ORIGINAL ARTICLE

ROLE OF CHEWING HABITS AND CIGARETTE SMOKING IN DIFFERENTIATION OF ORAL SQUAMOUS CELL CARCINOMA

Shumaila Younus[1], Naila Irum Hadi[2], Farah Ahmed[3], Haya Mohammad[1]
[1]Department of Oral Pathology, Ziauddin University and Hospitals, Karachi Pakistan.
.[2]Department of Pathology, Ziauddin University and Hospitals, Karachi Pakistan.
[3]Department of Community Health Sciences, Ziauddin University and Hospitals, Karachi Pakistan.

ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) is one of the most common cancers in the world. Cigarette smoking, gutka and pan are the most common addictions in Pakistan. This study is conducted to evaluate the frequency of these risk factors (cigarette smoking, gutka and pan) and correlate it with histopathological grade of OSCC.The objective of the study is to evaluate the role of cigarette smoking, gutka and pan consumption in the histopathological differentiation of Oral Squamous Cell Carcinoma.

Methods: A cross-sectional study was conducted. OSCC patients (n= 138) were recruited through purposive sampling technique from Dental OPD of Ziauddin Dental College, Ziauddin Hospital, Karachi. A detailed questionnaire was filled with information regarding patient's medical history along with the daily consumption of cigarette, gutka and pan. Co-relations were studied between various continuous variables. Cross-tabulations were performed between frequency of habits and different histopathological grades of OSCC.

Results: In the present study, 138 OSCC patients, 108 (78.3%) males and 30 (21.7 %) females, were enrolled and histopathologically diagnosed into well differentiated, moderately differentiated and poorly differentiated OSCC. Association of histopathological grades of OSCC with different age groups revealed moderately differentiated OSCC as the predominant histopathological differentiation in 30-39 years age group (46.3%). Majority of OSCC cases with cigarette smoking and gutka consumption used these products with frequency of > 15 packets per day, while, most of the pan consumers used 5-15 pan per day. All these patients had poorly differentiated OSCC i.e. 48.7% cigarette smokers, 64.1% gutka and 43% pan consumers.

Conclusion: This study concludes that most of OSCC patients with habits of gutka, cigarette smoking and pan were associated with poorly differentiated carcinoma with more cases occurring in younger age group. This point to an alarming situation and se¬rious thought should be given to early detection and prevention.

KEY WORDS: Oral squamous cell carcinoma, frequency, cigarette smoking, gutka, pan, grading.

INTRODUCTION

Oral cancer is a major cause of cancer death and oral squamous cell carcinoma is the most common type [1]. In 2015 it is estimated that approximately 45,750 individuals will be diagnosed with oral cancers and 8,650 are expected to die [2].The prevalence of oral cancer in Pakistan is around 10% [3]. Most of the cases have been diagnosed in the middle and in older age groups, but in recent years, many studies have shown increased incidence in young age individuals also [4]. Males are affected more than females with male to female ratio of 1.5:1. Major risk factors are betel quid (paan), areca nut, naswar, pan masala (gutka, mawa) and poor nutrition [5]. Tobacco is the main etiological factor for oral cancer and cigarette smoking is the main form of tobacco use. Tobacco contains N-nitroso compounds, well-known carcinogens that plays an important role in the malignant transformation of oral cancer [6]. Gutka is a powdered combination of arecanut, tobacco and slaked lime with many spices and flavoring agents[7]. Pan also known as betel quid, has four major ingredients: areca nuts, tobacco and slaked lime wrapped in betel leaf. It also contain flavoring agent's cardamom, cloves,

