British Microbiology Research Journal
11(1): 1-11, 2016, Article no.BMRJ.21921
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Oral Candidiasis: Complication of Concomitant Chemo-radiotherapy in Patients with Oral Squamous Cell Carcinoma

Sadia Minhas[1*], Muhammad Kashif[1], Wasif Altaf[2] and A. H. Nagi[1]

[1]Department of Morbid Anatomy and Histopathology, University of Health Sciences, Lahore, Pakistan.
[2]Fauji Foundation Hospital Lahore, Pakistan.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/21921
Editor(s):
(1) Kai Zhang, Department of Oral Biology, The State University of New York, Buffalo, USA.
Reviewers:
(1) Anirudh Bhattacharya, Critical Care and Trauma Hospital, India.
(2) Chen Yuk-Kwan, Kaohsiung Medical University, Taiwan.
Complete Peer review History: http://sciencedomain.org/review-history/11830

Original Research Article

Received 9th September 2015
Accepted 30th September 2015
Published 16th October 2015

ABSTRACT

Objectives: To investigate oral candidiasis clinically and cytologically in patients with oral squamous cell carcinoma cancer before and at the end of concomitant chemo-radiation therapy and to explore its association with clinical oral symptoms and days of Chemo-radiation. Secondary objective was to describe the oral *candidiasis* incidence in Pakistani patients with oral squamous cell carcinoma undergoing chemo-radiotherapy.
Subjects and Methods: 85 patients who received concomitant chemo-radiotherapy (CCRT) for the treatment of oral squamous cell carcinoma (OSCC) first time were included in this study. Patients were examined for signs and symptoms of oral *candidiasis*, mucositis and xerostomia before and at the end of CCRT. Oral scrapings were obtained from contralateral normal buccal mucosa and peri-tumoural area on the selected days i.e. before and at the end of CCRT. Cytological examination was carried out using H&E stain and GMS stain. Mucositis, oral hygiene and xerostomia were assessed by self-reporting questionnaires and also subjectively. Associations among oral candidiasis and these symptoms were evaluated. Age, gender and addictive habits of patients were recorded in a specially designed proforma.

**Corresponding author: E-mail: sadiawasif81@gmail.com;*

Results: The incidence of oral *candidiasis*, mucositis and xerostomia were significantly higher at the end of CCRT in these patients. Cytological examination reveals an increase in incidence of fungal colonization at the end of therapy smears on both sites (contralateral normal buccal mucosa and peri tumoural area). Significant association was observed at the end of CCRT between *candidiasis* and radiotherapy dosage (p = 0.000), oral hygiene and *candidiasis* (p = 0.012) (p =0.001), addictive habits of patients and candidiasis (p = 0.012), xerostomia and *candidiasis* (p = 0.000). Similarly significant association was observed among days of CCRT and fungal colonization on both sites of smears i.e. contralateral normal buccal mucosa and peri- tumoural area (p = 0.000).

Conclusion: Oral squamous cell carcinoma patients who have received concomitant chmeo-radiotherapy had a high incidence of fungal colonization in the oral cavity. As a result, prophylaxis to decrease *candidal* infection in these patients is requisite. This might improve the quality of life of patients who have be given chemo- radiation treatment for malignant oral cancers.

Keywords: Oral cancer; OSCC; CCRT; Candidiasis; mucositis; xerostomia; oral hygiene.

1. INTRODUCTION

Oral cancer comprised of a group of neoplasm's affecting every area of the oral cavity, salivary glands and pharyngeal regions. Though, this word is likely to be used conversely with oral squamous cell carcinoma (OSCC), which characterizes the most common of all oral neoplasms. It is expected that more than 90% of all oral malignancies are OSCC [1].

OSCC is mainly a disease of elderly people, but in recent years there has been a raise in the occurrence of the disease in people under the age of 45 years [2,3] and yet under the age of 40 years [4]. OSCC more commonly has an effect on men than women most likely because more men than women indulge in high-risk habits [2].

In Asia, chewing-tobacco promote a high incidence of oral cancers [5] whereas, the most important risk factor for oral cancer in the western world is the use of alcohol and tobacco [6,7]. Epidemiological studies have revealed that more than half the people with oral squamous cell carcinoma are smokers [8]. It is essential to consider further identified risk factors, such as betel quid chewing. Betel quid chewing is well-liked in Taiwanese and Indian populations and is related with a considerably increased risk of oral cancer [9].

