

ORIGINAL ARTICLE

NASWAR DIPPING, SHIFT IN BEHAVIOR IN MULTI ETHNICITY SETTING

Haya Naveen¹, Saeeda Baig², Wasfa Farooq², Hira Attique³

¹Dorset County Hospital, NHS Foundation Trust, UK, ²Department of Biochemistry,

³Department of Physiology, Ziauddin University

ABSTRACT

Background: Easy availability of various chewable options in big cities has led to a shift in behavior in the ethnicity specific habit of smokeless tobacco (SLT). This study was designed to find out the usage of naswar and other SLTs in naswar specific ethnicity as well as non naswar ethnicities and frequency of Human Papilloma virus (HPV) in their oral cavities.

Methods: Oral rinse (20-40ml) was collected and questionnaire was filled by 293 multiple ethnicity subjects habitual of naswar dipping, from Karachi and Peshawar after an informed consent. They were given dental floss with brush to gently sweep around oral cavity and over lesions. Floss was left in oral rinse and stored at 4°C. DNA was extracted and PCR was performed using HPV consensus primers Gp5+/Gp6+.

Results: The 293 subjects selected were divided into 3 groups; i. Original naswar users (56, Pathan residents of Peshawar), ii. Settler naswar users (79, Pathan residents of Karachi), and iii. adapted naswar users (158, various non naswar ethnicities). Peshawar subjects were not positive for leukoplakia or rough mucosa compared to rough mucosa in 43(54.4%) and leukoplakia in 30(37.9%) in subjects living in Karachi. Genetic testing for HPV showed 15(18.9%) samples from Karachi and 20(35.7%) samples from Peshawar, positive for HPV with significance difference of p-value of 0.029.

Conclusion: Epidemiological shift in behavior occurred due to change in ecology. Naswar dippers (Pathans) (40%) developed other addictions, whereas, subjects (22%) of other ethnicities became habitual to naswar. More comparative studies are required to further prove this.

KEYWORDS: Mouth Neoplasms; Tobacco; Smokeless; Carcinogens; Global health; Nicotine; Non-cigarette tobacco products

Corresponding Author:

Dr. Saeeda Baig,

Department of Biochemistry,

Ziauddin University, Karachi.

Email: saeeda.baig@zu.edu.pk

INTRODUCTION

Worldwide popularity of chewable tobacco is ethnicity specific and this habit is part of global history, tradition, and culture. A paradigm shift in chewing habit has been observed globally due to availability of multiple chewable tobacco formulations. Studies on lifestyle habits and high risk of oral cancer in UK and US, report that chewable habits are infiltrating into other ethnicities and can be a cause of oral cancer.¹ The situation of SLT consumption is grave due to easy availability of

these products where peer pressure (63.2%) and curiosity (14.4%) are two main reasons for starting the habit.²

In Pakistan, Naswar dipping is interwoven into the cultural fabric of a particular sect of Pakistani population from times unknown, belonging to Khyber Pakhtoon Khaw (KPK). Karachi being mini Pakistan represents all 4 provinces including a large number of settlers from KPK. All ethnicities live alongside in various squatter settlements, yet they are glued to their legacy and customs including

chewing habits.²

Naswar, unlike other chewable tobacco, is used by 'dipping' placing a pinch either under the lower lip or tongue, or inside the cheek from few minutes to hours. Naswar is made from sundried tobacco leaves of specie *Nicotina rustica*. After fine grinding these are mixed with calcium oxide and wood ash. Later cardamom and menthol are added for flavor and finally packed in the size of small tea bags. During the course of the day a pinch of Naswar is placed in the mouth several times for about 30 min before being spat out.³

Naswar is commonly used in India, Iran, Afghanistan and South Africa⁴ whereas a very similar product is marketed in Sudan known as toombak and in Sweden as snus. These products have a high pH and contain unionized nicotine and carcinogenic tobacco-specific N-nitrosamines (TSNAs), which impact negatively on oral and general health.⁵ World Health Organization (WHO) analyzed 53 oral tobacco products from 5 regions worldwide and they also found that some products have very high pH and high concentrations of unionized nicotine and its stimulated nitrosamines (e.g., gul powder, chimó, toombak, zarda, khaini).⁶

Due to easy availability of various chewable options in big cities, SLT is also not ethnicity restricted anymore and a shift is being observed in the ethnicity specific habit of smokeless tobacco (SLT). This study was designed to find out the usage of naswar and other SLTs in naswar specific ethnicity as well as non naswar ethnicities and frequency of Human Papilloma virus (HPV) in their oral cavities.