Corresponding Author: Shumaila Younas*

coconut and sugar. Areca nut used in pan and gutka is known to cause oral squamous cell carcinoma due the presence of arecoline which consists of specific nitrosamines that are carcinogenic [7]. Tobacco in any form, either smoked or smokeless, can cause a wide spectrum of oral mucosal changes leading to oral cancer. The type and location of the lesion varies with the type of tobacco used, the way it is used, and the frequency of consumption [8]. Cancer of the oral cavity involves gingiva, alveolar ridge, buccal mucosa, the floor of the mouth, tongue, hard palate, soft palate and uvula. Among the Asian population, buccal mucosa is the most commonly involved site in the oral cavity due to betel quid/tobacco chewing. Tongue is the most common site among European and US populations, amounting to 40%–50% of Oral cancers. According to the degree of keratinization, mitotic activity, cellular and nuclear pleomorphism, OSCC is divided into three categories: well differentiated, moderately differentiated and poorly differentiated. The histopathological grade reflects the aggressiveness of the tumour [9]. However, previous studies were not specifically designed to look into the role of cigarette smoking, gutka and pan consumption in the histopathological differentiation of Oral Squamous Cell Carcinoma (OSCC). With these considerations in mind we hypothesize that tobacco smoking and consumption of chewable tobacco products (gutka and pan) can affect the histopathological differentiation of OSCC. Hence, the aim of this study was to evaluate the frequency of these risk factors (gutka, cigarette smoking and pan) and correlate it with histopathological grade of OSCC.

METHODS

This cross sectional study was carried out in the department of Oral Pathology, Ziauddin University from March 2014 to June 2015. There were 138 subjects recruited by purposive sampling from Dental OPD of Ziauddin Dental College, Ziauddin Hospital, Karachi. A questionarre was filled with details regarding patient's medical history along with frequency of cigarettes, gutka and pan. The study was approved by Research and Advocacy Committee (RAC), Ethics Review Committee (ERC), Board of Advanced studies and Research (BASR), Ziauddin University.

Histopathologically diagnosed cases of OSCC, 18 years and above, both male and female, belonging to all ethnic groups without socioeconomic discrimination, with smoking and /or chewing habits were included in this study. Malignancies other than Oral Squamous Cell Carcinoma were excluded. Histological grading was done according to the protocol of College of American Pathologists (CAP) as follows; Grade 1 (well differentiated), Grade 2 (moderately differentiated) and Grade 3 (poorly differentiated) [10].

Patients were divided into 5 groups according to their ages at diagnosis: Group 1 (20–29 years), group 2 (30–39 years), group 3 (40–49 years), group 4 (50–59 years) and group 5 (60–69 years). For frequency of habits subjects were grouped into those who smoked 5-15 and more than 15 cigarettes per day and who consumed gutka packets and pan 5-15 and more than 15 per day.

The collected data was sorted, tabulated and statistically analysed. The analysis was performed to determine the frequency of risk factors with the histopathological grading in OSCC patients. The independent variables were age, gender, and frequency of habits (cigarette, gutka and pan). P-values were estimated with p-value of < 0.05 termed as statistically significant.

RESULTS

One Hundred and thirty eight Oral Squamous Cell Carcinoma (OSCC) patients were included in this research with an age range of 20 to 69 years. According to age- wise distribution, maximum number of cases i-e 41 (29.7%) were reported in 30-39 years age group followed by 37 (26.8%) in 40-49 years, 28 (20.3%) in 50-59 years and 25 (18.1%) in 60-69 years age group. Only 7(5.1%) patients were found in 20 -29 years age group. This study included more male patients (108; 78.3%) than females (30; 21.7%). According to ethnicity, most of the OSCC patients were Mohajirs (77; 55.8%) followed by Balochis (26; 18.8%), Sindhis (23; 16.7%) and Pathans (5; 3.6%) respectively. (Table 1). In this study most common site of OSCC was buccal mucosa (58.0%) followed by tongue (17.4%), lip (8.0%), palate and alveolar ridge (6.5%) and labial mucosa (3.6%) as shown in figure 1.