Typical treatment for locally advanced head and neck cancer comprises of a combination of radiation and surgery. Though, survival of OSCC patient population has not better during the past 20 years. Numerous multimodal treatment plans have been planned and chemotherapy is often used with the aim of organ preservation [10]. A concurrent chemoradiotherapy regimen represents the best current standard therapy operation for many patients with regionally advanced solid tumors, and improves the probability of cure. The general clinical goal of administering chemotherapy and radiation simultaneously is to improve both loco regional and systemic tumor control [11].

The purpose of concomitant chemo-radiotherapy is to destroy malignant cells; however it can also cause damage to normal cells, which can last after treatment [12,13]. In addition to mucositis, exposure of the head and neck region to ionizing radiation has an effect on the natural immunological barriers. This can lead to local infections in head and region as well as systemically and even may cause morbidity and mortality [14,15].

Oral *candidiasis* is an opportunistic infection of the oral cavity. It is frequent and diagnosed between the elderly, predominantly in those who wear dentures and in various cases is preventable with a good oral care routine. It can also be a sign of systemic disease, for example diabetes mellitus and it is a frequent problem between the immuno-compromised patients. An overgrowth or infection of the oral cavity by yeast-like fungus is a causative for oral *candidiasis* [16,17]. Oral *candidiasis* is the most frequent fungal infection of human particularly in early and later life [18].

Candidal infections in immuno-compromised patients are rapidly progressive, frequently severe and hard to treat and such patients have an ultimate possibility of developing oral *candidiasis* whereas, yet the constituents of the normal oral flora may develop into pathogenic conditions. *Candida albican* is the most frequent isolate from the oral cavity [19].

Overgrowth of *Candida* though can cause an altered taste sensation, local discomfort and dysphagia from esophageal overgrowth resulting in slow recovery and poor nutrition. In immuno-compromised patients, infection can extend through the upper gastrointestinal tract or bloodstream creating a severe infection with a remarkable mortality and morbidity [20].

The occurrence of oral candidiasis among AIDS patients is expected to be between 9% and 31%, and studies have documented clinical confirmation of oral candidiasis in nearly 20% of cancer patients [21].

Colonization of the yeast on damaged tissue (mucous membrane) can intensify the symptomatic effects of radiation on the mucosa. The practitioner should be aware of the multiple presentation of *Candida* including pseudo-membranous, chronic hyperplastic and chronic cheilitis [22].

Considering the above mentioned research findings of many researchers about OSCC, CCRT and their association with oral candidiasis, we had planned this study to evaluate the fungal colonization in oral scrapings obtained from the oral mucosa and peri-tumoural area of the patients of OSCC receiving CCRT on H&E and Giemsa stains.

2. SUBJECTS AND METHODS

Patients who were clinically and histologically proven cases of OSCC with first time treatment on concomitant chemo-radiotherapy were included in this study. Their personal information and demographic details, including their age gender, and habits were noted on a structured proforma. The patients of both gender and all age groups (above 18 years) were included in this study. Patients, included in this study, with OSCC underwent CCRT with 33 fractions of radiation 5 days/week for 7 weeks with or without hyper-fractionation with cisplatin only or combination (5-Fu and cisplatin) chemotherapy drugs for every week as advised by radiotherapist and chemotherapist.

Clinical details of every patient (mucositis, xerostomia and candidiasis) were measured subjectively during treatment and recorded on clinical proforma. Mucositis was graded according to WHO criteria (Table 1).

Xerostomia was calculated on observer-based toxicity scoring which is usually based on the Radiation Therapy Oncology Group (RTOG)/ European Organization for Research and Treatment of Cancer (EORTC) grading scale. Though, as xerostomia is defined as a symptom, it is evenly essential to estimate the subjective rating of oral dryness by the patient. Xerostomia questionnaire was developed to permit patient self-reporting. It has been suggested that this questionnaire is more accurate in estimating the severity of xerostomia compared with the RTOG/EORTC grading system (Table 2) [23].