METHODS

This study was carried out in the Research Laboratory of Ziauddin University, Karachi, from December 2013 to 2015. Approval was taken from Ziauddin Ethics Review Committee before the start of study. Prior to samples collection approval and consent was taken from the subjects and a questionnaire was filled. All subjects exposed to chewable tobacco with or without oral lesions having no febrile pathological conditions were included in the study. Subjects addicted to stuff other than chewable tobacco were excluded.

Sampling: trained medical volunteers were employed to record data regarding demographics and SLT history through a structured Questionnaire. Any kind of oral pathology was recorded after

thorough examination of oral cavity.

Oral rinse was collected from 998 subjects in Karachi and Peshawar who were habitual of chewable tobacco after an informed consent. Samples of oral-rinse were collected by giving the subjects 20 ml distilled water to swish in their mouth for one minute and then spit in the 50 ml corning tubes. A dental floss with a small brush was handed over to subjects to gently sweep around oral cavity and over lesions to collect mucosal cells. This floss was left in the corning tube which was stored at 4°C till DNA extraction.

PCR for general HPV was done using Gp5+/Gp6+. DNA was extracted and PCR was performed as previously reported.⁷ The primers, Gp5+/Gp6+, for amplification of HPV were purchased from Gene Link, USA.

Data was entered on Statistical Package for Social Sciences (SPSS) version 20.0. Frequencies and percentages were taken out for the qualitative data. Association between the qualitative variables was calculated using Pearson chi-square. At 95% confidence level, p-value less than 0.05 was taken as significant.

RESULTS

The 293 subjects selected were divided into 3 groups; i. Original naswar users (56, Pathan residents of Peshawar), ii, settler naswar users (79, Pathan residents of Karachi), iii. secondary naswar users (158, divided ethnicity naswar users, Bengali 31, Punjabi 51, Sindhis 32, Balochi 25, Muhajir 13, others (Kachi, Burmi) 06) (Table 1).

The Pathan naswar users were further assessed about habits involving other forms of chewable tobacco along with their naswar dipping habit (Table 2). The symptoms and lesions present in naswar users was compared between Pathan subjects from Karachi and Peshawar (Figure 1).

None of the subjects living in Peshawar were positive for leukoplakia or rough mucosa. In comparison, subjects from Karachi had rough mucosa in 43(54.4%) and leukoplakia in 30 (37.9%).

Genetic testing for HPV was done on each sample. Among specific ethnicity naswar 15(18.9%) samples from Karachi and 20(35.7%) samples from Peshawar were found positive for HPV. Chi square applied to HPV testing done on samples of Pathan naswar users from Karachi and Peshawar showed a significance difference with a p-value of 0.0288.

Table 1: Naswar dipping among different ethnicities living in Karachi with frequency of HPV in their oral cavity.