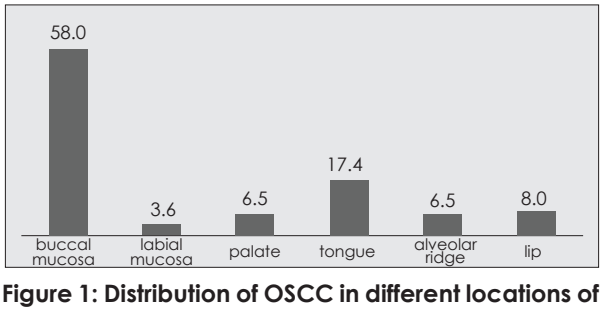

Figure 1: Distribution of OSCC in different locations of oral cavity

Table 1: Characteristics of OSCC patients.

Characteristics of OSCC patients	n (Percentage)
Age Range (years)	
20-29	7 (5.1%)
30-39	41 (29.7%)
40-49	37 (26.8%)
50-59	28 (20.3%)
60-69	25 (18.1%)
Gender	
Male	108 (78.3%)
Female	30 (21.7%)
Ethinicity	
Muhajir	77 (55.8%)
Balochi	26 (18.8%)
Sindhi	23 (16.7%)
Punjabi	7 (5.1%)
Pathan	5 (3.6%)

Differences in histopathological grading of OSCC with age is shown in Table 2. Moderately differentiated OSCC was predominant histopathological differentiation in 30-39 years (19; 46.3%), 40-49 years (16; 43.2%), 60-69 years (12; 48%) and 20-29 years (7; 100%) age groups. While poorly differentiated OSCC was predominantly seen (17; 60.7%) in 50-59 years age group (Table 2).

Table 2: Associtation of histopathological grades of OSCC with different age groups

Age in years	Well Differntiated (W) n=32 n(%)	Moderately Differntiated (M) n=60 n(%)	Poorly Differntiated (P) n=46 n(%)	P value
20-29 years	0(0%)	7(100%)	0(0%)	≤0.05
30-39 years	7(17.1%)	19(46.3%)	15(36.6%)	
40-49 years	13(35.2%)	16(43.2%)	8(21.6%)	
50-59 years	5(17.9%)	6(21.4%)	17(60.7%)	
60-69 years	7(28%)	12(48%)	6(24%)	

Table 3 shows the correlation between frequencies of cigarettes, gutka and pan consumption and histopathological differentiation. Moderately differentiated OSCC was more common in patients who smoked 5-15 cigarettes per day (24; 40%) and those who consumed 5-15 packets of gutka per day (39; 49.4%). For pan consumers (with frequency of 5-15 pans per day) both moderately differentiated (39; 42%) and poorly differentiated OSCC (40; 43%) had equal distribution.

Poorly differentiated OSCC was the predominant histopathological grade for cigarette smokers (> 15 cigarette / day) (20; 48.7%) and gutka users (> 15 packets / day) (25; 64.1%). For pan users (> 15 pan / day), both well differentiated (9; 40.9%) and moderately differentiated OSCC (8; 36.4%) showed equal distribution (Table 3).

Table 3: Correlation between frequency of cigarettes, gutka, pan consumption and histopathological differentiation in OSCC patients.

Frequency of risk factors		Well Differentiated (W) n=46 n(%)	Moderately Differentiated (M) n=60 n(%)	Poorly Differentiated (H) n=32 n(%)	*p value*
Cigarettes /day	5-15	16(27.6%)	24(40%)	18(30%)	≤0.05
	>15	9(22%)	12(29.3%)	20(48.7%)	
Gutka /day	5-15	22(27.8%)	39(49.4%)	18(22.8%)	≤0.05
	>15	3(7.7%)	11(28.2%)	25(64.1%)	
Pan /day	5-15	14(15%)	39(42%)	40(43%)	≤0.05
	>15	9(40.9%)	8(36.4%)	5(22.7%)	

DISCUSSION

Gender, wise distribution in our study revealed male preponderance with 108 (78.3%) male and 30 (21.7%) female OSCC patients. This could be due to higher consumption of gutka, pan and cigarette smoking in male gender which is consistent with findings of other studies [11, 12]. In contrast, a study conducted in Lahore observed an equal prevalence of OSSC in both genders [13]. However, in some parts of the world the incidence of OSCC in females is increasing, possibly due to of their increasing indulgence in high-risk habits [14].

