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Alteration in salivary parameters lead to oral lesions among chewable tobacco users

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Abstract
Background: Salivary flow and composition alters under deleterious chemical irritants present in chewable tobacco. Apart from inflammation and deterioration in health of oral mucosa it can also affect the major and minor salivary glands and cause a decline in salivary flow rate. The objective of the study was to find out alteration in salivary parameters that lead to oral lesions among chewable tobacco users
Methods: A total of 354 healthy male subjects, consuming any form of chewable tobacco product, belonging to low socioeconomic areas of Karachi were selected for this cross sectional study. A questionnaire was used to collect demographic data and details of chewing habits (using since, pack/day, duration of exposure etc.). Resting saliva of every subject was collected for 5min and Resting Salivary Flow Rate (RSFR) was expressed in ml/min. Salivary pH was determined by using pH strips (pH 0-14). Oral examination was done for the presence or absence of oral lesions. Data was analyzed on SPSS version 20.
Results: Out of the 354 subjects included, 27.4% consumed gutka, 24.3% niswar, 24.3% paan and 24% were multiple users. Mean RSFR was 0.52±0.34 ml/min and pH 6.58±0.78. Among these 96 (27.1%) had oral lesions with highest frequency observed among subjects who had hyposalivation (40%) and those having acidic pH (40%). A significant decrease in RSFR and pH and increase in frequency of oral lesions is observed with increased duration of exposure, duration of usage and increased number of tobacco packs consumed per day.
Conclusion: Consumption of chewable tobacco leads to decrease in RSFR and pH and hence increase in frequency of oral lesions

Keywords: Saliva; tobacco chewing; resting salivary flow rate, salivary pH, oral lesions

Introduction
Salivary glands produce saliva which plays an important role in maintaining oral health of teeth and soft tissues. The health of oral mucosa depends on oral clearance which, under resting conditions, depends on salivary parameters i.e. salivary flow rate and its pH [1, 2]. Salivary flow and composition alters under both physiological and pathological conditions [3]. Besides diseased conditions, habits such as drinking alcohol, smoking and chewing tobacco are also associated with altered salivary compositions and dental health [4, 5].
Chewable tobacco, an integral cultural component of majority South East Asian countries, is a cause of corrosive consequences on oral mucosa [6, 7]. Besides altering the functions of multiple body systems, tobacco in chewable form, is also a known culprit of causing oral soft tissue lesions [8-10]. The deleterious components of tobacco and their metabolites act as local chemical irritants, which along with the mechanical irritation of coarse betel quid fibers results in inflammation of oral tissues and also deteriorates the health of major and minor salivary glands [11, 12].
Oral Mucosal Lesions, due to chewing different types of tobacco products, are of wide variety and their occurrence due to chewable tobacco use and conversion of these lesions into malignancies is well documented [8, 13, 14]. Along with directly affecting the oral mucosa, tobacco use may alter salivary parameters which in turn damages the mucosa leading to oral lesions. However, as not much literature is available on the relationship that oral lesions develop because of altered salivary flow and pH due to tobacco use, the current study was conducted.

Method
A cross sectional study was designed in which 354 tobacco chewers, who fulfilled the inclusion and exclusion criteria, were selected from different low socio-economic localities of Karachi. Chewable tobacco products widely consumed in Pakistan are gutka, paan and niswar, which is why people who used any of these products were recruited. Tobacco, sun-dried roasted, finely chopped or powdered, is the main ingredient of these products combined with other ingredients such as ash, oil flavoring agents, lime, areca nut and betel leaf varying from product to product. These are either chewed constantly such as gutka and paan or placed and held under the tongue or in buccal mucosa for long periods of time such as niswar.

Inclusion Criteria
Study participants selected were healthy males between the ages of 18 to 50 years, consuming at least one form of chewable tobacco (gutka, pan or niswar) for at least a year.

Exclusion Criteria
People who were healthy male tobacco chewers but were suffering from any acute or chronic diseases of oral mucosa and teeth or salivary glands, complained of any systemic disease,

had received therapeutic radiation or medications which can affect saliva production were excluded from the study. Individuals consuming chewable tobacco for less than a year and who smoked or were addicted to any other substance were also excluded from the study.

