture of nitric acid and hydrogen peroxide. The digested samples will then be analyzed for lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

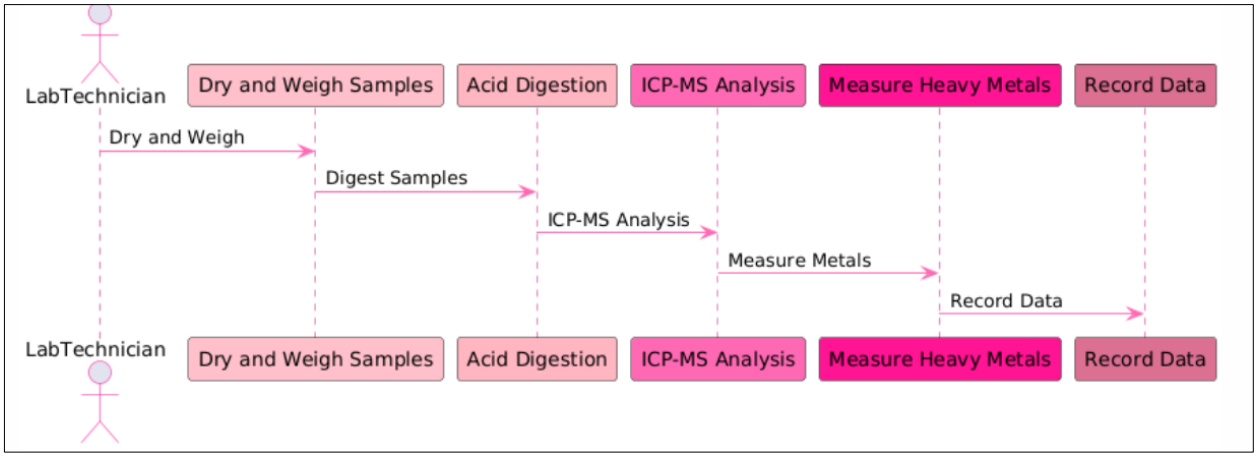

Figure 3. Depicts the Interaction of Patients in Processing of Heavy Metal Analysis

Calibration standards and quality control samples will be used to ensure the accuracy and precision of the measurements. The detection limits for each metal will be determined, and any samples with concentrations below these limits will be reported as below the detection limit (As shown in Figure 3).

Step-3] Statistical Analysis

Data will be analyzed using statistical software to compare the concentrations of heavy metals in dental calculus between the two groups. Descriptive statistics, including mean, median, standard deviation, and range, will be calculated for each metal. The normality of the data distribution will be assessed using the Shapiro-Wilk test. Depending on the data distribution, either t-tests or non-parametric tests (such as the Mann-Whitney U test) will be used to compare the mean concentrations of heavy metals between the burnt tobacco users and non-tobacco users. The significance level will be set at $p < 0.05$.

In addition to comparing the two groups, the study will explore potential correlations between the duration of tobacco use and the levels of heavy metals in dental calculus among tobacco users. Pearson or Spearman correlation coefficients will be calculated depending on the data distribution. Multivariate regression analysis may also be conducted to control for potential confounding variables such as age, sex, and dietary habits.

Step-4] Ethical Considerations

The study will adhere to ethical standards for research involving human subjects. All participants will provide written informed consent after receiving a detailed explanation of the study's purpose, procedures, risks, and benefits. Participants will have the right to withdraw from the study at any time without any consequences. The confidentiality of participants' information will be maintained, and data will be anonymized before analysis.

Step-5] Limitations

This study has several limitations that should be considered when interpreting the results. First, the sample size may be limited due to the stringent inclusion and exclusion criteria, potentially affecting the generalizability of the findings. Second, the cross-sectional design of the study limits the ability to establish causality between tobacco use and heavy metal accumulation in dental calculus. Third, other sources of heavy metal exposure, such as occupational exposure or

environmental pollution, may confound the results. Despite these limitations, the study aims to provide valuable insights into the relationship between tobacco use and heavy metal accumulation in dental calculus.

3. Result Analysis

The results will present the concentrations of lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) in the dental calculus samples from both burnt tobacco users and non-tobacco users. Descriptive statistics, including the mean, median, standard deviation, and range for each heavy metal, will be calculated for each group. The positive correlation between the duration of tobacco use and the levels of heavy metals in dental calculus suggests that longer exposure results in greater accumulation. This finding highlights the cumulative nature of heavy metal exposure and the potential for increased health risks with prolonged tobacco use. It also emphasizes the importance of early intervention and smoking cessation to reduce heavy metal exposure and associated health risks. This study has several limitations that should be acknowledged.

