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Study To Evaluate and Correlate the Levels of Heavy Metals in Dental Calculus of Burnt Tobacco (Mishri) Users and Non-Tobacco Users with Their Periodontal Status Using Atomic Absorption Spectrometry (Aas)

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Abstract

The use of burnt tobacco products, such as Mishri, is associated with numerous health risks, including periodontal diseases. Heavy metals present in tobacco can accumulate in dental calculus, potentially exacerbating periodontal conditions. This study aimed to assess and establish a correlation between the levels of heavy metals present in dental calculus among burnt tobacco (Mishri) users and non-tobacco users, in relation to their periodontal health status. Utilizing Atomic Absorption Spectrometry (AAS), heavy metal concentrations were measured in dental calculus samples collected from both user groups. The study included comprehensive periodontal assessments to evaluate the periodontal status of participants. Results revealed significant differences in heavy metal levels between tobacco users and non-users. Additionally, correlations between heavy metal concentrations and periodontal parameters were explored. This research sheds light on the potential oral health implications of burnt tobacco use and highlights the importance of monitoring heavy metal exposure in dental calculus as a potential biomarker for periodontal health assessment.

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INTRODUCTION

Periodontitis is a long lasting multi-factorial inflammatory disease that Affects the gingiva, periodontal ligament, and alveolar bone. This eventually results in its destruction and tooth loss in adults. Dye et al. stated periodontitis to be among the most common diseases around the globe. [1] The affected individuals initially show an impact of destruction. The disease then results in more significant progression to the adjoining supporting structures, starting up with gingival recession. It is typically associated with or without dentinal hypersensitivity in its early stages. Subsequently, the condition progresses, leading to tooth mobility, pathological migration, and ultimately, tooth loss. This directly affects chewing ability, speech functions, aesthetic characteristics, psychological aspects and quality of life. [2,3] Periodontitis, being a multi-factorial disease, is not caused by single risk element alone.

Several etiological factors have been proposed that are responsible for periodontitis including dental plaque, dental calculus, and systemic diseases. The deleterious habits like smoked or smokeless forms of tobacco, betel nut chewing, etc are also counted among them. Dental plaque and calculus are the main causative agents responsible for the initiation and progression of periodontal disease. [4,5] Dental plaque is defined as a distinct but variable accumulation of microorganisms, saliva-derived substances, and debris on tooth surfaces, restorations, and other oral structures. It predominantly comprises desquamated epithelial cells, mucin, debris, and microorganisms encased in an extracellular matrix. (WHO 1961). [6] Dental calculus, also known as tartar, is the calcified deposit that forms on the surfaces of natural teeth and dental prostheses as a result of mineralization of bacterial plaque.[7] Dental calculus (tartar) is a hard mineralized and calcified form of dental plaque, which acts as plaque reservoir that results in gingival inflammation, gingival recession and ultimately periodontitis [8].

Dental calculus is readily available and usually discarded during any periodontal procedure. It is a "medical waste" which is always preliminarily eliminated during every periodontal treatment. As far as we know, the diagnostic capability of dental calculus for tracking oral heavy metal (HM) exposure has not been investigated. Considering that tobacco is a major origin of toxic heavy metals, conducting quantitative elemental analysis of dental calculus from individuals with prolonged exposure could offer insights into its capacity to indicate HM levels. Thus, based on this premise, the present study aims to identify levels of harmful heavy metals in dental calculus of burnt tobacco (mishri) users and non-tobacco users using an atomic absorption spectrometer analyzer, and to correlate these levels with their periodontal status. This shall promote knowledge and awareness of oral and associated fatal health problems due to mishri intake to reduce the relative risk for readers' benefit.

The study seeks to assess and contrast the concentrations of heavy metals in dental calculus among users of burnt tobacco (mishri) and individuals who do not use tobacco, utilizing Atomic Absorption Spectrometry (AAS) analysis. Specifically, it seeks to analyze the heavy metal levels in dental calculus of burnt tobacco users, assess the heavy metal levels in dental calculus of control individuals with no history of tobacco use, and compare the heavy metal levels in dental calculus between burnt tobacco users and non-tobacco users, examining any potential correlations with their periodontal status.