The habits of smoking, gutka and pan are also popular in younger age groups due to attractive appearance, low prices and easy availability of these products, supported by deficiency of knowledge about their devastating effects. In the present study, the age of patients ranged from 20 to 69 years with the peak age range of OSCC occurrence in the 30-39 years (41; 29.7%). This finding is in agreement with a study carried out in Taiwan [15]. Similarly, a study in India reported a high incidence of OSCC below 40 years of age [16]. In contrast a research conducted in an Indian population reported greater incidence in the age range of 51-60 years [17]. While another study reported the average age of diagnosis of oral malignancy as 62 years [18].

Majority of OSCC patients were Muhajirs (Urdu speaking people) (55.8%). Urdu speaking people have migrated from India at the time of partition and have retained their social and cultural heritage [19]. This is in accordance with the researches done in Karachi, which also confirms that malignancy was predominant amongst the Mohajirs [14, 20].

In our study the most common site of involvement was the buccal mucosa (58.0%) followed by tongue (17.4%). These findings are consistent with other studies carried out in this region [21, 22]. In some Asian countries such as Thailand, Taiwan and India, buccal mucosa was also found to be the most common site of involvement [23]. In contrast, tongue is the most commonly affected site of oral cancers in western countries as tobacco smoking is more prevalent than tobacco chewing (9). Similarly, in Iran, UK, and Srilanka, the most common site of involve-

ment was the tongue [24,25,26]. This difference seems to be related to different habits [27].

The histopathological distribution of OSCC in our study indicated a clear predominance of moderately differentiated carcinoma (n=19) in 30-39 year age group. A research done in Thailand revealed that most of the OSCC tumors were moderate to well differentiated carcinoma, in patients younger than 45 year [28]. Similarly, a study conducted in Karachi revealed equal distribution of well-differentiated and moderately differentiated carcinoma with age ranges between 21-40 years [29]. In contrast a study conducted in Iran reported majority of cases were diagnosed as well differentiated carcinoma with age ranges of 20-40 years [24].

In our study risk factors were smoking, gutka and pan. Tobacco smokers have a 27 fold higher risk of developing Oral squamous cell carcinoma as compared to non-smokers [30]. Tobacco-specific nitrosamines (N-nitroso compounds) play a major role in the malignant transformation of oral squamous cell carcinoma [6]. In our study subjects who consumed more than 15 cigarettes per day 48.7% (n=20) are significantly associated with poorly differentiated carcinoma. This is consistent with a study conducted in Brazil, demonstrating 18.8% of smokers who consumed more than 20 cigarettes per day had poorly differentiated carcinoma (40.4%) [31]. Muange et al., reported cigarette smoking in 60% of cases, with poorly differentiated carcinoma (48.8%) [32]. Degree of low differentiation risk increased with increasing number of cigarette smoked [33].

An industrially prepared mixture of areca nut, slaked lime, catechin containing substance, sandalwood fragrance and tobacco is known as Gutka which is kept in the buccal pouches for long periods and its juices are sucked in. This leads to significant exposure of the subjected mucosa to carcinogens [7]. In our study subjects who consumed gutka more than 15 packets per day are significantly associated with poorly differentiated carcinoma (25; 64.1%). In contrast, a study conducted in Karachi reported 60% of cases as well differentiated carcinoma [34] .Similar findings were seen in a study where, gutka users (70%) showed 66% of well differentiated carcinoma [35]. While another Indian study conducted by Bhat et al., showed 52% of gutka consumers in which 47% were confirmed as well differentiated carcinoma [36]. These studies have not mentioned the details about frequency and duration of consumption of these products and we assume that prolonged use of these products in our patients has resulted in poorly differentiated OSCC[37]. People get addicted to gutka as it is reported to have stimulant and relaxation effects. When a person chews gutka, the mixture directly enters the system through the oral cavity [38]. While in the case of smoking, the harmful chemicals reach the lungs and so smoking is 90% related with lung cancer [39].