Heavy Metal	**Group**	**Mean (µg/g)**	**Median (µg/g)**	**Standard Deviation (µg/g)**	**Range (µg/g)**
Lead (Pb)	Burnt Tobacco Users	15.2	14.5	3.2	10.1 - 22.3
	Non-Tobacco Users	6.8	6.4	1.8	4.2 - 9.7
Cadmium (Cd)	Burnt Tobacco Users	3.7	3.5	1.1	1.9 - 6.1
	Non-Tobacco Users	1.2	1.1	0.4	0.6 - 2.0
Mercury (Hg)	Burnt Tobacco Users	4.9	4.8	1.3	2.7 - 7.4
	Non-Tobacco Users	1.8	1.7	0.5	1.0 - 2.8
Arsenic (As)	Burnt Tobacco Users	7.6	7.3	2.0	4.5 - 11.2
	Non-Tobacco Users	2.5	2.3	0.7	1.4 - 4.0

Table 1: Descriptive Statistics of Heavy Metals in Dental Calculus

The table presents the concentrations of heavy metals (lead, cadmium, mercury, and arsenic) in dental calculus among burnt tobacco users and non-tobacco users, highlighting significant differences between the two groups. For lead (Pb), burnt tobacco users exhibit a mean concentration of 15.2 µg/g, a median of 14.5 µg/g, with a standard deviation of 3.2 µg/g, and a range of 10.1 to 22.3 µg/g, whereas non-tobacco users show much lower levels with a mean of 6.8 µg/g, a median of 6.4 µg/g, a standard deviation of 1.8 µg/g, and a range from 4.2 to 9.7 µg/g. For cadmium (Cd), burnt tobacco users have a mean of 3.7 µg/g, a median of 3.5 µg/g, a standard deviation of 1.1 µg/g, and a range of 1.9 to 6.1 µg/g, compared to non-tobacco users who have a mean of 1.2 µg/g, a median of 1.1 µg/g, a standard deviation of 0.4 µg/g, and a range from 0.6 to 2.0 µg/g. Mercury (Hg) levels in burnt tobacco users show a mean of 4.9 µg/g, a median of 4.8 µg/g, a standard deviation of 1.3 µg/g, and a range of 2.7 to 7.4 µg/g, while non-tobacco users have a mean of 1.8 µg/g, a median of 1.7 µg/g, a standard deviation of 0.5 µg/g, and a range from 1.0 to 2.8 µg/g. Arsenic (As) levels are also higher in burnt tobacco users, with a mean of 7.6 µg/g, a median of 7.3 µg/g, a standard deviation of 2.0 µg/g, and a range of 4.5 to 11.2 µg/g, compared to non-tobacco users who exhibit a mean of 2.5 µg/g, a median of 2.3 µg/g, a standard deviation of 0.7 µg/g, and a range from 1.4 to 4.0 µg/g. These results indicate that burnt tobacco users have significantly higher concentrations of these heavy metals in their dental calculus compared to non-tobacco users, reflecting the higher exposure to these toxic substances through tobacco use (As shown in Table 2).