Users	Ethnicity	Chewable tobacco							HPV +ive
		Niswar alone	Niswar + Gutka	Niswar + paan	Niswar +Areca Nut	Naswar +paan +gutka	Niswar + Cigarette	Naswar +paan +gutka +cig	
Naswar Specific Ethnicity (n=135)	Pathan (Karachi) (n=79)	45 (57.0%)	07 (9.21%)	16 (20.2%)	04 (5.0%)	02 (2.5%)	03 (6.3%)	02 (2.5%)	15 (18.9%)
	Pathan (Peshawar) (n=56)	42 (75.0%)	0 (0.0%)	2 (3.6%)	0 (0.0%)	0 (0.0%)	13 (23.2%)	0 (0.0%)	20 (35.7%)
NonNaswar Ethnicities of Karachi N=158	Bengali (n=31)	01 (3.2%)	01 (3.2%)	02 (6.45%)	0 (0.0%)	21 (67.7%)	0 (0.0%)	06 (19.3%)	06 (19.3%)
	Punjabi (n=51)	15 (29.4%)	13 (25.4%)	02 (4.0%)	03 (5.8%)	07 (13.7%)	03 (5.8%)	09 (17.6%)	14 (27.4%)
	Sindhi (n=32)	06 (18.7%)	12 (37.5%)	03 (9.3%)	03 (9.3%)	06 (18.7%)	01 (3.1%)	01 (3.1%)	11 (34.3%)
	Balochi (n=25)	06 (24.0%)	04 (16.0%)	01 (4.0%)	02 (8.0%)	04 (16.0%)	03 (12.0%)	05 (20.0%)	04 (16.0%)
	Urdu speaking (n=13)	04 (30.7%)	02 (15.4%)	04 (30.7%)	0 (0.0%)	01 (7.7%)	0 (0.0%)	02 (15.4%)	03 (23.0%)
	Others* (n=06)	01 (16.0%)	01 (16.0%)	0 (0.0%)	0 (0.0%)	04 (66.6%)	0 (0.0%)	0 (0.0%)	02 (33.3%)
Total	293								

Table 2: Contrast in SLT habits of original naswar dippers in Peshawar and settlers in Karachi.

PATHAN NASWAR USERS	Niswar alone	Niswar + Gutka	Niswar + Paan	Niswar + Areca Nut	Naswar +paan +gutka	Niswar + Cigarette	Naswar +paan +gutka +cig	P value	HPV positive	P value
Karachi N=79	45 (57.0%)	07 (9.21%)	16 (20.2%)	04 (5.0%)	02 (2.5%)	03 (6.3%)	02 (2.5%)	0.000	15 (18.9%)	0.028
Peshawar N=56	42 (75.0%)	0 (0.0%)	2 (3.6%)	0 (0.0%)	0 (0.0%)	13 (23.2%)	0 (0.0%)		20 (35.7%)	

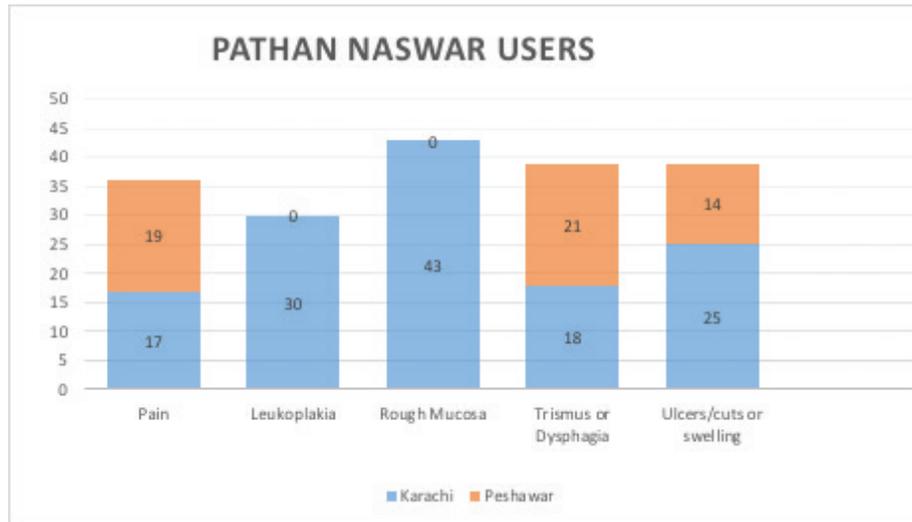


Figure 1: Disparity in oral lesions observed among naswar dippers of two cities

The oral lesions in Peshawar naswar users showed Trismus or dysphagia [21(35.6%)], ulcers, cuts or swelling [14(25.0%)] and pain [19(33.9%)]. Karachi had high frequency for leukoplakia [(30(37.9%)), Rough mucosa [43(54.4%)], Pain [17(21.5%)], Trismus or dysphagia [18(22.8%)] and ulcers, cuts or swelling [25(31.6%)].

