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ORIGINAL ARTICLE

Oral Candidal Carriage and its Correlation with Salivary Oxidative Stress in Smokeless Tobacco Users

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ABSTRACT

Background: Smokeless tobacco use, particularly the consumption of Naswar, is prevalent in Pakistan and has been associated with various chronic diseases, including oral cancer. Naswar is known to contain toxic heavy metals, carcinogenic substances, and microbial contaminants. This study aims to assess the oral Candida carriage and biofilm formation in Candida albicans isolates from Naswar users and its association with salivary oxidative stress.

Methods: A cross-sectional study was conducted involving 150 participants, divided into Naswar users (n=75) and Non-users (n=75). Socio-demographic data, oral health, hygiene practices, and Naswar usage patterns were collected through a standardized questionnaire. Salivary samples were collected, and Candida strains were identified using culture techniques. Salivary oxidative stress was assessed using a DPPH solution-based free radical system.

Results: The mean age of participants was 30 ± 7.3 years for Naswar users and 30.2 ± 7.1 years for non-users. No significant differences were observed in oral health and hygiene practices between the two groups. Among Naswar users, the most common type of product used was green moist Naswar (77.3%). Oral Candida carriage was significantly higher in Naswar users, particularly those with fungal infections of C. albicans, C. krusei, or both. These participants also exhibited elevated levels of salivary oxidative stress.

Practical Implication: Public health interventions and oral health education programs should be implemented to raise awareness about the risks associated with Naswar and promote good oral hygiene practices among users.

Conclusion: This study reveals significant differences in oral Candida carriage and salivary oxidative stress between Naswar users and non-users, indicating potential implications for the oral health of Naswar users. These findings underscore the need for larger prospective studies to validate the detrimental effects of Naswar use. Public health interventions targeting smokeless tobacco users should prioritize oral health promotion and emphasize the risks associated with Naswar consumption.

Keywords: Candida species, smokeless tobacco, oral health, oxidative stress

INTRODUCTION

Smokeless tobacco (ST) use is a prevalent habit in many regions of the world and is associated with numerous health risks. Unlike traditional tobacco products, ST is not burned but rather consumed orally, delivering nicotine through passive absorption [1]. The diverse forms of ST include chewing tobacco, snuff, and moist snuff, each posing potential health hazards due to the presence of carcinogens and heavy metals. In South Asia, ST products such as Naswar, Gutkha, Zarda, Khaini, and Tambaku Paan are popular, contributing to a significant burden of ST use in the region [2].

ST use has been linked to various oral health issues, including oral candida carriage, oxidative stress, and associated oral diseases. C. albicans, a common fungal species found in the oral cavity, has been associated with oral leukoplakic lesions and an increased risk of oral carcinoma. The presence of Candida species, particularly C. albicans, has been recognized as a major risk factor for oral mucosal lesions, chronic hyperplastic candidiasis, and potentially malignant disorders[3, 4]. Furthermore, ST users have been found to exhibit a higher ratio of oral candida carriage compared to non-users, indicating the potential influence of ST on oral Candida colonization[5].

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and antioxidants in the body, plays a significant role in the pathogenesis of various diseases [6]. The oral cavity is exposed to both endogenous and exogenous sources of ROS, including physiological metabolism and external factors like food, radiation, air pollution, and tobacco use [7]. ST use has been shown to contribute to oxidative stress in the oral cavity, leading to cellular damage and increasing the risk of oral diseases such as oral cancer, dental caries, lichen planus, and chronic periodontitis[8, 9].

Understanding the interplay between ST use, oral Candida carriage, oxidative stress, and oral health is crucial for developing effective preventive strategies and interventions. This comprehensive review aims to synthesize the existing literature on the association between ST use and its impact on oral Candida carriage, oxidative stress, and oral health. By examining the available evidence, this review seeks to provide insights into the mechanisms underlying the relationship between ST use and oral health outcomes.

METHODOLOGY

Study design and setting: A cross-sectional study was conducted between November 2020 and July 2021 among Naswar users and non-users. The study was carried out at the Outpatient Department of Sardar Begum Dental College and Institute of Basic Medical Sciences, Khyber Medical University, Peshawar.

Ethical issues and approval: Ethical approval was obtained from the ethical review board of Khyber Medical University. Written permissions were obtained from the heads of the participating centers. Written informed consent was obtained from each study participant, and they had the option to withdraw from the study at any time.

Agreement with hospital: Written agreements were signed with the head of Sardar Begum Dental College and Hospital, Gandhara University, Peshawar, for sample collection and study execution. Administrative approval from the hospital incharge was obtained before starting data and sample collection.