LITERATURE REVIEW

1. Krishnamurthy et al. [9] Conducted a preliminary study on 178 pregnant females, the research explored the impacts of maternal smokeless tobacco (mishri) consumption. The findings unveiled potential associations between maternal mishri use during pregnancy. The researcher highlighted the importance of confirming the elevated peril of low birth weight in female child compared to male child of tobacco users, suggesting it could serve as a compelling incentive for women, particularly those longing for a male child, to discontinue tobacco usage.
2. Wray A et al. [10] conducted a retrospective study, in which they concluded that there is a significant correlation between oral cancer and smokeless tobacco use, highlighting an increased cancer risk. Therefore, implementing early intervention strategies is imperative to assist individuals in quitting this harmful habit.
3. Yetkin-Ay Z et al. [11] researchers conducted a study in which they revealed a significant relationship between the clinical periodontal parameters and oxidative stress/damage indices in apprentices who were indirectly exposed to low levels of lead.
4. Jacob V et al. [12] in a cross-sectional population-based study in which their results from six sextants showed that individuals who did not use tobacco exhibited greater periodontal health and lower rates of bleeding on probing, but they also had fewer periodontal pockets compared to tobacco users.
5. Sumanth et al. [13] in their cross-sectional study in which they underscored the detrimental effects of betel nut on the periodontium, emphasizing that the synergistic effect of adding tobacco to betel nut exacerbates the impact on periodontal tissues.
6. Parmar et al. [14] in their case-control investigation they concluded that a notably higher proportion of quid chewers experienced bleeding from the gums. Additionally, the oral hygiene

status of quid chewers was significantly inferior. The study further revealed that the impact of quid chewing on the periodontium, including the incidence of periodontal pockets, gingival lesions, and gum recession, was markedly more pronounced in quid chewers compared to non-chewers.

7. Arora M et al. [15] explored the correlation between environmental cadmium (Cd) exposure and periodontal disease in adults residing in the United States Findings revealed that the mean urine Cd concentration (micrograms per gram creatinine) was notably elevated among individuals with periodontal in comparison to those without periodontal disease.

MATERIALS AND METHODS

The present cross-sectional study was carried out at the Department of Periodontology, School of Dental Sciences (SDS), Krishna Institute of Medical Sciences Deemed to be University (KIMSDU), Karad. Approval from the Institutional Ethical Committee was obtained (Project IEC approval number: KIMSDU 0326/2018-2019). Ninety patients aged between 18 and 80 years, attending the Department of Periodontology, were enrolled in the study upon meeting the designated inclusion criteria.

Criteria For Selection of The Study Subjects:

Inclusion criteria

1. Individuals of age group 18 - 80 years.
2. Individuals who have not undergone periodontal therapy within the past twelve months.
3. Systemically healthy individuals without any dietary supplements.
4. Individuals who use burnt tobacco (mishri) since a time of 5 - 10 years.
5. Individuals who have never chewed tobacco (for control group).
6. Individuals having supra-gingival dental calculus.
7. Individuals having fully erupted all permanent teeth.

Exclusion criteria

1. Individuals with metallic restorations (including amalgam or cast restorations and removable prostheses with metallic frameworks).
2. Individuals with occupational heavy metal exposure (farmers, metal workers, mine workers, smelters).

Materials Required

1. ***For clinical examination***:

- Mouth mask.
- Oral mirror.
- Oral Explorer.
- (University of North Carolina) UNC-15 Periodontal Probe (Hu-Friedy, Chicago, IL 60618-5935, United States).
- Disposable gloves.

2. ***For sample collection:***

- Universal 15/30 scaler (Hu-Friedy, Rockwell St. Chicago, IL 60618-5935, United States).
- Cumine scaler (GDC, New Delhi, India).
- Tweezer.
- 1.5 ml sterile eppendorf tube (Microtubes 3810X, Banglore, India).