DISCUSSION

In our study we observed that 42% of Pathans settlers in Karachi developed SLT habits of other ethnicities, whereas, 21% subjects of other ethnicities became habitual to naswar who have a totally different culture and SLT practices. Karachi is the biggest metropolitan city of Pakistan and people from all four provinces have migrated to Karachi for better livelihood. Their chewable tobacco habits have also crossed borders with them and have become part of shops in their comfort zones, where all these products such as Gutka, Naswar, Paan and Arica nut are available, generally in the squatter settlements around the city. This movement has also been reported from other countries showing how human globalization has compelled ethnicity specific SLT customs to travel with them. In London, borough Naswar of Pakistani origin was found freely available in 15 different outlets and purchasers were men of 32±10 years of age with limited education who had little or no knowledge of health related issues of Naswar, its dependency or devastation related to behavioral and socio-demographic factors.⁸

Since, all these chewable products such as Gutka, Naswar, Paan and Arica nut contain Nicotine and hence people fall easy prey to its dependence, especially the new generations. In Pakistan, the unraveled popularity of Naswar in the Pathan

culture from times unknown is due to the reason that there is a general perception that it is completely harmless.⁹ Naswar, unlike other chewable tobacco products is not chewed, rather placed in oral vestibule where after continuous exposure an oral wart develops. Since oral warts are not painful naswar dippers consider them harmless and do not accept its association with oral or lung cancer.^{9,10} Nicotine along with other carcinogens in these products, corrode the oral mucosa making the surroundings favorable for viruses such as HPV to settle in the epithelium and start replicating in synchrony with the S-phase of the host. Oral cancer is becoming an epidemic in Pakistan affecting people younger than the ones earlier reported.

A recent study on naswar in KPK revealed 68% of oral cancer in men and 38% in women are attributed to naswar dipping.¹¹ Comparative HPV testing in this study done on oral rinse samples of Pathan naswar users from Karachi and Peshawar also revealed a declining trend in pathogenesis, 18.3% in Karachi and 35.7% in Peshawar with a significant difference (p-value <0.02). However, HPV frequency was found higher in subjects who developed trismus after continuous use of SLT suggesting a high risk of oral squamous cell carcinoma.¹²

Naswar has also been found to cause adverse cardiovascular affects by altering various biochemical markers when compared to controls. Such as levels of serum total cholesterol, LDL-C, triglycerides and LDL-C/HDL-C ratio have been found significantly increased (P<0.01), whereas, Glutathione per oxidase, super oxide dismutase and HDL-C significantly reduced (P<0.01) in naswar dippers.¹³ Naswar dipping compromises the Immune system by significantly lowering the levels

of interleukin 1 β , however, no differences in the levels of serum IL-6 were observed by the researchers. Similarly, Naswar was also found to alter thyroid function by significantly increasing serum FT3 and FT4 levels.¹⁴ Regarding levels of trace elements in blood, serum copper levels in naswar addicts were significantly raised with a mean of $5.1 \pm 2.4 \mu\text{g/dl}$ compared to mean of $2.6 \pm 0.1 \mu\text{g/dl}$ in healthy control group ($P = 0.005$).¹⁵

Evaluation of toxicity of multiple heavy and carcinogenic metals, noxious ingredients in naswar of 30 Pakistani brands showed that calculated lifetime risk of cancer from the ingredients present in these SLT products was much higher than the 'target range' for potentially hazardous substances, according to the US Environmental Protection Agency.³

Other studies on other SLT products in Pakistan showed a significant rise ($p < 0.001$) in the levels of heavy metals such as Cd, Ni, and Pb, in their blood.^{16,17}