Sample size and sampling procedure: A total of 150 participants were included in the study, with 75 Naswar users (cases) and 75 non-users (controls). Non-probability convenient sampling technique was used for participant selection.

Study participants: Naswar users were individuals using Naswar at least once or twice daily for one year. Controls were healthy individuals with no history of tobacco use. Inclusion criteria included males aged 18 to 50 years.

Data and samples collection: Socio-demographic data, tobacco habits, and oral health information were collected using a structured questionnaire. Clinical and oral health examinations were performed by a trained dentist. Saliva samples and oral rinse were collected for further analysis.

Sample collection procedure: Participants visited the dental college and hospital in the morning without oral hygiene procedures. Saliva samples and oral rinse were collected. Saliva was collected by asking participants to spit into a sterile falcon tube for two minutes. Oral swabs were collected using commercially available sterile Bio oral swabs. Samples were stored and transported to the microbiology laboratory for further processing.

Solution preparation for determination of antioxidant activity: A 0.1mM solution of diphenylpicrylhydrazl (DPPH) was prepared. The DPPH solution was added to the sample extracts, and the absorbance was measured at 517 nm using a UV-VIS spectrophotometer.All tests were performed three times, DPPH radical scavenging 517nm activities of the digested products of saliva were calculated by using the following formula:

$$\mathrm{AA\%} = 100 - \frac{(\mathrm{Abssample} - \mathrm{Absblank}) \times 100}{\mathrm{Abscontrol}}$$

Free radical generating system: A 96-well plate free radical system was generated using DPPH solution. The color changes in the reaction mixture were observed, and the absorbance at 517 nm was measured using a UV-VIS spectrophotometer. The antioxidant activity (AA%) was calculated using a formula.

Fungal Candidal Species Identification using Sabouraud Dextrose Agar (SDA) and CHROM-Agar Media: Oral rinse samples were streaked on Sabouraud Dextrose Agar (SDA) plates and incubated for 48 hours at 37°C. Additionally, CHROM-Agar media was prepared and poured into petri dishes. The inoculated plates were incubated at 37°C for two days.After incubation, Candida colonies on the plates were counted and identified based on their colony structure and color produced by the chromogenic reaction. Candida species were identified as C. albicans and C. krusei based on colony colors.

Statistical analysis: The collected data was initially transferred to Microsoft Excel and carefully examined for potential errors and missing values. Subsequent statistical analysis was conducted using SPSS (Statistical Package for Social Science) version 24 (SPSS Inc., Chicago, Ill., USA). Descriptive statistics, including the calculation of means and standard deviations, were performed to summarize the data. To determine the appropriate statistical test, the distribution of the data was assessed. P-values below 0.05 were regarded as statistically significant, indicating a significant difference or relationship.

This rigorous statistical analysis facilitated the exploration of patterns, trends, and associations within the dataset, thereby enhancing the validity and reliability of the research findings.

RESULTS

This study examined the combined impact of Naswar use and oral candida carriage on oral health compared to a control group. Physiological characteristics, including salivary oxidative stress, were affected by Naswar and Candida albicans. Demographic parameters and clinical investigations were compared between the test and control groups, providing insights into their association with oral health. The findings highlight the combined influence of candida and Naswar on oral health and present concise comparative outcomes for each contributing factor in both groups.

Characteristics of participants: The age groups did not significantly differ (P≥0.05) between the two groups, with mean ages of 30.49±7.349 and 30.27±7.129 for Naswar users and non-users, respectively. The control group had a significantly higher average monthly income compared to the Naswar user group (P = 0.017).

Participants belonged to various professional domains, including businessmen, daily wagers, drivers, government employees, shopkeepers, students, and tailors in both groups. Non-Naswar users had a higher frequency in the business group (16%) compared to Naswar users (5.3%). Among Naswar users, daily wagers accounted for 33.3%, while non-Naswar users accounted for 20%. No significant differences were observed between the two groups based on occupation, ethnicity (all Pashtun), religion, or country of birth.

Self-rated oral health status: Participants, both Naswar users and non-users, were asked to rate their oral health as good, fair, or poor. The results are presented in Table 1. The majority of participants in both groups rated their oral health as good or very good. However, 7 Naswar users (9.3%) reported poor or very poor oral health, while no participants in the control group reported the same. The self-rated oral health status was found to be significantly different between the two groups (P = 0.017).