3. ***For sample processing:***

- Agate mortar pestle.
- Borosil glass test tube 27 ml (18×150).
- 10 ml borosil glass pipette with rubber tit.
- 6 ml - 68% Nitric acid (HNO_3).
- 2 ml - 37% Hydrochloric acid (HCL).

- Test tube holder.
- Test tube metal rack.
- Hot air oven (CIE8050, Meta lab scientific Industries, Mumbai, India).
- 10 ml distilled water.
- 10 ml sterile disposable syringe.
- 50 ml sterile centrifuge tube (546041, Tarsons products private. Ltd, Kolkata, India).

4. ***For sample analysis:***

- Atomic absorption spectrometer (AA8000, Lab India Analytical Instruments private limited, Thane, India).

Method of Collection of Data

The enrolled subjects were then interviewed, and the history of tobacco usage was recorded on the dental chair in the Department of Periodontology.

Based on the history of tobacco intake the study subjects were categorized in the following two subgroups with 45 subjects in each group as follows:

1. GROUP 1 (n = 45) (Control group) - Comprised of (Non-tobacco users).
2. GROUP 2 (n = 45) (Test group) - Comprised of burnt tobacco (mishri) users in the past 5-10 years.

RESULTS

This current cross-sectional study aimed to evaluate and correlate the levels of heavy metals in dental calculus between burnt tobacco (mishri) users and non-tobacco users with their periodontal status, utilizing an atomic absorption spectrometer.

Table 1. Distribution of the Study Population by Gender.

Gender	Non-tobacco users	Percentage (%)	Mishri users	Percentage (%)
MALE (M)	22	48.8%	25	55%
FEMALE (F)	23	51.2%	20	45%
TOTAL	45	100%	45	100%

Forty-five study subjects in each group were included in study. Out of the forty-five subjects enrolled for non-tobacco users' group, 22 (48.8%) were males and 23 (51.2%) were females. Among the mishri users' group 25 (55%) males and 20 (45%) females were present respectively. In the non-tobacco users group the number of male and female subjects were equal while in the mishri users' group more male subjects were noted in Table 1.

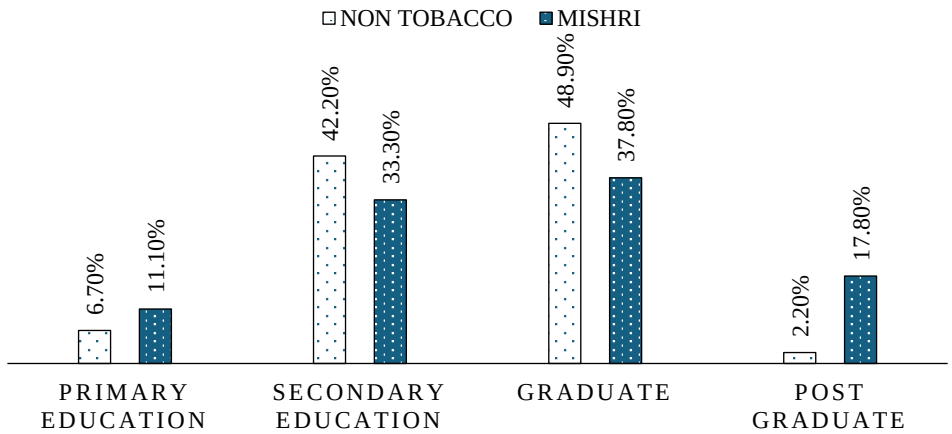

Figure 1. Bar diagram representing education wise distribution of study population.

The level of education of all the ninety subjects enrolled in both the study groups ranged from primary education to post graduation with no statistical significance. The graduates were noted to be more in non-tobacco users group with 48.9% with just 37.8% in the mishri users group. The post-graduate participants were more in the mishri users groups with 17.8% as compared to the non-tobacco users group with just 2.2%. The level of secondary education was noted to be less with 33.3% in mishri users group with 42.2% in non-tobacco users group shown in Figure. 1. Primary education was noted with 11.1% in mishri user group and 6.7% in non-tobacco group respectively.