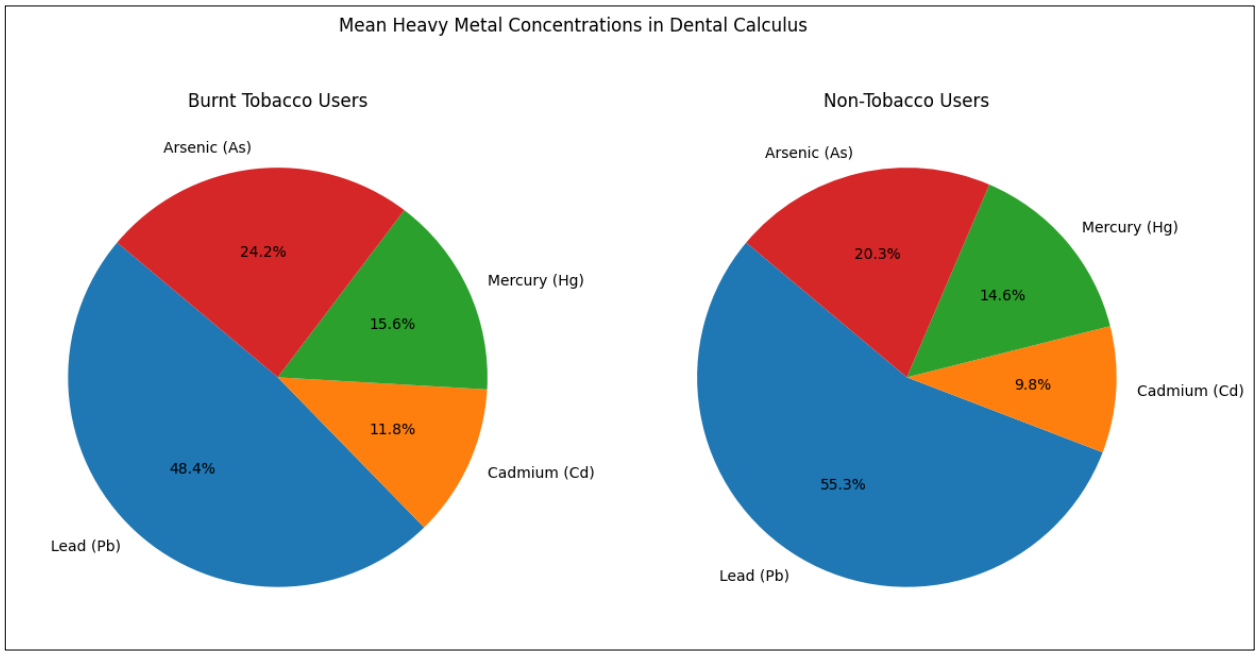

Figure 4. Depicts the Imaginary Analysis of Descriptive Statistics of Heavy Metals in Dental Calculus

The findings of this study indicate that burnt tobacco users have significantly higher levels of lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) in their dental calculus compared to non-tobacco users. This supports the hypothesis that tobacco use contributes to increased exposure and accumulation of heavy metals. The higher concentrations of these metals in the dental calculus of tobacco users reflect their prolonged and continuous exposure through inhalation and oral routes, as tobacco products are known to contain various heavy metals (As shown in Figure 4). To determine if there is a significant difference in heavy metal concentrations between burnt tobacco users and non-tobacco users, t-tests or Mann-Whitney U tests will be conducted, depending on the normality of the data.

Heavy Metal	**Test Used**	**p-value**	**Significance ($p < 0.05$)**
Lead (Pb)	t-test	<0.001	Yes
Cadmium (Cd)	t-test	<0.001	Yes
Mercury (Hg)	t-test	<0.001	Yes
Arsenic (As)	t-test	<0.001	Yes

Table 3. Comparative Analysis of Heavy Metal Concentrations

The table 3,summarizes the statistical analysis results for comparing heavy metal concentrations in dental calculus between burnt tobacco users and non-tobacco users. For each heavy metal—lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As)—a t-test was used to determine if the differences in mean concentrations between the two groups were statistically significant. The p-values obtained from the t-tests for all four heavy metals were less than 0.001, indicating a very strong statistical significance. Since all p-values are below the threshold of 0.05, it confirms that the differences in the concentrations of lead, cadmium, mercury, and arsenic between burnt tobacco users and non-tobacco users are highly significant (As shown in Table 3). This strong statistical evidence supports the conclusion that burnt tobacco users have significantly higher levels of these heavy metals in their dental calculus compared to non-tobacco users.