Table 1:Self-reported oral health of the participants

Variables	Category	Participant groups		P-values
		Naswarusers(%)	Non users(%)	
Self-reported oral health status	Good	47 (62.7%)	56 (74.7%)	0.017
	Fair	21 (28.0%)	17 (22.7%)	
	Poor	7 (9.3%)	0 (0.0%)	

Self reported oral health problems: When asked about presence of experience of different oral health issues in the recent past (one year), both Naswar users and non users reported presence of multiple oral health related issues as presented in Table2. Tooth ache, sensitivity, bleeding gums and carious lesion were all highly prevalent in Naswar users although the difference was not significant between the two groups.

Table 2: Self-reported oral health problems among participants

Parameters	Subjects		Control		P-value
	n %		n%		
	Yes	No	Yes	No	
Ache	64(85.3%)	11(14.7%)	7(9.3%)	68(90.7%)	0.315
Sensitive	16(21.3%)	59(78.7%)	10(13.3%)	65(86.7%)	0.196
Bleeding	7(9.3%)	68(90.7%)	5(6.7%)	70(93.3%)	0.547
Caries	2(2.7%)	73(97.3%)	0(0.0%)	75(100.0%)	0.155
No Problem	46(61.3)	29(38.7%)	53(70.7%)	22(29.3%)	0.228

Spitting habits among Naswar users: Naswar use is commonly associated with spitting habits among users, as swallowing the saliva containing Naswar extracts can have health implications. In the study, it was observed that 67 Naswar users (89.3%) were likely to spit the saliva during Naswar usage, while only 8 participants (10.7%) reported swallowing the saliva (Table 3).

Table 3: Spitting habits among naswar users

Saliva in mouth	Frequency (n)	Percentage(%)
Spit	67	89.3
Swallow	8	10.7
Total	75	100.0

Naswar dip frequency, duration, and amount of money spent on the habit: The average number of daily dipping units of Naswar and its duration were also found to be different among Naswar users. On average, Naswar users consumed around 12 units/dips per day (range: 3 - 40). The average duration of Naswar usage ranged from as low as 2 minutes to as high as 60 minutes among Naswar users in our study. The monthly average amount spent to purchase Naswar packets ranged from 100 to 1200 rupees, with an average of 349.67 rupees. These findings are further summarized in Table 4.

Table 4: Frequency and comparism of daily number of dips, average duration and amount spend.

Parameters	Minimum	Maximum	Mean+Sd.
Daily number of unit/dips	3	40	12.73+6.35
Average duration of dip	2 (minutes)	60 (minutes)	6.43+7.8
Amount spend on Naswar use	100 (rupees)	1200 (rupees)	394.67+230.97

Association between self-reported oral health and oxidative stress among the participants: The comparison of oral health status with oxidative stress was analyzed and is shown in Table 5. The table shows that there is no significant difference in the oxidative stress of the participants with good oral health. However, participants who reported fair oral health status were found to have a highly significant difference. This indicates that participants with

fair oral health status have a higher level of oxidative stress. Due to the lack of availability of participants in the control group, a significant difference in poor oral health could not be determined.

Table 5: Comparison of oxidative stress with self reported oral health parameters

Oral health	Subjects Mean+Sd.	Control Mean+Sd.	P-value
Unknown	-	44.2 +19.1	-
Fair	28.1+5.67	35.2+11.01	0.016
Good	28.8+8.45	42.8+43.98	0.051
Poor	26.000+8.0454	-	-
Very good	25.1+7.86	33.5+11.01	0.079

Oral Candida carriage among Naswar users and non-users: Naswar users had a significantly higher percentage of C. albicans (13.3%) compared to non-users (1.3%). However, non-users had a higher proportion of C. Krusei species (82.7%) compared to users (73.3%). Both groups had participants infected with both fungal species. The P-value ($P < 0.05$) indicates a low level of significance. Figure 1 presents the fungal species found in Naswar users and non-users, specifically C. albicans and C. Krusei.