Table 1. WHO criteria for oral mucositis

Mucositis grade	Oral toxicity scale (WHO)
Grade 0	**No alterations**
Grade I	Pain and erythema
Grade II	Erythema and ulcers
Grade III	Ulcers (liquid diet only)
Grade IV	Unable to feed

Table 2. Xerostomia questionnaire

	The xerostomia questionnaire (XQ)
1	Rate your difficulty in talking due to dryness
2	Rate your difficulty in chewing due to dryness
3	Rate your difficulty in swallowing solid food due to dryness
4	Rate the frequency of your sleeping problems due to dryness
5	Rate your mouth or throat dryness when eating food
6	Rate your mouth or throat dryness while not eating
7	Rate the frequency of sipping liquids to aid swallowing food
8	Rate the frequency of sipping liquids for oral comfort when not eating

Xerostomia grade was determined before and end of CCRT at the radiotherapy department by 2 researchers at each visit. Each one of the observers allocates the score separately. The scores ranged from 0 to 2.

0. None
1. Mild mouth dryness/ slightly thickened saliva altered taste such as metallic taste/ these changes not reflected in alteration in baseline feeding behaviour, such as increased use of liquids with meals.
2. Moderate to complete dryness/thick, sticky saliva/ markedly altered taste.

Table 3. The scoring of OH on plaque and calculus debris

Score	Criteria for classifying debris
0	No debris or stain present
1	Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of surface area covered
2	Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface.
3	Soft debris covering more than two thirds of the exposed tooth surface
	Criteria for classifying calculus
0	No calculus present
1	Supra-gingival calculus covering not more than third of the exposed tooth surface
2	Supra-gingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of sub-gingival calculus around the cervical portion of the tooth or both
3	Supra-gingival calculus covering more than two third of the exposed tooth surface or continues heavy band of sub-gingival calculus around the cervical portion of the tooth or both.

The oral hygiene was assessed using the simplified Oral hygiene Index (OHI-S). The scoring was based on plaque and calculus deposits and describe in the given tables (Table 3). Scores for calculus and debris were recorded and the index values are calculated and were graded as good, moderate and poor OH.

The scrapings were taken from contralateral normal buccal mucosa and peri- tumoural area before therapy and at the end of therapy (CCRT). The wooden spatula was scraped firmly against contralateral normal buccal mucosa and peri-tumoural area; the smears were then transferred onto the frosted glass slide and were fixed in absolute alcohol. These smears were stained with H&E and GMS stains. Cytological details of the smears were recorded. Data was analyzed by using SPSS version 20.0. Frequencies and percentages were computed for presentation of all categorical variables including age, gender and personal habits. The study was approved and certified by Institutional Ethical Review Committee and Advanced Studies & Research Board of the University of Health Sciences Lahore, Pakistan.

3. RESULTS

This study comprised of 85 patients of histologically confirmed OSCC, 41.2% of patients were from age group between 46-55 years. Males were predominantly presented with OSCC in approximately 64.7 % and 35.3% females with a male to female ratio of 1.8:1.

Smoking was the most common habit reported in n=31 (36.5%) cases, followed by smoking plus paan in n=13 (15.3%), paan in n=11 (12.9%) and naswar in n=2 (2.3%) cases. Another interesting observation was that n=28 (33%) patients had no such history of addictive habits.

Oral hygiene was assessed both in before therapy cases and at the end of CCRT. It was divided into good, moderate and poor and patients mostly presented with poor oral hygiene (48.2%) followed by moderate (40.2%) and good (11.8%) accordingly at the end of treatment. Whereas in before CCRT cases good oral hygiene was predominantly observed in n = 49 (57.6%) of cases (Table 4).

Considering the fractions of radiotherapy dosages, patients received 70Gy, 90Gy and 119Gy. Most of the patients (52.9%) received 90Gy dose of radiotherapy (Table 4). While considering the chemotherapy most of the patients n=69 (81.2%) received combination drug therapy (Cisplatin and 5-Flurouracil) whereas in n=16 (18.8%) patients received cisplatin only (Table 4).

As far as the side effects of combined chemotherapy and radiotherapy treatment, among 85 patients, all patients complained of mucositis of varying degree during and at the end of CCRT, however, no signs of mucositis were observed in before therapy. All the patients also complained of different degrees of radiotherapy induced xerostomia, mild (10.6%), moderate (42.4%) and severe (47.1%), though no signs of xerostomia were observed in before treatment.