Participants were asked to submit a written consent of voluntary participation and the demographic data, comprising details of type of chewable product used, how long they have been using it (years), number of packs consumed per day and duration of exposure in min (i.e. how long it is kept in the mouth), was obtained through a questionnaire. Sampling was done after taking approval from Ethical Review Committee, Ziauddin University.

Measurement of Salivary Parameters (RSFR and pH)

To avoid diurnal variations, unstimulated (resting) whole saliva samples from all subjects were collected in the morning between 9 a.m. to 12 a.m. Subjects were instructed to refrain from eating, drinking, and chewing of tobacco for at least 1 hour prior sample collection. Subjects were comfortably seated, in relaxed state and were asked to avoid swallowing and spit all the saliva they produced for 5 minutes into a graduated test tube, through a glass funnel. The whole volume collected was then measured and expressed as Resting Salivary Flow Rate (RSFR) ml/min.

pH of the samples was immediately measured using pH indicator strips (pH 0-14, universal indicator, Merck, Germany), following the instructions provided with the kit. The strip was dipped for 5sec in the collected saliva and the color change was noted and assessed by comparing it with the standard color chart given. The corresponding pH was then noted.

Identification of Oral Lesions

Intraoral examination of all subjects was done by two trained examiners, using mirror and gauze, oral mucosa was checked for the presence or absence of oral lesions. Criteria for deciding the presence of lesion is based on Axell *et al.* [15] and Greer & Poulson [16] criteria. They classified Grade 1 oral lesions *as: slight, superficial wrinkling of the mucosa. Color of the mucosa may range from normal to pale white or gray. Mucosa does not appear to be thickened.*

So in this study, any subject falling in above criteria was considered as having a lesion. Those subjects who did not have any of the above mentioned signs were said as not having any lesions [17].

Data was entered and analyzed using SPSS version 20. Frequencies and percentages were taken out for categorical variables. Mean and standard deviation were calculated for numerical variables. Association between categorical data is observed by Chi Square test. Difference of means among groups is assessed by independent t- test and ANOVA. P value less than 0.05 was taken as significant.

Results

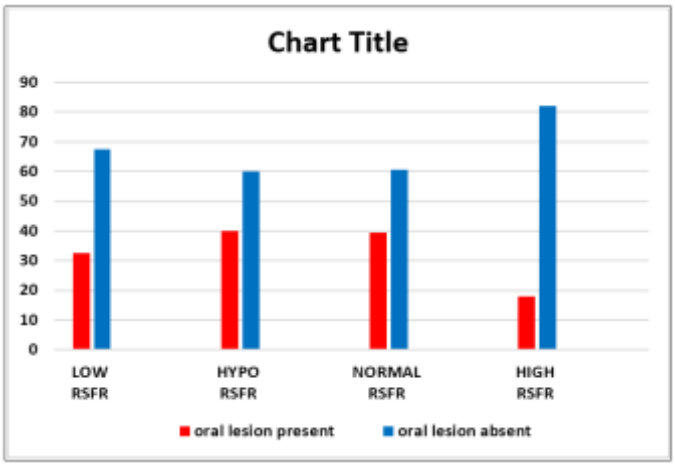
Out of the 354 subjects, 27.4% consumed gutka, 24.3% niswar, 24.3% paan and 24% were multiple users (consumed more than one chewable tobacco product) with mean number of tobacco packs consumed per day was 9.27 ± 9.52, duration of exposure (min) 17.32 ± 14.32 and mean duration of usage (years) 10.09 ± 7.29. Subjects belonged to different ethnicities and the mean age group was 29.3±9.3 years.

The mean Resting Salivary Flow Rate (RSFR) was 0.52±0.34 ml/min, pH 6.58±0.78 and 27.1% had oral lesions. RSFR and pH among chewable tobacco users decreases with increase in number of packs consumed per day, duration of exposure and duration of usage (Table 2 and 3). Table 1 shows significant difference in mean RSFR and pH between subjects with and without oral lesions. For further understanding of the data RSFR was divided into 4 different categories, according to standard reference ranges mentioned in the literature as: Hyposalivation (< 0.1ml/min), Low RSFR (0.1 – 0.29 ml/min), normal RSFR (0.3 – 0.5 ml/min) and Hyper RSFR (> 0.5ml/min). Similarly salivary pH was also categorized as acidic (< 6.8), normal (6.8 – 7.8) and alkaline pH (>7.8).