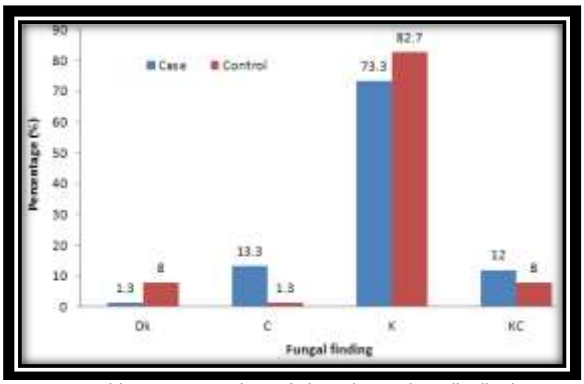

Figure 1: Graphic representation of fungal species distribution among participants

Comparison of oxidative stress with fungal species among participants: The mean values of both groups exhibit a slight difference. However, participants with fungal infections of C.albicans, Krusei, or both displayed a significant difference ($P < 0.001$) in oxidative stress levels. This suggests that Naswar users with any fungal infection are more likely to have elevated oxidative stress. Notably, no significant difference in oxidative stress was observed in participants with unknown fungal species. Table 6 presents the relationship between oxidative stress and fungal infections in Naswar users and non-users.

Table 6: Comparison of oxidative stress of fungal species among participants

Fungal finding	Patients Mean+Sd.	Control Mean+Sd.	P-value
Unknown	31.300	35.917+7.58	0.597
C. albicans	31.289+10.147	57.900	0.038
C. Krusei	27.80+7.46	39.50+37.74	0.026
Both	25.80+6.00	42.36+9.32	0.001

DISCUSSION

Smokeless tobacco, particularly Naswar, is a significant risk factor for oral cancer, especially in South Asian countries [10,11]. Despite this, there has been limited research on the impact of Naswar on cancer risk [12]. This study aimed to investigate the effects of Naswar usage on oral health, including oxidative stress, fungal infections, and dental health.

The study ensured that the age and professional groups of the control and subjective groups were similar, except for minor differences. The findings showed that over 80% of oral cancer patients were Naswar users, consistent with previous studies [13]. The study also examined different types and brands of Naswar and found that they had similar impacts on oral health.The placement site of Naswar in the mouth, particularly on the upper right or left side, has been observed to be common among users. This may have implications for oral health, such as tumor or cancer development. Tobacco use in the oral cavity can lead to keratotic changes, and certain patterns have been observed in association with smokeless tobacco placement [14].

Naswar users tend to spit more frequently than non-users, which may contribute to oral health differences. The number of Naswar dips per day and the duration of each dip varied among users. Previous studies have indicated a high percentage of oral cancers attributed to Naswar dipping. Oral hygiene habits, including the frequency and timing of teeth cleaning, were found to affect oral health and oxidative stress[15]. Naswar users who cleaned their teeth once a day or in the morning had significantly higher levels of oxidative stress compared to the control group. It is important to note that Naswar consumption may lead to oxidative stress and an increased risk of oral and gastrointestinal cancer[16]. The majority of Naswar users had poor oral hygiene, as evidenced by their lack of cleaning aids and irregular visits to dentists. The proportion of individuals who never visited the dentist was high in both groups. Visiting the dentist occasionally or not knowing the last visit was associated with significant changes in oxidative stress. This highlights the need for more oral health education programs to raise awareness about the importance of oral hygiene. Regarding dental clinical investigation, Naswar users reported more toothaches compared to the control group. There is limited evidence linking smokeless tobacco use to oral pain, but it may have implications for periodontal diseases, gingival recession, attachment loss, dental caries, and tooth loss.

Fungal infections, particularly Candida species, were more prevalent among Naswar users compared to the control group. However, previous studies have reported conflicting results on the relationship between smokeless tobacco use and Candida carriage. Poor oral hygiene and lower salivary flow rate are potential contributing factors[17]. Naswar usage is associated with an increased risk of Candida albicans infection, which, in turn, is linked to diabetes mellitus[18]. Medical resources, diagnosis, and treatment must improve in developing countries. There are limited resources: access to medical and health resources; knowledge about disease; awareness, trainings, and awareness about health. Health literacy is mandatory for any disease and facilitates the patients access to resources, databases, and trainings about the disease in print and electronic (hybrid) format[19-26].

In summary, this study provides valuable insights into the impact of Naswar usage on oral health. It highlights the association between Naswar use and oral cancer, oxidative stress, fungal infections, poor oral hygiene, and dental problems. Public health interventions and oral health education programs should be implemented to raise awareness about the risks associated with Naswar and promote good oral hygiene practices among users.

CONCLUSION

The findings of this study indicate that Naswar use may contribute to an elevated risk of oral candida carriage and salivary oxidative stress, thereby impacting oral health. These results emphasize the importance of conducting larger-scale, prospective cohort studies to validate and further elucidate the deleterious effects of Naswar use. By enhancing our understanding of the potential harms associated with Naswar, we can develop targeted preventive measures and interventions to improve the oral health outcomes of individuals engaged in this habit.

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