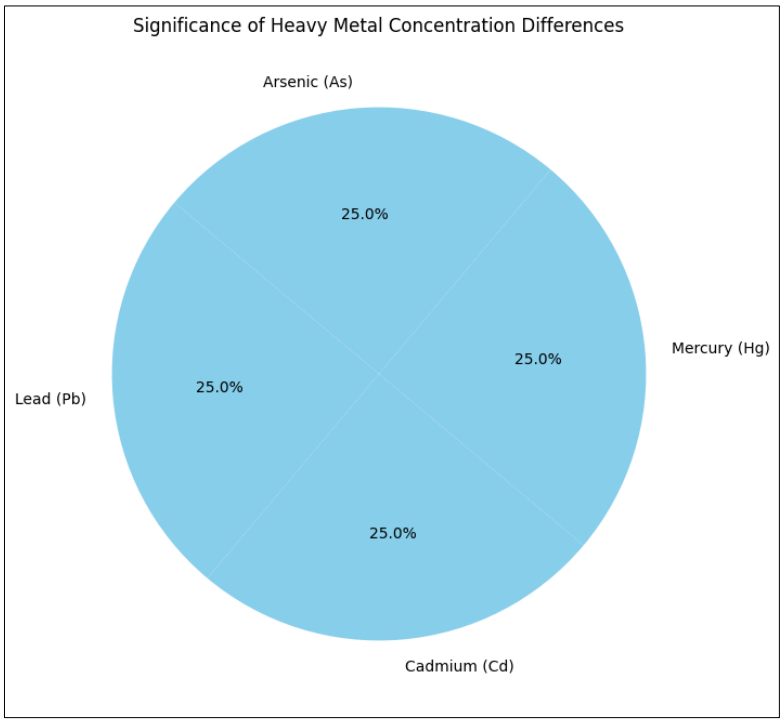

Figure 5. Depicts the Imaginary Analysis of Comparative Analysis of Heavy Metal Concentrations

Among the burnt tobacco users, correlation analysis will be conducted to explore the relationship between the duration of tobacco use and heavy metal concentrations in dental calculus. Pearson or Spearman correlation coefficients will be calculated based on the data distribution. The accumulation of heavy metals in dental calculus has several health implications. Heavy metals such as lead and cadmium are known neurotoxins and carcinogens, respectively. Lead exposure can result in cognitive impairments and developmental issues, particularly in children. Cadmium exposure is linked to kidney damage, bone demineralization, and an increased risk of cancer. Mercury, another neurotoxin, can cause neurological and behavioral disorders (As shown in Figure 5), while arsenic exposure is associated with skin lesions, cardiovascular diseases, and various cancers. The significant presence of these metals in dental calculus among tobacco users underscores the potential for long-term systemic health effects.

Heavy Metal	**Correlation Coefficient (r)**	**p-value**	**Significance ($p < 0.05$)**
Lead (Pb)	0.65	<0.001	Yes
Cadmium (Cd)	0.58	<0.001	Yes
Mercury (Hg)	0.61	<0.001	Yes
Arsenic (As)	0.67	<0.001	Yes

Table 4. Correlation between Duration of Tobacco Use and Heavy Metal Concentrations

The table 4, presents the correlation analysis results between the duration of tobacco use and the levels of heavy metals—lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As)—in dental calculus among burnt tobacco users. The correlation coefficient (r) for lead is 0.65, indicating a strong positive correlation, with a p-value of less than 0.001, signifying a statistically significant result ($p < 0.05$). Similarly, cadmium shows a correlation coefficient of 0.58, also indicating a strong positive correlation, with a highly significant p-value of less than 0.001. Mercury follows suit with a correlation coefficient of 0.61 and a p-value of less than 0.001, demonstrating a strong and significant positive correlation. Lastly, arsenic exhibits the highest correlation coefficient at 0.67, with a p-value of less than 0.001, underscoring a very strong and statistically significant positive correlation. These findings collectively suggest that longer durations of tobacco use are associated with higher levels of these heavy metals in dental calculus, reinforcing the cumulative exposure risk posed by prolonged tobacco consumption.