Table 4. Frequency of clinical signs and chemo-radiotherapy dosages

Oral hygiene (end of CCRT)	Oral hygiene (before CCRT)	Candidiasis (before CCRT)	Candidiasis (end CCRT)	Radiotherapy dosage	Chemotherapy drug
Good (11.8%)	Good (57.6%)	Yes (23.5%)	Yes (75.3%)	70Gy (30.6%)	Combination (81.2%)
Moderate (40.0%)	Moderate (35.3%)	No (76.5%)	No (24.7%)	90Gy (52.9%)	Cisplatin (18.8%)
Poor (48.2%)	Poor (7.1%)			110Gy (16.5%)	

Clinical signs of candidiasis were seen at the end of CCRT in n = 64 (75.3%) and were absent in n = 21 (24.7%) cases, though at the start of CCRT clinical signs of candidiasis were observed in only n = 20 (23.5%) of cases and absent in the remaining of cases (Table 4).

By applying Fisher Exact test upon clinical and microscopic findings before CCRT, significant associations were observed among oral hygiene and clinical signs of candidiasis (p = 0.012), radiotherapy dosage and candidiasis (p = 0.000), addictive habits of patients and candidiasis (p = 0.012). Similarly, by applying Fisher Exact test upon the findings at the end of CCRT, significant associations were observed among oral hygiene and candidiasis (p = 0.001) and xerostomia and candidiasis (Fig. 1) (p = 0.000). However insignificant associations were observed between Chemotherapy drugs and candidiasis (p = 0.336) and candidiasis (before therapy) and gender (p = 0.301) by applying Fisher Exact test.

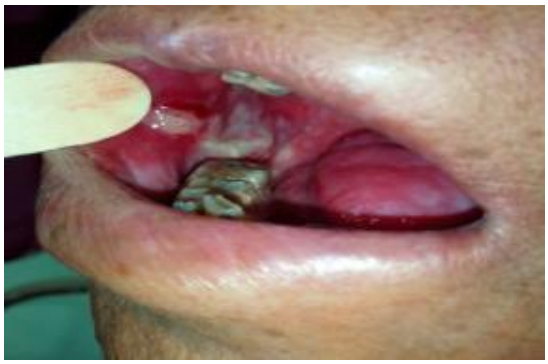

Fig. 1. OSCC patient with poor oral hygiene, xerostomia, mucositis and clinical evidence of oral candidiasis on contralateral buccal mucosa (red and white patches)

Overall, a total of 170 smears from normal buccal mucosa on respective days of CCRT from n= 85 patients, fungus was evident in n= 50 (29.4%) and it was absent in the remaining smears (Fig. 1). Among 85 smears from normal buccal mucosa before CCRT fungus was seen in only n= 4 (4.7%) smears and absent in n= 81 (95.3%) smears. Total of 85 smears from normal buccal mucosa at the end of treatment fungus was evident in n= 30 (35.3%) smears and absent in n=55 (64.7%) smears (Figs. 2, 3).

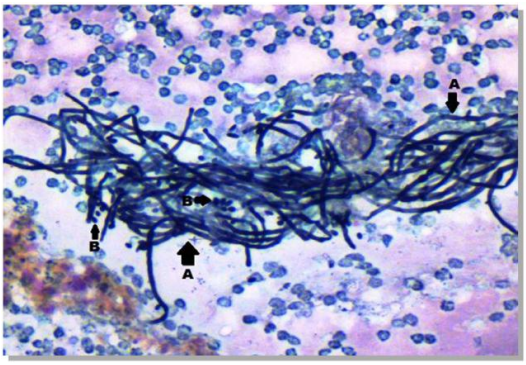

Fig. 2. Photomicrograph from contralateral normal buccal mucosa at end of CCRT from OSCC patient showing hyphae of fungus (A arrow) and spore (B arrow) (GMS x 200)