The frequency of oral lesions among different RSFR and pH groups is shown in Figure 1 and 2, respectively, with the highest number of lesions in subjects having hyposalivation (40%) and in those having acidic pH (40%). Highest frequency of oral lesions (39.6%) is found in subjects consuming multiple types of chewable products (Figure 3). Figure 4, 5 and 6 shows frequency of oral lesions among different types of chewable tobacco product users due to various contributing factors.

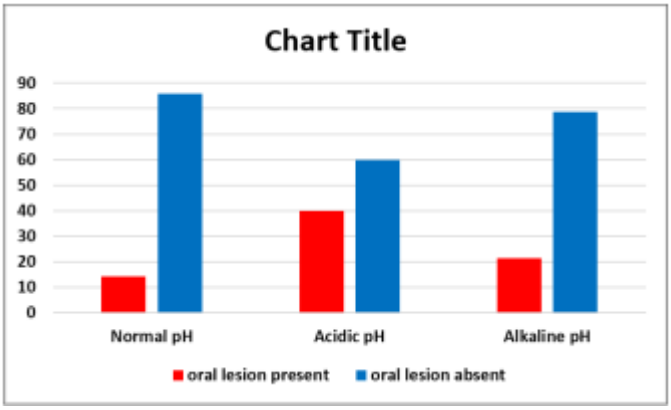
Table 1: Mean RSFR and pH of Subjects With and Without Oral Lesions.

Oral Lesion	n	RSFR ml/min (Mean± SD)	pH (Mean± SD)
Present	96	0.45 ± 0.34	6.27 ± 0.81
Absent	258	0.55 ± 0.34	6.69 ± 0.74
P-Value		0.012	0.0001