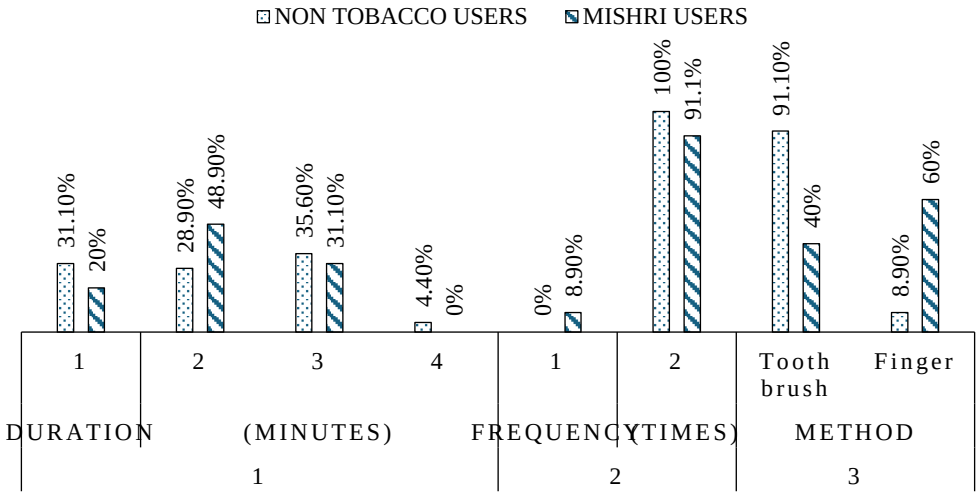

Figure 2. Bar Diagram Representing Teeth Brushing Habit (Duration, Frequency and Method) of Study Population.

When asked about the oral hygiene habits, the enrolled participants in both the study groups gave a history of brushing their teeth with toothbrush as well as finger. Among the non-tobacco user group 91.1% and just 40% in mishri user group use toothbrush for cleaning their teeth. In the mishri users group 60% subjects and just 8.9% in non-tobacco user group use their finger instead of toothbrush to clean the teeth with statistical significance difference (p value < 0.001). The teeth brushing duration ranged from 1 to 4 minutes while the frequency ranged from 1 to 2 times daily. It was observed that 100% subjects from non-tobacco user group and 91.1% from mishri user group brush their teeth twice daily. Brushing teeth for a time span of three minutes daily was noted by 35.6% of non-tobacco group with 31.1% in mishri users group. In non-tobacco user group 28.9% and 48.9% in mishri user group cleaned their teeth for two minutes daily. Brushing teeth for one minute was observed in 31.1% of non-tobacco users group with just 20% in mishri users group depicted in Figure.2.

Table 2. Mishri Intake Habit (Duration, Frequency and Method) of Study Population.

Mishri habit	Duration(minutes)	Number	Percentage (%)
	2	13	28.9
	3	14	31.1
	4	18	40
	Frequency(times)	Number	Percentage (%)
	5	9	20
	6	17	37.8
	8	19	42.2

All the subjects of mishri users group used finger or digits of the hands for application of mishri. The mishri application ranged from 5 - 8 times in frequency and 2 - 4 minutes in duration daily. In the mishri users group 19 (42.2%) subjects applied mishri for 8 times a day, 17 (37.8%) subjects for 6 times and

9 (20%) subjects for 5 times daily. The duration of application of mishri for 4 minutes was observed in 18 (40%) subjects, 3 minutes in 14 (31.1%) subjects and 2 minutes in 13 (28.9%) subjects respectively which is shown in Table 2.

Table 3. Oral Hygiene Index – Simplified of Study Population.

Ohi-s index		Non-tobaccousers	MishriuserS	P value
Debris	Fair	43	4	<0.001*
	Poor	2	41	
Calculus	Good	1	0	<0.001*
	Fair	42	2	
	Poor	2	43	
Overall	Good	0	0	<0.001*
	Fair	32	0	
	Poor	13	45	
Interpretation	Fair	32	0	<0.001*
	Poor	13	45	

To assess the oral hygiene status of both study groups, the OHI-S index was evaluated in Table 3. The results revealed the score of debris index as well as calculus index to be higher in mishri users as compared to non-tobacco user group with mathematically noTable difference (p value < 0.001). The overall inference was found to be poor in mishri users as relative to the non-tobacco user group and the difference between them was found to be notably (p value < 0.001). Thus, the final interpretation was also found to be poor in mishri users as relative to the non-tobacco users' group and the difference between them was found to be notably (p value < 0.001).