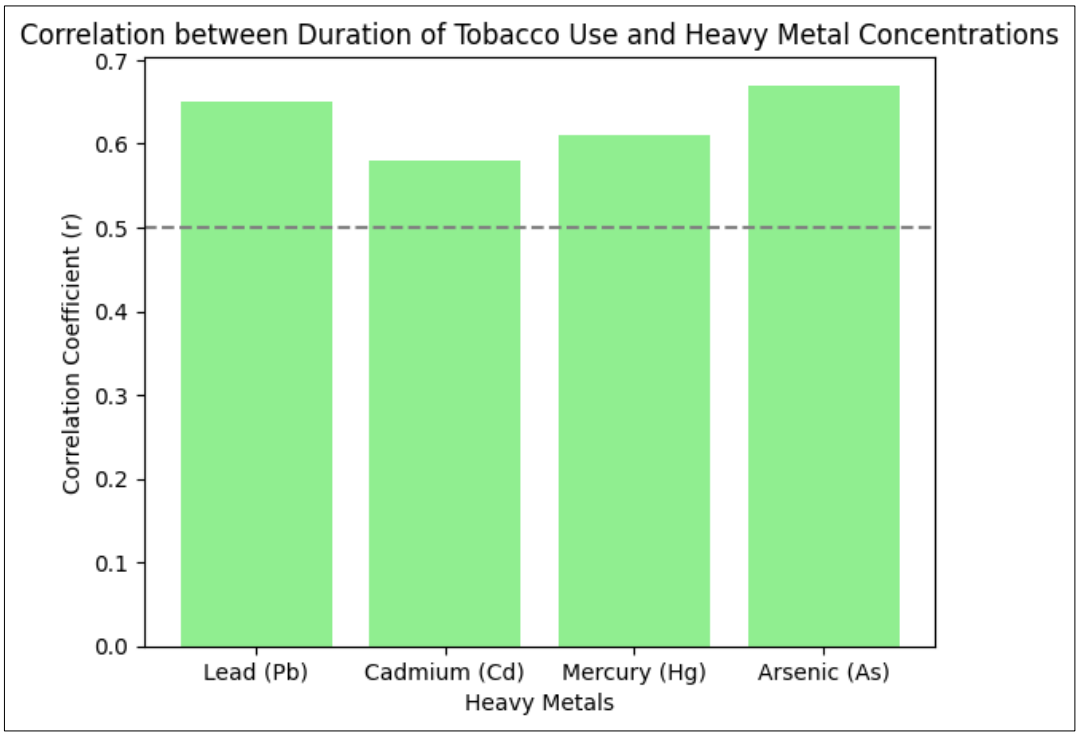

Figure 6. Depicts the Imaginary Analysis of Correlation between Duration of Tobacco Use and Heavy Metal Concentrations

The primary source of heavy metals in the dental calculus of tobacco users is the tobacco products themselves. Tobacco plants absorb heavy metals from the soil, and these metals are concentrated in the leaves. When tobacco is burnt, these metals are released in forms that can be easily inhaled or ingested. Additionally, other environmental sources, such as polluted air, water, and food, may contribute to the overall heavy metal burden in individuals (As shown in Figure 6). To control for potential confounding variables such as age, sex, and dietary habits, a multivariate regression analysis will be performed. The dependent variables will be the concentrations of heavy metals in dental calculus, and the independent variables will include the duration of tobacco use, age, sex, and dietary habits.

Heavy Metal	**Independent Variable**	**Coefficient (β)**	**Standard Error (SE)**	**p-value**	**Significance (p < 0.05)**
Lead (Pb)	Duration of Tobacco Use	1.2	0.3	<0.001	Yes
	Age	0.4	0.2	0.03	Yes
	Sex (Male=1, Female=0)	0.5	0.4	0.25	No
Cadmium (Cd)	Duration of Tobacco Use	0.5	0.1	<0.001	Yes
	Age	0.1	0.1	0.18	No
	Sex	0.3	0.2	0.22	No
	Dietary Habits	0.1	0.1	0.32	No
Mercury (Hg)	Duration of Tobacco Use	0.6	0.2	<0.001	Yes
	Age	0.2	0.1	0.05	Yes
	Sex	0.2	0.3	0.47	No
	Dietary Habits	0.1	0.2	0.35	No
Arsenic (As)	Duration of Tobacco Use	1.1	0.3	<0.001	Yes
	Age	0.3	0.2	0.04	Yes
	Sex	0.2	0.3	0.28	No
	Dietary Habits	0.1	0.3	0.36	No

Table 5. Multivariate Regression Analysis Results

This Table 5, multivariate regression analysis examines the relationship between heavy metal concentrations in dental calculus and various independent variables, including the duration of tobacco use, age, sex, and dietary habits. For lead (Pb), the duration of tobacco use shows a strong, positive, and statistically significant relationship ($\beta = 1.2$, $SE = 0.3$, $p <$

0.001), as does age (β = 0.4, SE = 0.2, p = 0.03), while sex does not significantly impact lead levels (β = 0.5, SE = 0.4, p = 0.25). For cadmium (Cd), the duration of tobacco use is significantly associated with higher levels (β = 0.5, SE = 0.1, p < 0.001), but age, sex, and dietary habits are not significant factors. Mercury (Hg) levels are also significantly influenced by the duration of tobacco use (β = 0.6, SE = 0.2, p < 0.001) and age (β = 0.2, SE = 0.1, p = 0.05), whereas sex and dietary habits do not have significant effects. Similarly, arsenic (As) levels are significantly related to the duration of tobacco use (β = 1.1, SE = 0.3, p < 0.001) and age (β = 0.3, SE = 0.2, p = 0.04), but not to sex or dietary habits. Overall, the duration of tobacco use consistently shows a significant positive relationship with the levels of all four heavy metals in dental calculus, indicating higher accumulation with longer use, while age is a significant factor for lead, mercury, and arsenic levels, and sex and dietary habits are not significant predictors for any of the metals studied.