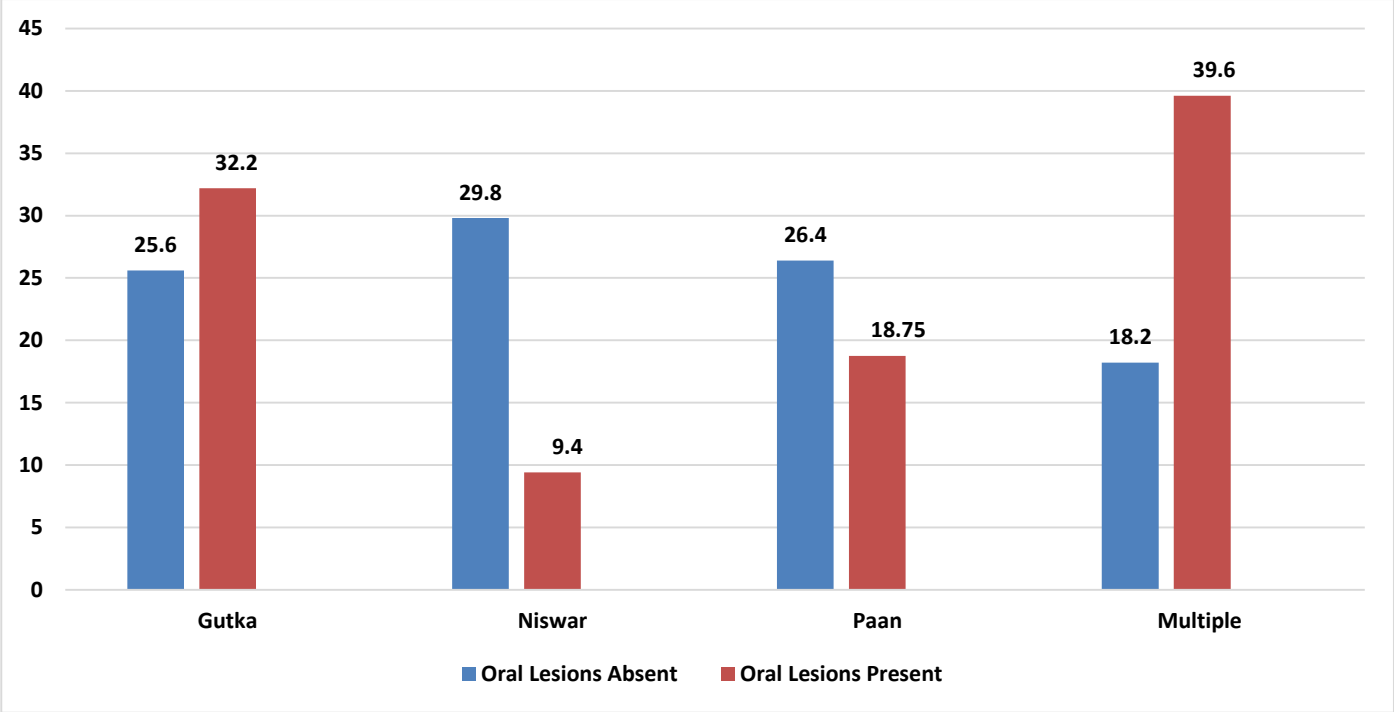
P- Value 0.001

Fig 1: Frequency of Oral Lesions in Different RSFR Groups



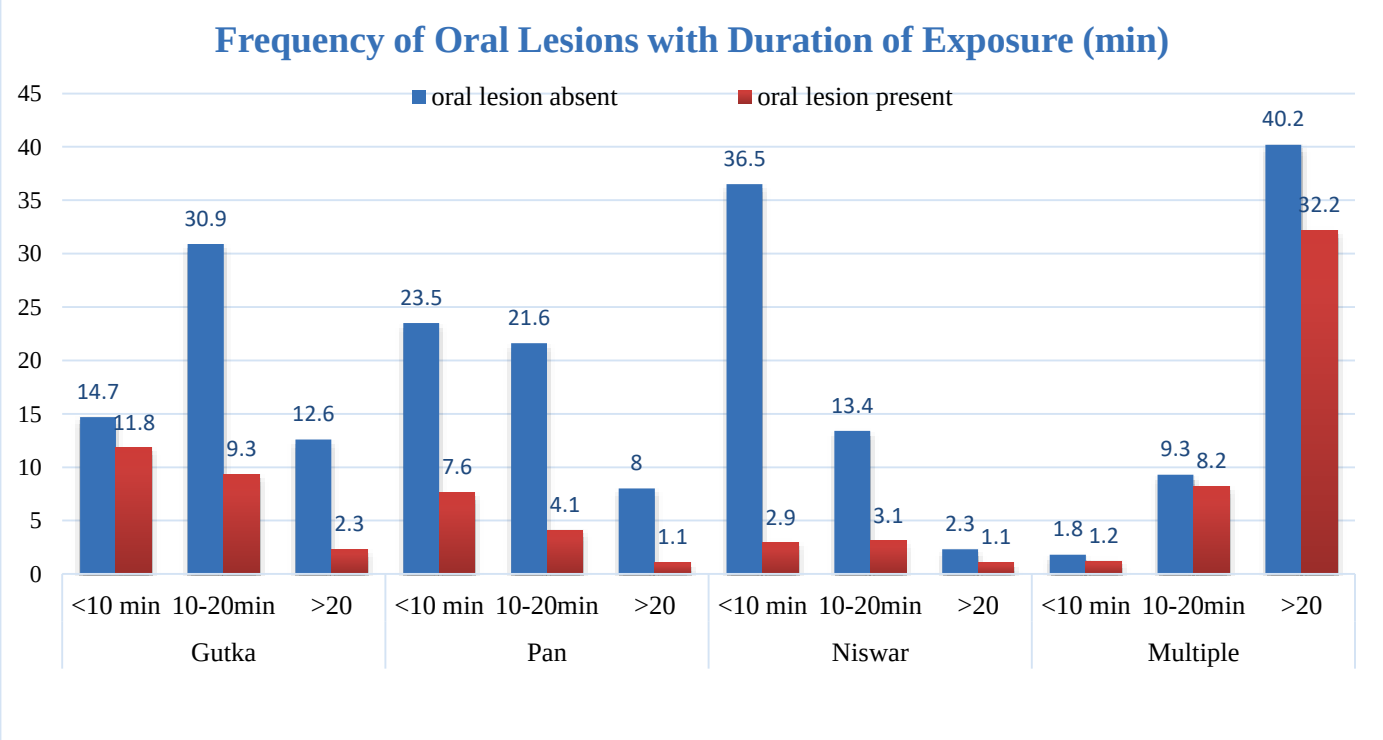
P- Value 0.000