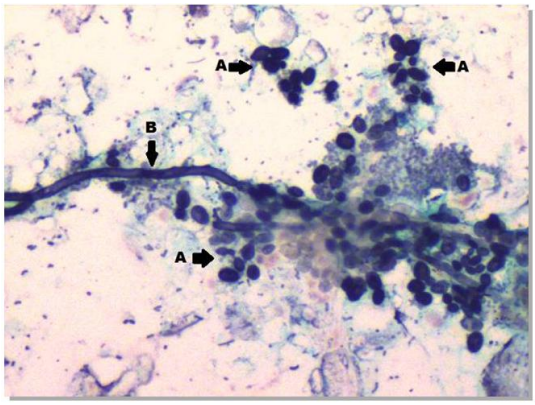

Fig. 3. Photomicrograph showing fungus invaded squamous epithelial cell in smear from contralateral normal buccal mucosa at the end of treatment from patients receiving CCRT as OSCC treatment spores (A arrow) and hyphae (B arrow) (H&E x 400)

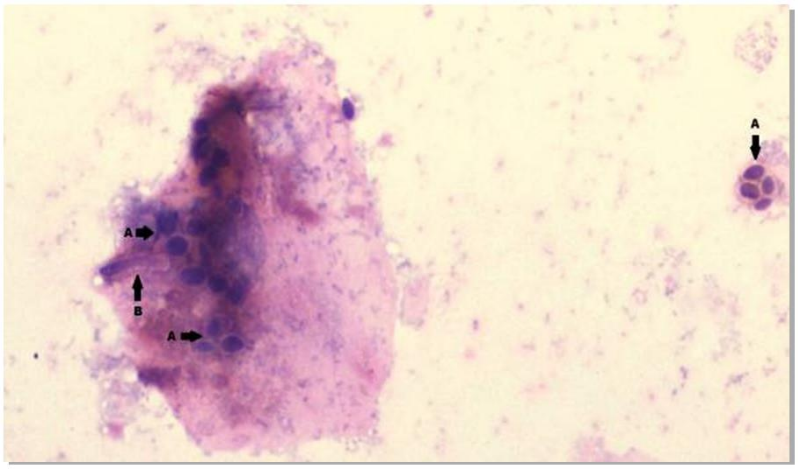

Fig. 4. Photomicrograph showing fungus invaded squamous epithelial cell in smear from peri-tumoural area at the end of treatment from patients receiving CCRT as OSCC treatment spores (A arrow) and hyphae (B arrow) (H&E x 400)

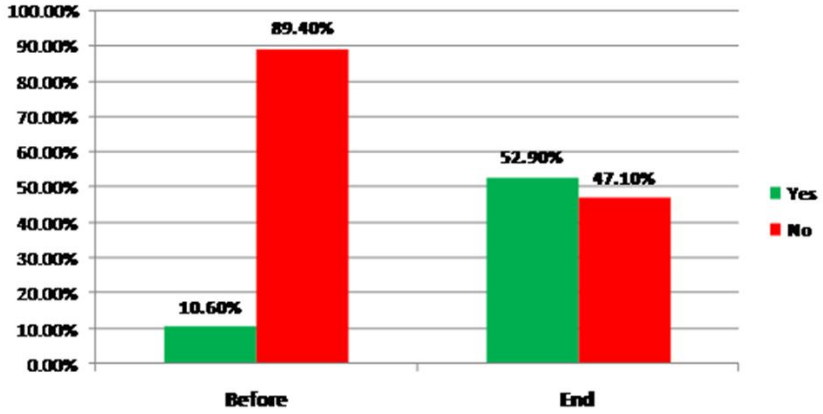

Fig. 5. Frequency of fungus on peri-tumoural area at different days of smears

Similarly, a total of 170 smears from peri-tumoural area on all particular days of CCRT from n= 85 patients, fungus was present in n= 60 (35.2%). No such changes were seen in n= 110 (64.7%) smears. Among 85 smears from peri-tumoural area on each day i.e. before and end of CCRT fungus was seen in n = 9 (10.6) and n = 45 (52.9%) smears respectively and absent in the remaining smears (Figs. 4 and 5 above). A significant association observed between evidence of fungus and days of treatment by applying Chi-square test for both normal buccal mucosa (p = 0.000) and peri-tumoural area (p = 0.000).

4. DISCUSSION

The mean age of the patients in present study was 50.50±11.1. This is in accordance with the results of other studies i.e. a study conducted in Karachi, Pakistan reported that the ages of patients differ from 25 to 80 years with mean age being 47.84±12.18 [24]. Another study conducted by Wang et al, also documented that the age range from 24 to 83 years with mean age 58.5 years [25]. However the study conducted in Iran reported that the mean age of OSCC patients was 30.19±4.24 [26].