Betel quid (BQ) chewing is an ancient ethnic practice in Southeast Asian countries. Betel quid is chewed owing to its medicinal properties [37] and as a symbol of social life. Betel Quid in Pakistan and India is called 'Pan'. The components of pan are betel leaf (Piper betel), areca nut (Areca catechu) also called betel nut and slaked lime (calcium hydroxide). Further use of tobacco and other spices are dependent on the individual's choice. The major components of the areca nut are fats, carbohydrates, fiber, proteins, polyphenols (flavonolsandtannins), minerals and alkaloids [40]. Nitrosamines are generated by nitrosation of the alkaloids in stored dried nuts and when in the mouth, especially in the acid environment, in the presence of nitric oxide generated by bacteria [41]. BQ chewing forms reactive oxygen species (ROS) that is harmful to oral mucosa under an alkaline environment during the auto oxidation of areca nut (AN) polyphenols. Slaked lime is mainly calcium hydroxide. In coastal areas of Sri Lanka, slaked lime is produced by heating sea shells and in the islands of the Pacific it is discovered from limestone. When it is added in pan it creates erosion of oral mucosa and allow penetration of betel quid carcinogens through mucous membrane [42].

In our study subjects who consumed 5- 15 pans per day were significantly associated with poorly differentiated carcinoma (40; 40%). In contrast, Znoar et al., in India reported majority of subjects with well differentiated carcinoma (48.3%) [43]. This is in accordance with a study conducted in Karachi, in which 86.7% of patients had history of pan consumption, and well-differentiated type was prevalent histological pattern [29]. OSCC patients with pan habits showed a greater tendency for structural changes in the oral epithelium and hence may be at a higher risk of getting converted to malignancy.

CONCLUSION

Outcomes of the present study revealed association between frequency of risk factors (cigarette, gutka and pan) with histopathological pattern. In Pakistan the usage of gutka, pan along with cigarettes is prevalent because they are easily available from roadside shops for a very cheap amount. There is an essential requirement for establishing regarding awareness campaign in all age groups, against the consumption of these addictive products, in order to lower the incidence of OSCC.

REFERENCES

1. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer .Oral Oncology 2009; 45: 309–16.
2. Siegel RL, Miller KD, Jemal A. CA Cancer J Clin 2015; 65(1):5-29.
3. Siddiqui IA, Farooq MU, Siddiqui RA, Rafi SMT. Role