Fig 2: Frequency of Oral Lesions in Different pH groups



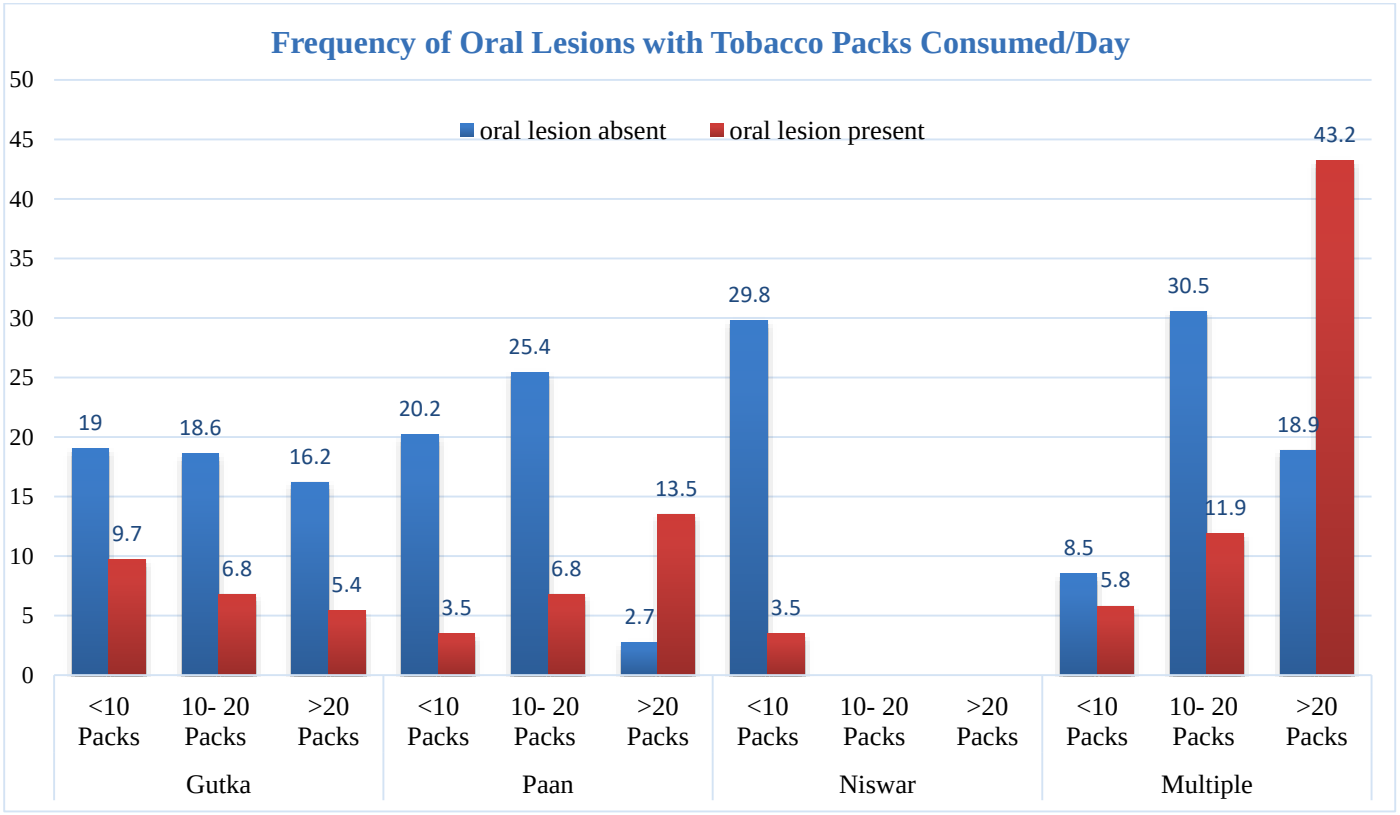
P- value 0.000

Fig 3: Frequency of Oral lesions Among Types of Chewable Tobacco Users



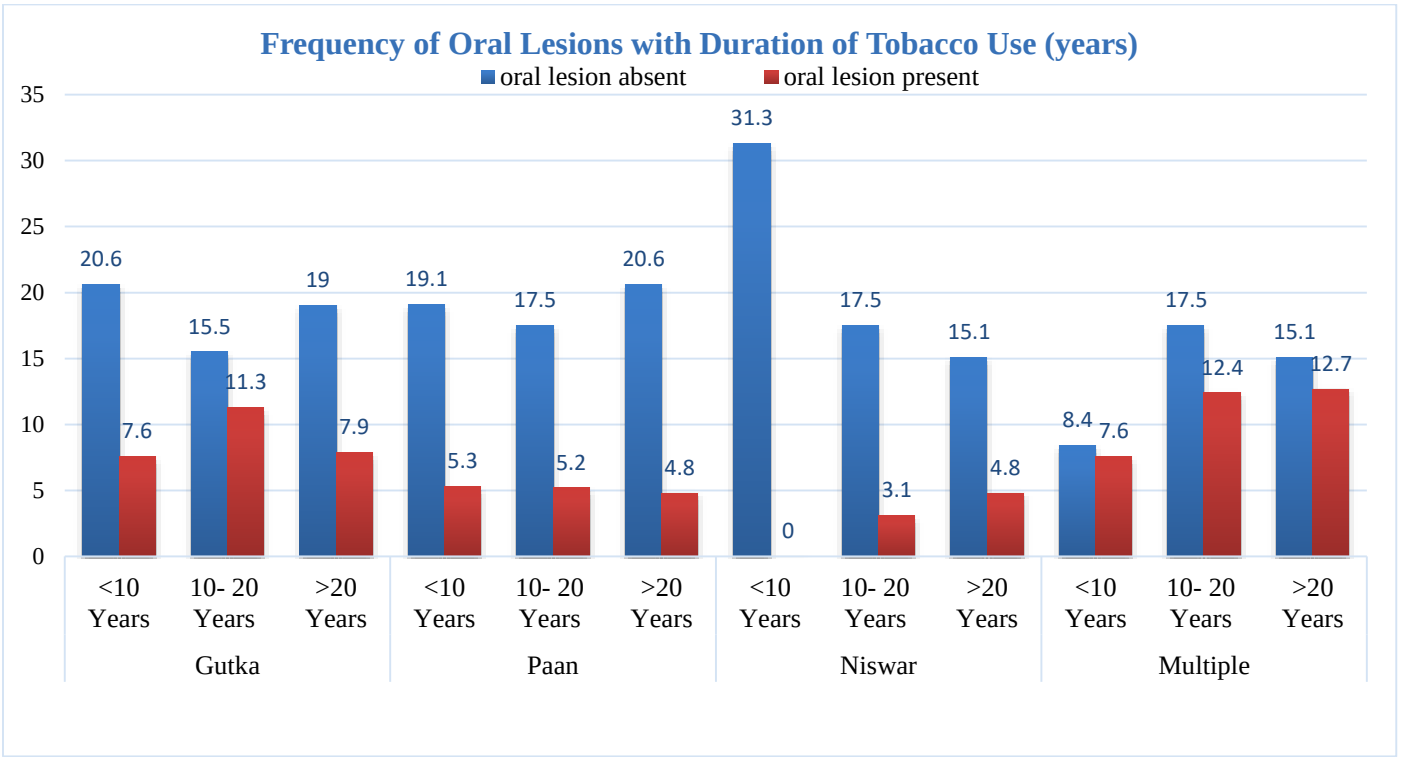
P-value (<10min: 0.000 10-20min: 0.115 >20min: 0.103)

Fig 4



P-value (<10packs: 0.000 10-20 packs/day: 0.865 >20packs/day: 0.041)

Fig 5



P-value (<10yrs: 0.000 10-20yrs: 0.113 >10yrs: 0.091)