In present study, male predominance was observed which is in consistent with the other studies conducted in Libya, Mexico and a number of local studies [27-31]. The deviation from gender related findings of present study, a study conducted in Lahore which reported female predominance with a ratio of 1.5: 1 [32]. The higher frequency of OSCC among males in all these studies, is due to addictive habits i.e.

smoking, chewing habits and snuff consumption. While in our society females less commonly indulge in tobacco smoking.

In current study the patients were presented predominantly with smoking habit. The habits varies according to regions, for example in Punjab, the most common habit for the OSCC is smoking whereas in Karachi gutka and in NWFP the naswar is mostly used by people, hence the most common cause of oral cavity tumour. In western countries the most common aetiological factors for the development of OSCC are smoking and alcohol. The international studies conducted in Taiwan and Sweden is in compliance with the present study [33,34]. Whereas some international studies stated that chewing tobacco was the most common cause of OSCC [35,36]. Aetiological factors related findings of present study are not very different from that of other local and international studies. Our study highlight one of the most important factor that the increasing incidence of oral squamous cell cancer in females with no obvious habitual risk factors, an area of increasing concern. Published data in this particular group are inadequate.

In present study all patients received radiotherapy treatment with different radiotherapy dosages with 2 Gy daily for 5 days/week, for 7 weeks and these may also have boost plan according to the response of tumour to the CCRT. The study conducted in Taiwan reported that the 60-66 Gy was administrated for post operative radiotherapy in oral squamous cell carcinoma patients [37]. The study conducted in USA reported that radiation therapy consists of a total dose of 50–70 Gy administered in fractionated doses (2.0 Gy/d × 5 d) over 5–7 weeks for the treatment of head and neck cancers [38]. The radiotherapy dosage depends on the tumour staging and patient health.

The most common drug regimen used in present study was combination drug therapy. The study conducted in Chicago and Israel by Taylor et al. and Zidan et al. stated that the patients with unresectable or recurrent stage III or IV disease or locally advanced head and neck tumours were treated with either concurrent cisplatin or 5-FU based chemoradiotherapy and they demonstrated a high complete response rate and survival possibility [39,40]. Losrado and his associates documented that concurrent chemo-radiation gives good locoregional control for locally advanced head and neck cancers. Chemoradiotherapy appears to have an emerging role in the primary management of head and neck cancers [10].

Mucositis is defined as the inflammation of oral mucosa. The study conducted by Volpato in Poland reported that there was increased incidence of mucositis as the chemotherapy and radiotherapy intensity was increased and in some cases they appear as a "wave-like" pattern throughout irradiation period [41,42]. It is now concluded that the incidence of mucositis with radiotherapy was somewhat lower but when chemotherapy was added with radiotherapy the incidence and severity of mucositis was increased. And as mucositis is constant variable i.e. it was observed in all patients with slightly different intensities. Hence its significance with the fungal colonization is difficult to calculate. But it is a significant factor in enhancing the opportunistic infections in oral cavity.

Xerostomia is a dry mouth resulting from absent or reduced saliva flow. The studies conducted in Netherland and USA reported that patients experienced the moderate to severe degree of xerostomia during radiotherapy and salivary flow was decreased significantly after radio-chemotherapy [43,44].

Clinical signs of candidiasis were seen in 75.3% patients receiving CCRT. A study carried out in China by Xu and his associates reported that Chemotherapy and radiotherapy, especially combined radio and chemotherapy, resulted in more oral infections compared with palliative care and surgery [45]. Similarly the study conducted by Panghal and his associates reported that *C. albicans* was the most important pathogens in radiotherapy and radio-chemotherapy cases [46]. The study carried out by Dahiya et al. and Jham et al. in USA documented that there was increased incidence of oropharyngeal *candidiasis* in patients receiving combined chemo radiotherapy as compared to radiotherapy alone [47,48]. Redding and his colleagues narrated that patients on radiation therapy for head and neck cancer frequently develop oral mucosal Candidasis [49]. The study conducted in Japan reported that the incidence of oral candidiasis during Radiotherapy was significantly higher in the oral cavity irradiated than in the oral cavity non-irradiated group [50]. The study from USA reported that radiotherapy and chemotherapy treatments for head and neck tumours are independently associated with a significantly increased risk for oral fungal

infection [51]. Here we concluded from all these studies and present study that the incidence of oral candidiasis/oral fungal infection in head and neck/ oral cancers are common finding and this is increased in patients receiving both treatments concomitantly (radiotherapy and chemotherapy).