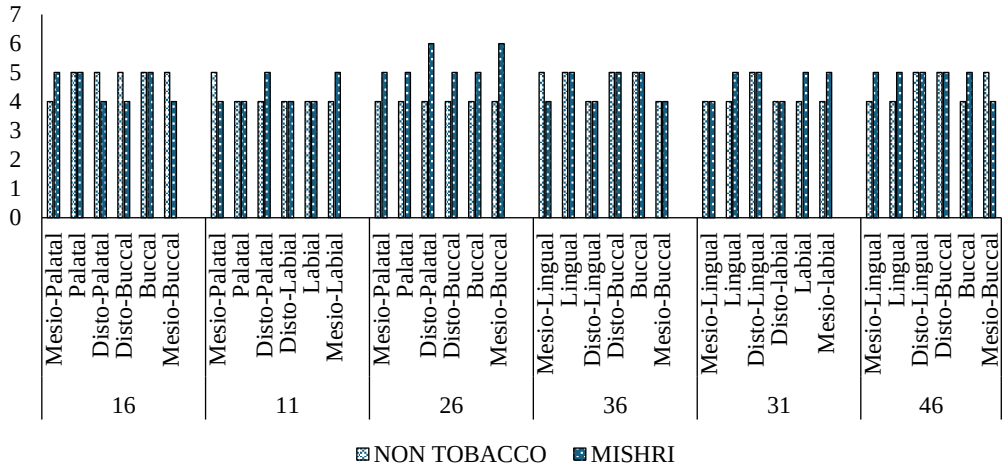

Figure 3. Bar Diagram Representing Probing Pocket Depth (PPD) of Study Population.

The probing pocket depth score for tooth 46 on the mesio-lingual and disto-buccal surfaces was greater in mishri users, and this difference was statistically noTable (p value < 0.001). The scores on the other surfaces were similar, with no major difference which shown in Figure. 3.

Table 4. Clinical attachment level (CAL) of study population.

Tooth number	Tooth surfaces	Non-tobaccousers	Mishriuse RS	P values
16	Mesio-Palatal	5(5-6)	7(7-7)	<0.001*
	Palatal	5(5-6)	6(6-7)	<0.001*
	Disto-Palatal	6(6-6)	7(6-7)	<0.001*

	Disto-Buccal	5(5-6)	7(7-8)	<0.001*
	Buccal	5(5-7)	7(6-7)	<0.001*
	Mesio-Buccal	6(6-7)	7(6-7)	<0.001*
11	Mesio-Palatal	6(5-6)	7(6-7)	<0.001*
	Palatal	5(5-6)	6(6-7)	<0.001*
	Disto-Palatal	6(5.5-6)	6(6-6.5)	.346
	Disto-Labial	6(5-6)	7(6-7)	<0.001*
	Labial	5(5-6)	7(6-7)	<0.001*
	Mesio-Labial	6(6-6)	6(6-6)	<0.001*
26	Mesio-Palatal	5(4-6)	7(6-7)	<0.001*
	Palatal	6(4-6)	7(7-7)	<0.001*
	Disto-Palatal	5(5-6)	7(6-7)	<0.001*
	Disto-Buccal	5(4-6)	7(6-7)	<0.001*
	Buccal	6(6-6)	7(6.5-7)	<0.001*
	Mesio-Buccal	5(5-6)	7(6-7)	<0.001*
36	Mesio-Lingual	6(6-6)	7(6-7)	<0.001*
	Lingual	6(6-6)	7(6-7)	<0.001*
	Disto-Lingual	6(5-6.5)	7(7-7)	<0.001*
	Disto-Buccal	6(5.5-6)	7(6-8)	<0.001*
	Buccal	6(6-6)	7(6-7)	<0.001*
	Mesio-Buccal	6(5-7)	7(7-8)	<0.001*
31	Mesio-Lingual	5(4-5)	6(6-7)	<0.001*
	Lingual	5(5-5.5)	7(7-7)	<0.001*
	Disto-Lingual	5(5-6)	6(6-7.5)	<0.001*
	Disto-Labial	4(4-5.5)	6(5.5-7)	<0.001*
	Labial	5(5-5)	7(7-7)	<0.001*
	Mesio-Labial	5(4.5-6)	6(6-7)	<0.001*
46	Mesio-Lingual	4(4-5)	7(6-7)	<0.001*
	Lingual	5(5-6)	7(6-7)	<0.001*
	Disto-Lingual	6(5-6)	7(6-7.5)	<0.001*
	Disto-Buccal	4(4-5)	7(6-7)	<0.001*
	Buccal	5(5-6)	7(6-7)	<0.001*
	Mesio-Buccal	6(5-6)	7(6-7)	<0.001*
Mann-Whitney u statistics was performed. * - Significant at p≤ 0.05CAL was expressed in mm.				