Fig 6

Table 2

Mean RSFR in different Tobacco Products according to various contributing factors					
		RSFR Mean ±SD			
		Gutka n= 97	Paan n= 86	Niswar n= 86	Multiple User n= 85
Using Since	<5 years	0.54 ± 0.38	0.73 ±0.36	0.65 ±0.32	0.57 ±0.31
	5-10 years	0.38±0.29	0.65 ±0.45	0.76 ±0.41	0.37 ±0.20
	>10 years	0.27 ±0.11	0.56 ±0.37	0.58 ±0.22	0.35 ±0.21
	Overall	0.41 ± 0.30	0.65 ±0.37	0.66 ±0.32	0.41 ±0.25
P- value		0.0001			
Packs/day	<10 packs	0.46 ±0.32	0.72±0.40	0.66±0.32	0.46±0.29
	10-20 packs	0.26 ±0.12	0.52±0.33	-	0.44±0.22
	>20 packs	0.17 ±0.06	0.31±0.19	-	0.29±0.16
	Overall	0.41 ± 0.31	0.64 ± 0.39	0.66±0.32	0.41 ±0.25
P- value		0.0001			
Duration of Exposure	<10 min	0.52±0.37	0.68±0.41	0.72±0.32	0.60±0.47
	10-20 min	0.31±0.14	0.62±0.41	0.45±0.25	0.56±0.31
	>20 min	0.35±0.28	0.46±0.15	0.41±0.17	0.35±0.18
	Overall	0.41±0.31	0.64±0.39	0.67±0.32	0.41±0.25
P- value		0.0001			

Table 3

Mean pH in different Tobacco Products according to various contributing factors					
		pH Mean ±SD			
		Gutka n= 97	Paan n= 86	Niswar n= 86	Multiple User n= 85
Using Since	<5 years	7.19 ±0.66	7.03± 0.89	6.97 ± 0.52	6.52± 0.67
	5-10 years	6.34±0.75	6.59 ± 0.79	7.0 ± 0.56	6.20 ± 0.49
	>10 years	6.17 ±0.57	6.31 ± 0.93	6.68 ± 0.56	5.9 ± 0.65
	Overall	6.6± 0.79	6.65 ± 0.93	6.89 ± 0.55	6.16 ± 0.65
P- value		0.0001			
Packs/day	<10 packs	6.74 ±0.78	6.91 ±0.81	6.89 ±0.55	6.32 ± 0.66
	10-20 packs	6.26 ±0.70	6.21 ±0.92	-	6.24 ± 0.59
	>20 packs	6 ± 0.76	5.33 ±0.51	-	5.8 ± 0.57
	Overall	6.6 ±0.79	6.66 ± 0.93	6.89 ±0.55	6.16 ± 0.65
P- value		0.0001			
Duration of Exposure	<10 min	6.75 ±0.80	6.86 ±0.87	7.0 ± 0.49	6.6 ± 0.54
	10-20 min	6.48 ±0.72	6.44 ±0.86	6.5 ± 0.63	6.3 ± 0.78
	>20 min	6.46 ±0.96	5.87 ±0.99	6.66 ± 0.57	6.07± 0.60
	Overall	6.6 ±0.79	6.65 ± 0.93	6.89 ± 0.55	6.16 + 0.65
P- value		0.001			

Discussion

Tobacco consumption, in either smoked or smokeless form, is a leading preventable cause of death and a modifiable risk factor for major non-communicable diseases [18]. According to an estimate, around 600 million people (10-25%) of the world's population use tobacco in the smokeless form. Multiple studies conducted on the prevalence and trends of chewable tobacco use in different cities of Pakistan reported that its popularity is due to its easy availability, social acceptability, cheap price, sweet flavor, ability to relieve toothache, headache and stress, and apparently having no ill health effects [19-21]. A recent nationwide survey conducted in 2012 showed that 34.9% males and 5.1% females in Pakistan consume tobacco in any form, smoked or smokeless and minimum mean starting age of 11.5years [22]. Also, studies [23-25] have reported that the chewing of tobacco products is more common among males more specifically those belonging to low socioeconomic group of the society which is the reason why we selected only males, having tobacco chewing habit, belonging to this under privileged group for our study. Baig *et al.* [25] and Khawaja *et al.* [26] observed that chewing habit is more common among younger adults and this corresponded with our results as the mean age group of our study participants was found to be 29.3±9.3 years with 43%subjects in age group of 20-29 years and 29% in age group of 30-39 years.