of toluidine blue in early detection of oral cancer. Pak J Med Sci 2006; 22:184-87.
4. Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: implications of therapy. J Dent Res.2008; 87:14-32.
5. Haq ME, Abid H, Hanif MK, Warraich RA, Mahmood HS, Saddique K. Frequency and Pattern of Oral and Maxillo-facial Carcinomas. Annals KEMU 2009; 15:171-75.
6. Patel BP1, Rawal UM, Shah PM, Prajapati JA, Rawal RM, Dave TK, et al. Study of tobacco habits and alterations in enzymatic antioxidant system in oral cancer. Oncology 2005; 68:511-19.
7. Khan MA, Saleem S, Shahid SM, Hameed A, Qureshi NR, Abbasi Z, et al. Pak. Prevalence of oral squamous cell carcinoma (OSCC) in relation to different chewing habits in Karachi, Pakistan. Pak. J. Biochem. Mol. Biol 2012; 45(2): 59-63.
8. Daftary DK, Murti PR, Bhonsle RB, Gupta PC, Mehta FS, Pindborg JJ. Oral precancerous lesions and conditions of tropical interest. In: Oral diseases in the tropics. Oxford Medical Publications 1992:402-28.
9. Omar AE. The outline of prognosis and new advances in diagnosis of oral squamous cell carcinoma (OSCC): Review of the literature. Journal of Oral Oncology 2013; 1-13.
10. Richardson M S, Barnes L, Carison D L, Chan J, Ellis G, Harrison LB, et al. Protocol for the examination of specimens from patients with carcinomas of the lip and oral cavity. CAP 2012:1-27.
11. Zini A, Czerninski R, Cohen SG. Oral cancer over four decades: epidemiology, trends, histology, and survival by anatomical sites. J Oral Pathol Med 2010; 39: 299–305.
12. Bhurgri Y1, Rahim A, Bhutto K, Bhurgri A, Pinjani PK, Usman A, et al. Incidence of carcinoma of the oral cavity in Karachi – district south. J Pak Med Assoc 1998; 48(11):321-5.
13. Zulfiqar A, Nagi AH, Nasim N. A clinicopathological study of orofacial squamous cell carcinoma in local population. Biomedica 2013; 29: 147-50.
14. Johnson NW, Jayasekara P, Amarasinghe AA. Squamous cell carcinoma and precursor lesions of the oral cavity: Epidemiology and aetiology. Periodontol 2000 2011; 57:19-37.
15. Chiang W F, Yen C Y, Liu S Y. Squamous cell carcinoma of the oral cavity in young patients. Chin J Oral Maxillofac Surg 2005; 16: 8-16.
16. Udeabor SE, Rana M, Wegener G, et al. Squamous cell carcinoma of the oral cavity and the oropharynx in patients less than 40 years of age: a 20-year analysis. Head Neck Oncol 2012; 4:1-7.
17. Dhar PK, Rao TM, Nair NS et al. Identification of risk factors for specific subsites within the oral andoropharyngeal region- a study of 647cancer patients. Indian J cancer 2000; 37: 114-22.
18. Dias GS, Almeida AP. A histological and clinical study on oral cancer: descriptive analyses of 365 cases. Med Oral Patol Oral Cir Bucal 2007; 12(7):474-8.
19. Bhurgri Y1, Bhurgri A, Hussainy AS, Usman A, Faridi N, Malik J, et al. Cancer of the oral cavity and pharynx in Karachi - Identification of potential risk factors. Asian Pac J Cancer Prev 2003; 4(2):125-30.
20. Mazahir S, Malik R, Maqsood M, Merchant K A, Malik F, Majeed A, et al. Socio-demographic correlates of betel, areca and smokeless tobacco use as a high risk behavior for head and neck cancers in a squatter settlement of Karachi, Pakistan. Bio Med Central 2006; 1:1-6.
21. Zakai MA, Aziz M, Jafri F. A profile of oral cancer presenting at ASH. An Abbassi Shaheed Hosp Karachi Med Dent Coll 2002; 7: 352-53.
22. Wahid A, Ahmad S, Sajjad M. Pattern of carcinoma of oral cavity reporting at dental department of Ayub medical college. J Ayub Med Coll Abbottabad 2005; 17(1):65-6.
23. Kaminagakura E,Villa LL,Andreoli MA, Sobrinho JS, Vartanian JG, Soares FA.High-risk human papillomavirus in oral squamous cell carcinoma of young patients. Int J Cancer 2012; 130(8):1726-32
24. Falaki F, Dalirsani Z, Pakfetrat A, Falaki A, Saghravanian N, Nosratzehi T, Clinical and histopathological analysis of oral squamous cell carcinoma of young patients in Mashhad, Iran: A retrospective study and review of literatures. Med Oral Patol Oral Cir Bucal 2011; 16 (4):473-7.
25. Sasaki T, Moles DR, Imai Y, Speight PM. Clinico-pathological features of squamous cell carcinoma of the oral cavity in patients <40 years of age. J Oral Pathol Med 2005; 34:129-33.
26. Siriwardena BS, Tilakaratne A, Amaratunga EA, Tilakaratne WM. Demographic, aetiological and survival differences of oral squamous cell carcinoma in the young and the old in Sri Lanka. Oral Oncol 2006; 42:831-6.
27. Andisheh -Tadbir A, Mehrabani D, Heydari ST. Epidemiology of squamous cell carcinoma of the oral cavity in Iran. J Craniofac Surg 2008; 19:1699-702.
28. Iamaroon A, Pattanaporn K, Pongsiriwet S, Wanachantararak S, Prapayasatok S, Jittidecharaks S, et al. Analysis of 587 cases of oral squamous cell carcinoma in northern Thailand with a focus on young people. Int J Oral Maxillofac Surg 2004; 33:84-8.
29. Shafique S, Haider SM, Ali Z. Histological patterns and clinical presentation of oral squamous cell carcinoma. J Pak Dent Assoc 2010; 19(3): 171-76.
30. Jerjes W, Upile T, Radhi H, Petri A, Abiola J, Adams A. The effect of tobacco and alcohol and their reduction /cessation on mortality in oral cancer patients: short communication. Head Neck Oncol 2012; 4:1-5.
3. Bernardes VF, Gleber-Netto FO, Sousa SF, Rocha RM, Aguiar MC. EGFR status in oral squamous cell carcinoma: comparing immunohistochemistry, FISH and CISH detection in a case series study. BMJ Open 2013; 3(1):1-7.
32. MuangeP, Chindia M, Njiru W, Dimba E, MutaveR. Oral squamous cell carcinoma: A 6-MonthClinico-Histopathologic Audit in a Kenyan Population. Open Journal of stomatology 2014;