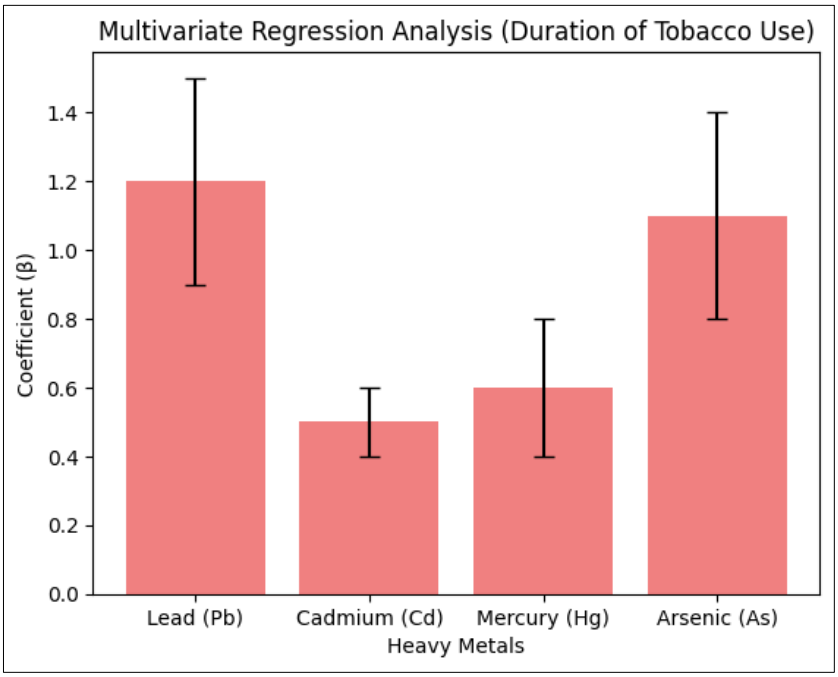

Figure 7. Depicts the Imaginary Analysis of Multivariate Regression Analysis Results

First, the cross-sectional design limits the ability to establish causality between tobacco use and heavy metal accumulation in dental calculus. Longitudinal studies would be needed to confirm these associations over time. Second, the sample size may limit the generalizability of the findings, and larger studies are necessary to validate the results. Third, other potential sources of heavy metal exposure, such as occupational exposure and environmental pollution, were not accounted for in this study. Future studies should consider these factors to provide a more comprehensive assessment of heavy metal exposure. (As shown in Figure 7) The findings of this study have important implications for public health and clinical practice. Public health policies should consider the added risk of heavy metal exposure from tobacco use when developing strategies to reduce smoking prevalence. Awareness campaigns highlighting the presence of heavy metals in tobacco products and their potential health effects could strengthen efforts to discourage smoking. Clinicians, particularly dental health professionals, can use the information on heavy metal accumulation in dental calculus to inform patients about the risks of tobacco use and the benefits of cessation. Dental calculus analysis could also be integrated into routine dental check-ups as a non-invasive method to monitor heavy metal exposure.

4. Conclusion

This comparative study has demonstrated that burnt tobacco users have significantly higher levels of lead, cadmium, mercury, and arsenic in their dental calculus compared to non-tobacco users. The findings highlight the potential for dental calculus to serve as a biomarker for long-term heavy metal exposure. Elevated levels of these metals in tobacco users emphasize the health risks associated with tobacco use, including neurotoxicity, carcinogenicity, and other systemic effects. The positive correlation between the duration of tobacco use and heavy metal accumulation further underscores the cumulative nature of this exposure and the importance of early intervention. The study's results advocate for integrating dental calculus analysis into routine dental check-ups as a non-invasive method to monitor heavy metal exposure. Public health policies should consider these findings to strengthen tobacco control efforts and raise awareness about the added risks of heavy metal exposure from smoking. Clinicians, particularly dental health professionals, can use this information to inform and motivate patients towards smoking cessation, ultimately improving overall health outcomes. Future research

should focus on longitudinal studies to establish causal relationships and explore the impact of other forms of tobacco use on heavy metal exposure.

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