Oral mucositis and xerostomia played an important role in oral cavity infections and the patients become more vulnerable to *Candida* infections and both of these together increases the discomfort to the patients on CCRT. Significant associations were observed among xerostomia and candidiasis (end of CCRT), radiotherapy dosage and candidiasis (end of CCRT) and oral hygiene and candidiasis. These all factors are correlated with each other i.e. initiation of one factor is the predisposing factor or the cause of other factor to initiate. Saliva reveals mechanical cleansing action in healthy persons with normal salivary glands function [52]. As we know that when salivary glands are included in the field of radiation, xerostomia occur which provides basis for progressive increase in oral c*andidal* colonization.

As *candidiasis* is a common opportunistic infection in immuno-compromised patient and radiation to the head and neck region alters the oral mucous membrane and produces xerostomia which in turn predispose them to colonization by *Candida* species [53]. Saliva has multiple potent antimicrobial agents and if salivary secretion is reduced or if salivary levels of the antimicrobial agents are declined, oral microbial overgrowth is permitted and oral mucosal infection such as c*andidiasis* is induced [52]. Similarly, radiotherapy generated xerostomia does have association with oral Candida overgrowth. Deng and his associates stated that oral *candidiasis* concurrent with oral mucositis due to radiotherapy may raise the oropharyngeal discomfort during radiotherapy [50]. But it is noticed that areas of mucositis attributable to radiation might be infected by *candida* secondarily and even if *candidal* infection does not take part in the etiology of oral mucositis, it might add to the discomfort and duration of mucositis. Similarly, irradiation does have an unfavorable outcome that present an additional favorable environment for the development of the fungi [54]. The study conducted by Suryawanshi and her associates stated that radiation absolutely increases incidence of occurrence of oral candidiasis. Radiation causes the weakness (fragility) of oral mucosa with development of specific lesions such as erythematous lesions, dryness of mouth and ulceration. This demonstrates statistically significant role in more vulnerability of oral mucosa to oral candidiasis with increased number of colony forming units [55]. These all studies are in accordance with the present study.

5. CONCLUSION

Oral squamous cell carcinoma is most commonly seen in fifth decade of life with male predominance. CCRT is directly related with fungus colonization in oral cavity of the OSCC patients. The data presented here show that concomitant chemo-radiotherapy might be an important factor for *Candida* species overgrowth. Therefore steps should be taken prophylactically to decrease *candidal* infection and treatment of oral *candidiasis*, since it may lead to severe clinical complications causing treatment interruption and even can lead to reduction in anti-tumor efficacy. This may improve the quality of life of the patients who receive concomitant chemo-radiation treatment for malignant oral cancers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: implications for therapy. J Dent Res. 2008; 87:14–32.
2. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncology. 2009;45(4):309-316.
3. Johnson NW, Jayasekara P, Amarasinghe AA. Squamous cell carcinoma and precursor lesions of the oral cavity: Epidemiology and aetiology. Periodontol. 2000;57:19-37.
4. Schantz SP, Yu GP. Head and neck cancer incidence trends in young Americans, 1973-1997, with a special analysis for tongue cancer. Arch Otolaryngol Head Neck Surg. 2002;128: 268-74.
5. Ranasinghe A, Macgeoch C, Dyer S, Spurr N, Johnson WN. Some oral carcinomas from Sri Lankan betel/tobacco chewers overexpress p53 oncoprotein but lack mutations in exons 5-9. Anticancer Res. 1993;13:2065-8.

