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Research Article

**ANALYSIS OF SALIVARY BIOMARKERS INTERLEUKIN-8  
LEVELS CORRELATED WITH DIFFERENT PARAMETERS  
OF NASWAR**<sup>1</sup>Dr Ameena Najam, <sup>1</sup>Dr Humaira Akhtar, <sup>1</sup>Dr Muhammad Farhan Raza  
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**Abstract:**

**Introduction:** Almost one-fifth of the tobacco used in the world is smokeless form. Smokeless tobacco products are highly addictive due to their high nicotine content. **Aims and objectives:** The basic purpose of this study was to evaluate the salivary biomarkers interleukin-8 levels correlated with different parameters of naswar. **Material and methods:** This cross sectional study was conducted in August 2018 to January 2019 at Nishter Dental College, Multan. The data was collected from 100 patients who were used naswar from last one year. The data was collected through a non-probability sampling technique. The age range for this study was 30 to 60 years. Age, duration of use of naswar, frequency of use, type of naswar used and site of placement of naswar in oral cavity were noted. A morning sample of unstimulated whole saliva was collected. Collected samples were tested for levels of interleukin-8 cytokine by enzyme-linked immunosorbent assay (ELISA) procedure. Frequency of usage of naswar were also noted. **Results:** The data was collected from 100 participants. The mean age of the participants was 35.65 years. Levels of salivary IL-8 were found from 173.48 pg/ml to 296.78 pg/ml with mean and standard deviation of 173.48±46.52pg/ml. Naswar dippers mostly belonged to the Pathan community (76.2%). Regarding educational status in naswar users and non-users, 59.5% and 36.4% were uneducated, respectively. Most common reason for using naswar was that they were just addicted to it. **Conclusion:** It is concluded that there was a high level of IL-8 were present in naswar users. There is a positive correlation between salivary biomarkers and IL-8 levels and frequency of naswar usage.

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## INTRODUCTION:

Almost one-fifth of the tobacco used in the world is smokeless form. Smokeless tobacco products are highly addictive due to their high nicotine content. These products also contain carcinogenic compounds such as Tobacco-Specific N-nitrosamines (TSNAs), which eventually lead to an increased risk of oral cavity, laryngeal and oesophageal cancer. An increased risk of mortality due to cardiovascular diseases has also been observed among smokeless tobacco users [1]. Around 10 million people are estimated to die of tobacco use in developing countries by 2030, and this figure is higher than the figures estimated for AIDS, drug abuse, road accidents, murder and suicide [2].

Naswar is categorized as one of the smokeless tobacco products (STPs), the ingredients of which are mainly sundried crushed local tobacco, ash, calcium oxide (slaked lime), and sometimes flavoring agents (e.g., cardamom, menthol) as well as coloring agents (indigo). It is commonly used in Pakistan, Afghanistan, Iran, Central Asia and South Africa [3]. Naswar usage is gaining popularity as it is now available and being consumed in different parts of the world including England. It is prepared and wrapped into small plastic bags which even lack safety warnings [4]. It is used mostly by applying and retaining it in the vestibular cavity adjacent to the buccal or labial mucosa or at times under the tongue. All the other types of STPs are consumed via chewing but naswar is never bitten because of its bad taste [5].

Smokeless tobacco (SLT) consumed orally or nasally has been in use for as long as other forms of tobacco. A conceptual model of SLT-associated carcinogenesis postulates that carcinogens present in SLT products are ingested and processed, leading to metabolic activation of carcinogens. The carcinogens cause formation of DNA adducts and subsequent mutations in *K-ras*, *p53* and other genes, leading to uncontrolled cell growth [6].

Other changes, including chronic local inflammation, oxidative stress and formation of reactive oxygen species, may also contribute to tumour promotion. Mechanisms such as activation of Akt and protein kinase A lead to reduced apoptosis and increased angiogenesis and cellular transformation [7]. Apart from TSNAs, other compounds present in SLT products such as polycyclic aromatic hydrocarbons and areca nut may also contribute to causation of

cancer in SLT users. Epigenetic pathways, such as promoter methylation of tumour-suppressor genes leading to unregulated proliferation, are also speculated to be involved in SLT-related carcinogenesis [8].

## Aims and objectives

The basic purpose of this study was to evaluate the salivary biomarkers interleukin-8 levels correlated with different parameters of naswar.

## MATERIAL AND METHODS:

This cross sectional study was conducted in August 2018 to January 2019 at Nishter Dental College, Multan. The data was collected from 100 patients who were used naswar from last one year. The data was collected through a non-probability sampling technique. The age range for this study was 30 to 60 years. Age, duration of use of naswar, frequency of use, type of naswar used and site of placement of naswar in oral cavity were noted. A morning sample of unstimulated whole saliva was collected. Collected samples were tested for levels of interleukin-8 cytokine by enzyme-linked immunosorbent assay (ELISA) procedure. Frequency of usage of naswar were also noted.

## Statistical analysis

The data was collected and analysed using SPSS version 21.0. Descriptive analysis was done for the age, IL-8 levels and duration of use of naswar. Frequencies were also calculated regarding the type, frequency of naswar used, and site of placement.

## RESULTS:

The data was collected from 100 participants. The mean age of the participants was 35.65 years. Levels of salivary IL-8 were found from 173.48 pg/ml to 296.78 pg/ml with mean and standard deviation of  $173.48 \pm 46.52$  pg/ml. Naswar dippers mostly belonged to the Pathan community (76.2%). Regarding educational status in naswar users and non-users, 59.5% and 36.4% were uneducated, respectively. Most common reason for using naswar was that they were just addicted to it.

**Table 01:** Descriptive statistics of patients and usage of naswar

	Age (years)	IL-8 levels (pg/ml)	Duration of use
<b>N</b>	45	35	3
<b>Mean</b>	35.65	173.48	5.42
<b>Std.deviation</b>	4.67	46.52	3.45
<b>Range</b>	27	173.20	25
<b>Min</b>	29	109.43	2
<b>Max</b>	50	296.78	35

**DISCUSSION:**

Salivary cytokines are produced during periodontal inflammation and tissue destruction. Smoking also increases cytokine levels in the saliva and gingival crevicular fluid, accelerates inflammation, and destroys periodontal tissue [9]. Therefore, many studies have focused on the effect of smoking on cytokines in periodontitis, and adult participants, including middle-aged and older people, have generally been targeted. In our current work, we studied periodontally healthy young adults to avoid the effects of the periodontal conditions on the saliva biomarker concentrations [10]. The primary findings of this study were that salivary IL-1 $\beta$  is associated with active smoking, independent of the amount smoked, and that salivary TNF- $\alpha$  levels positively correlate with the amount smoked [11].

The levels of IL-8 increased with the increased duration of use of naswar. The black and green types of naswar are more commonly used with different brand names, with blackone being stronger and more injurious to health as it contains more amount of nicotine and high pH value. A new form of naswar by the brand name of Tara is now circulating in the market which is even more filtered but serves the purpose [12]. As from the literature when tobacco is placed adjacent to the gingiva, gingival recession occurs frequently resulting in epithelial attachment loss [13]. Smokers experience widespread periodontal destruction but in case of SLT use adverse effects are seen at the site of placement resulting in gingival recession and appearance of white lesions [14].

**CONCLUSION:**

It is concluded that there was a high level of IL-8 were present in naswar users. There is a positive correlation between salivary biomarkers and IL-8 levels and frequency of naswar usage.

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