The powdered SLT popular in Ghana when tested for concentration of thirty-four elements showed potentially toxic levels of Cr, Cd, Sb and Cu in the samples which approximately was two to eight times higher than dried tobacco leaves.¹⁸

Looking at the typography of oral lesions in Naswar dippers in this study there were differences in lesions in dippers of two cities. Karachi dippers had high frequency of leukoplakia (37.9%) and rough mucosa (54.4%) which was not observed in Peshawar dippers. This probably was due to addiction to other SLT products such as Gutka, another advanced variety of SLT product which is a cause of high frequency of premalignant lesions, leukoplakia and erythroplakia.¹⁵ Rests of the lesions were the same in both cities with surprisingly similar frequency.

Clinical and radiographic periodontal parameters in Naswar dippers has been observed significantly elevated ($P < 0.01$) compared to control group with Raman spectrum sharpest band at 1260 cm^{-1} and Raman band at Amide I, whereas, rate of release from dentin matrices of C-telopeptides pyridinoline cross-links of type I collagen (ICTP) and C-terminal crosslinked telopeptide (CTX) from gingival collagen was found significantly higher ($P < .05$) compared to controls showing a higher degree of collagen breakdown in the connective tissue with both bone and attachment loss due to naswar dipping.¹⁹

In Sweden, snus is the most popular SLT which surprisingly resembles naswar in manufacturing and dipping practices. A comprehensive review on adverse effects showed that Snus dipping, leads to non-malignant lesions that are reversible and

disappear if its use is stopped.²⁰ The use of smokeless tobacco in Sweden has been found to be about 10-fold higher than the rest of Europe (17 countries) and more popular amongst men than women.²¹

Toombak is another naswar like product which is marketed in Sudan. Unlike naswar a lot of research has been found on Toombak where 40% of males are addicted to toombak dipping.²² Toombak like Naswar is used through dipping method. Similarly women users comprise only older women which make up 10% of the users. HPV research on Toombak revealed that its frequency of use is linked to the high risk HPV related oral cancer in Sudan.²³

Nass, another similar form of chewable tobacco, is marketed in northern Pakistan, Iran and the Central Asian Republics with the same mode of dipping. The manufacturing procedure is also quite similar except it is only partially cured, with addition of ash, cotton or sesame oil and lime.²⁴

CONCLUSION

Epidemiological shift in behavior occurred due to change in ecology. Naswar dippers (Pathans) (40%) developed other addictions, whereas, subjects (22%) of other ethnicities became habitual to naswar. HPV frequency was found higher in native chewers (35.7%) compared to settlers and non naswar ethnicities (18.9%), this may be due to their other social practices. More comparative studies are required to investigate this further. The medical, dental, and public health communities need to join forces to combat this emerging threat.²⁵

REFERENCES

1. McClave-Regan AK, Berkowitz J. Smokers who are also using smokeless tobacco products in the US: a national assessment of characteristics, behaviors and beliefs of 'dual users'. *Tob Control* 2011;20(3):239-42.
2. S Baig, MM Arif, M Obaid, Z Rubab. Pattern of substance abuse in multi ethnic groups in different localities of Karachi. *Pak J Med Dent* 2013; 1(1): 47-54.
3. Zakiullah, Saeed M, Muhammad N, Khan SA, Gul F, Khuda F, et al. Assessment of potential toxicity of a smokeless tobacco product (naswar) available on the Pakistani market. *Tob Control* 2012;21(4):396-401.
4. ARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 89. Smokeless tobacco and some tobacco-specific N-nitrosamines: International Agency for Research on Cancer; 2007.
5. Niaz K, Maqbool F, Khan F, Bahadar H, Ismail Hassan F, Abdollahi M. Smokeless tobacco (paan