4:475-83.
33- Wang X, Xu J, Wang L, Liu C, Wang H. The role of cigarette smoking and alcohol consumption in the differentiation of oral squamous cell carcinoma for the males in China. J Cancer Res Ther 2015; 11(1):141-5.
34- Akram S, MirzaT, Mirza MA, Qureshi M. Emerging patterns in clinico-pathological spectrum of Oral Canc. Pak J Med Sci 2013; 29(3): 783–87.
35- Memon IM, Iqbal SM, Hussain SI, Baig MN. Pattern of oral malignancies at tertiary care hospitals. Pak J Surg 2014; 30(3):268-71.
36- Bhat SP, Naik RCN, Swetadri GK, Souza HD, Jayaprakash C S, Bhat V. Clinicopathological spectrum of malignancies of oral cavity and oropharynx-our experience in a referral hospital. World articles in Ear, Nose and Throat 2010; 3 (2):1-5.
37-Raghavan V, Baruah HK. Areca nut: India's popular masticatory history, chemistry and utilization. Econ botany 1958; 12:315-45.
38- Willis DN, Popovech MA, Gany F, Hoffman C, Jason L. Blum J L, et al. Toxicity of Gutkha, a Smokeless Tobacco Product Gone Global: Is There More to the Toxicity than Nicotine. Int J Environ Res Public Health 2014; 11(1): 919–33.
39- White C. Research on smoking and lung cancer: a landmark in the history of chronic diseases. Yale Journal of Biology and Medicine 1990; 63(1):29–46.
40- Mack T. The new pan-Asian pan problem. Lancet 2001; 357:1638-9.
41- Rajani A, Boucher BJ, Gajalakshmi V, Gupta PC, Nair J, Chen TH, et al . Betel-quid and areca-nut chewing and some areca-nut related nitrosamines. IARC Monogr Eval Carcinog Risks Hum. 2004; 85:1-334.
42- Merchant A, Husain SS, Hosain M, et al. Pan without tobacco: an independent risk factor for oral cancer. Int J Canccr 2000; 86:128-31.
43- Znaor A, Brennan P, Gajalakshmi V, Mathew A, Shanta V, Varghese C. Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. Int J Cancer 2003; 10:681-86.