The measurement and comparison of the clinical attachment level for all the surfaces of six teeth in the mishri user's groups was found to be mathematically noTable (p value < 0.001) as relative to the non-tobacco user group. All the above shown in Table 4 where calibrations for CAL were performed using Mann-Whitney statistics.

Table 5. Comparison of Heavy Metals of Study Population.

Heavy metals	Non-tobacco users	Mishriusers	P value
Nickel*(M ± SD) (µg/L)	7.46 ± 2.69	14.22 ± 5.485	<0.001
Cobalt*(M ± SD) (ng/L)	498.39 ± 119.858	606.55 ± 168.82	<0.001
Manganese*(M±SD)(µg/L)	8.334 ± 2.685	73.54 ± 9.649	<0.001
Zinc** M (µg/L)	105.60(101.1,110.34)	166.9(133.025,174.43)	<0.001
Iron** M(µg/L)	0.87(0.38,1.39)	0.70(0.11,1.26)	0.659
Chromium** M(µg/L)	1.12(0.74,6.55)	3.97(2.23,6.55)	<0.001
Copper** M(µg/L)	7.75(5.22,9.48)	28.66(26.75,32.48)	<0.001
Lead** M(ng/L)	218.90(152.16,255.6)	769.35(579.17,966.53)	<0.001
Cadmium** M (ng/L)	8.90(4.09,13.72)	258.45(195.71,349.16)	<0.001
Aluminum** M(µg/L)	29.08(26.18,33.96)	72.71(68.91,84.80)	<0.001

The above comparative results shown in Table 5 for iron, zinc, chromium, copper, lead, cadmium and aluminium between the study populations was calibrated using the Mann-Whitney U statistics. Thus, it can be concluded from the above-mentioned observation and results that the concentration of nine heavy metals excluding iron was more or higher in mishri users' group as compared to non-tobacco users' group respectively with statistical significance difference (p value < 0.001).

CONCLUSION

The atomic absorption spectrometry (AAS) analysis conducted to investigate the elemental composition of dental calculus revealed the presence of harmful heavy metals (HM). The levels of these heavy metals were found to be significantly higher in the dental calculus of burnt tobacco (mishri) users compared to non-tobacco users, indicating a potential correlation between mishri use and alterations in dental calculus composition. Mishri, a form of smokeless tobacco, is implicated in affecting the elemental composition of dental calculus. Given that dental calculus is a readily available biological material categorized as "medical waste," it can be non-invasively collected and may serve as a useful tool for monitoring oral heavy metal exposure. Furthermore, observations of increased clinical attachment loss, probing pocket depth, halitosis, tooth abrasion, gingival recession, tooth discoloration, and habituation were noted alongside chronic mishri use, suggesting a link between mishri consumption, deteriorated periodontal status, and heightened accumulation of heavy metals in dental calculus. These results show the major contribution of mishri as a noTable risk factor for the deterioration and advancement of periodontal tissue damage.

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