- and gutkha) consumption, prevalence, and contribution to oral cancer. *Epidemiol Health* 2017; 39: e2017009.
- 6.Stanfill SB, Connolly GN, Zhang L, Jia LT, Henningfield JE, Richter P, et al. Global surveillance of oral tobacco products: total nicotine, unionised nicotine and tobacco-specific N-nitrosamines. *Tob Control* 2011;20(3):e2.
- 7.MH Lucky, S Baig. Isolation of DNA from Oral Rinse in HPV Positive Patients. *J Coll Phys Surg Pak* 2013; 23 (7): 455-8.
- 8.Basharat S, Kassim S, Croucher R. Availability and use of Naswar: an exploratory study. *J Public Health* 2011;34(1):60-4.
- 9.Ali S, Wazir K, Rahman MA, Qadir S. NASWAR. *Professional Med J* 2017;24(3).
- 10.Baig S, Z Rubab, MM Arif, MH Lucky. Chewable risk factors-Threatened oral cancer HPV's Looming Epidemic in Pakistan. *European J Biotechnol Biosci* 2015; 3(1): 39-45.
- 11.Khan Z, Dreger S, Shah SMH, Pohlabein H, Khan S, Ullah Z, et al. Oral cancer via the bargain bin: The risk of oral cancer associated with a smokeless tobacco product (Naswar). *PloS One* 2017;12(7):e0180445.
- 12.Z Rubab, AM Mughal, S Baig, MH Lucky, MA Khan. Relationship of Human Papilloma Virus with Trismus in Chewable Tobacco Users Pakistan. *J Med Dent* 2013; 2(01): 3-11.
- 13.Sajid F, Bano S. Effects of smokeless dipping tobacco (Naswar) consumption on antioxidant enzymes and lipid profile in its users. *Pak J Pharm Sci* 2015;28: 1829-33.
- 14.Sajid F, Bano S. Pro inflammatory interleukins and thyroid function in Naswar (dipping tobacco) users: a case control study. *BMC Endocr Disord* 2016;16(1):47.
- 15.Ullah A, Khan A, Iqbal Z, Khan I. Evaluation of Serum Copper Level in Naswar (Smokeless Tobacco) Addicts Using Flame Atomic Absorption Spectroscopy. *Arch Iran Med* 2017;20(10):649.
- 16.Kazi TG, Arain SS, Afridi HI, Naeemullah, Brahman KD, Kolachi NF, et al. Analysis of cadmium, nickel, and lead in commercial moist and dry snuff used in Pakistan. *Environ Monit Assess* 2013;185(6):5199-208.
- 17.Arain SS, Kazi TG, Afridi HI, Brahman KD, Naeemullah, Khan S, et al. Preconcentration and determination of lead and cadmium levels in blood samples of adolescent workers consuming smokeless tobacco products in Pakistan. *Environ Monit Assess* 2015;187(5):309.
- 18.MA Addo, J K Gbadago, H A Affum, T Adom, K Ahmed, GM Okley. Mineral profile of Ghanaian dried tobacco leaves and local snuff: A comparative study. *J Radioanal Nucl Chem* 2008; 277 (3): 517.
- 19.Daood U, Abduljabbar T, Al-Hamoudi N, Akram Z. Clinical and radiographic periodontal parameters and release of collagen degradation biomarkers in naswar dippers. *J Periodontal Res* 2018;53(1):123-30.
- 20.Ahlbom A, Olsson U, Parshagen G. Health hazards of moist snuff. Report of the National Board of Health and Welfare, Sweden, 1997.
- 21.Leon ME, Lugo A, Boffetta P, Gilmore A, Ross H, Schüz J. Smokeless tobacco use in Sweden and other 17 European countries. *Eur J Public Health* 2016;26(5):817-21.
- 22.Idris A, Prokopczyk B, Hoffmann D. Toombak: a major risk factor for cancer of the oral cavity in Sudan. *Preventive Med* 1994;23(6):832-9.
- 23.Ahmed HG. Aetiology of oral cancer in the Sudan. *J Oral Maxillofac Res* 2013; 4(2): e3.
- 24.Gupta PC, Ray CS. Smokeless tobacco and health in India and South Asia. *Respirology* 2003;8(4):419-31.
- 25.Changrani J, Gany F. Paan and Gutka in the United States: an emerging threat. *J Immigrant Health* 2005;7(2):103-8.