Resting saliva is a significant contributor to total saliva during diurnal cycle and by keeping the mucosa moist at all times it maintains oral health [27], therefore RSFR and pH were two salivary parameters assessed in the current study. It was found that mean RSFR significantly decreased among different types of tobacco chewers more specifically among gutka chewers and multiple tobacco product users. This was similar with studies of Rooban *et al.* [28], Kanwar *et al.* [29] and Rad *et al.* [30] who also found decrease in salivary flow rates with tobacco use.

We also found significant decrease in RSFR with increased number of packs consumed per day, duration of exposure and prolonged duration of usage among all groups of tobacco chewers' group with multiple tobacco users and gutka chewers consuming more than 10 packs/day and using for more than 10 years dominating the results. This finding is in accordance with the literature which shows that intense, long term smokeless tobacco use results in degenerative changes in more than 40% of the minor salivary glands that are in contact with tobacco [31]. This dose dependent change was however not much pronounced among niswar and pan chewers probably because niswar use does not involve much chewing and the tobacco content in these products may be less. Salivary pH is dependent on salivary flow rate, so a decrease in salivary flow rate also decreases its pH and vice versa [32]. Hence, salivary pH also showed significant decline and turns acidic with increased number of packs consumed per day, duration of exposure and prolonged duration of usage among all groups of tobacco chewers.

Frequency of oral lesions found in our study population was 27.12% and 40% of the people having oral lesions fell in the category of hyposalivation (Figure 1) suggesting that a fall in RSFR contributes to a decline in oral health and hence development of oral lesions. Studies have proposed that long term use of chewable tobacco products and consuming large quantities, lead to prolonged nicotine dependent activation of sympathetic nervous system, even in periods of non-chewing, which depresses saliva production [33-35]. Studies have also reported that comparable to smoked tobacco, nicotine from chewable tobacco is more readily absorbed and stays longer in blood stream [9]. Also the areca nut component of these products has tendency of eroding the oral mucosa which further contributes to development of rough mucosa [36] and together all these factors i.e. continued heavy chewing, depressed salivary gland function, nicotine and other carcinogenic metabolites, then help in progression of the rough mucosa to premalignant and malignant oral lesions.

Since salivary pH, both under resting and stimulated condition, is directly dependent on salivary flow rate [32, 37], a decrease in RSFR also decreases resting salivary pH. Our results showed a statistically significant decline between RSFR (p value: 0.012) and pH (p value: 0.0001) of subjects with and without oral lesions (Table 1). This finding is in contrast with findings of Zulkarnain *et al.* [38] who reported non-significant difference in RSFRs of subjects with and without oral lesions. However, our results of pH change matches with Zulkarnain's [38], who also found acidic pH among subjects having oral lesions. We also found that subjects in hyposalivation group and those having acidic pH had more frequency of oral lesions than others and this difference was statistically significant (figure 1 and 2 respectively). So from current findings, we can say that in periods of non-chewing, decreased salivary flow makes the oral pH acidic and this acidity may also contribute then in deteriorating oral mucosal health.

Frequency of oral lesions is highest among multiple product users (39.6%) and least in niswar users (9.4%) (Figure 3) probably because niswar product is simply kept in the cheek and sucked but not chewed. So the only factor that may develop an oral lesion in a niswar user is the nicotine content and other carcinogenic metabolites of tobacco which in turn depend on the frequency of its use and how long it has been used. We found a significant rise in the development of oral lesions among those who consumed niswar for more than 10 years (Figure 6). However, in multiple users, the frequency of oral lesions increased with increased duration of exposure of tobacco, packs consumed per day and duration of use (Figure 4, 5 and 6 respectively). These outcomes were similar with the findings of Maqsood *et al.* [39], Sujatha *et al.* [40], Yen *et al.* (41), Aruna *et al.* [42] and Kallischnigg *et al.* [43] who also found that increased frequency and recency of chewable tobacco use results in increased occurrence of oral lesions.

Conclusion

The deleterious components present in Chewable tobacco after a long term use, deteriorate the health of major and minor salivary glands by decreasing salivary gland function (RSFR and pH).

Further studies are required to understand the pathophysiology of the harmful effects of individual components presents in these formulations. Counseling programs and preventive strategies should be extended to the users and their families.

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