6. Chole RH, Patil RN, Basak A, Palandurkar K, Bhowate R. Estimation of serum malondialdehyde in oral cancer and precancer and its association with healthy individuals, gender, alcohol, and tobacco abuse. J Cancer Res Ther 2010;6:487-91.
7. Marichalar-Mendia X, Acha-Sagredo A, Rodriguez-Tojo MJ. Alcohol-dehydrogenase (ADH1B) Arg48His polymorphism in Basque country patients with oral and laryngeal cancer: Preliminary study. Anticancer Res. 2011;31:677-80.
8. Boyle P, Zheng T, Macfarlane GJ, et al. Recent advances in the etiology and epidemiology of oral cancer. Curr Opin Oncol. 1990;2:539-545.
9. Su CC, Yang HF, Huang SJ, Lianle B. Distinctive features of oral cancer in Changhua County: High incidence, buccal mucosa preponderance, and a close relation to betel quid chewing habit. J Formos Med Assoc. 2007;106:225-33.
10. Lasrado S, Moras K, Pinto GJ, Bhat M, Hegde S, Sathian B, Luis NA. Role of concomitant chemoradiation in locally advanced head and neck cancers. Asian Pac J Cancer Prev. 2014;15(10):4147-52.
11. Vokes EE, Kies MS, Haraf JD, et al. Concomitant Chemoradiotherapy as Primary Therapy for Locoregionally Advanced Head and Neck Cancer. J of Clinical Oncology. 2000;18(8):1652-1661.
12. Garfunkel AA. Oral mucositis the search for a solution. N Engl J Med. 2004;351: 2649-51.
13. Wright J, Feld R, Knox J. Chemotherapy-induced oral mucositis: New approaches to prevention and management. Expert Opin Drug Saf. 2005;4(2):193-200.
14. Bufarah CDHB. Fisiopatologia da mucosite oral- abordagem atual e perspectives futuras. Revisão de caso. Rev Soc Bras Cancerol. 2002;18:38-41.
15. Sonis ST. The pathobiology of mucositis. Nat Rev Cancer. 2004;4:277-84.
16. Epstein JB. Antifungal therapy in oropharyngeal mycotic infections. Oral Surg Oral Med Oral Pathol. 1990;69:32–4.
17. Guida RA. Candidiasis of the oropharynx and oesophagus. Ear Nose Throat J. 1988;67:832–40.
18. Abu-Elteen KH, Abu-Alteen RM. The prevalence of *Candida albicans* populations in the mouths of complete denture wearers. New Microbiol. 1998;21: 41–8.
19. Kantheti LPC, Reddy BVR, et al. Isolation, identification, and carriage of candidal species in PHLAs and their correlation with immunological status in cases with and without HAART. J Oral Maxillofac Pathol. 2012;16(1):38–44.
20. Fraser VJ, Jones M, Dunkel J, Storfer S, Medoff G, Dunagan WC. Candidemia in a tertiary care hospital: epidemiology, risk factors, and predictors of mortality. Clin Infect Dis. 1992;15:414–21.
21. Centers for disease control and prevention. Oral Candidiasis Statistics. Fungal diseases. Centers for disease control and prevention; 2014. Available:http://www.cdc.gov/fungal/diseases/candidiasis/thrush/statistics.html [Last Accessed: 25th May 2015].
22. Lucas J, Rombach DD, Joel Goldwein J. Effects of radiotherapy on the oral cavity. 20th Anniversary Oncolink; 2001. Available: <http://www. oncolink. org/treatment/article.cfm?c=157&id=17#ref_5> [Accessed 22 May 2015].
23. Meirovitz A, Schipper M, Jabbari S, et al. Evaluation of xerostomia: Is scoring by the physician adequate? Int J Radiat Oncol Biol Phys. 2005;63:S130.
24. Akram S, Mirza T, Aamir MM, Qureshi M. Emerging patterns in clinico-pathological spectrum of oral cancers. Pak J Med Sci. 2013;29(3): 783-7.
25. Wang B, Zhang S, Yue K, Wang XD. The recurrence and survival of oral squamous cell carcinoma: A report of 275 cases. Chin J Cancer. 2013;32 (11): 614-618.
26. Falaki F, Dalirsani Z, Pakfetrat A, et al. Clinical and histopathological analysis of oral squamous cell carcinoma of young patients in Mashhad, Iran: a retrospective study and review of literature. Med Oral Patol Oral Cir Bucal. 2011;16(4):473-7.
27. Jaber MA, Abu Fanas SH. The pattern of occurrence of oral squamous cell carcinoma in Libya. Ibnosina J Med BS. 2010;2(3):105-110.
28. Hernández-Guerrero JC, Jacinto-Alemán LF, Jiménez-Farfán MD, et al. Prevelance trends of oral squamous cell carcinoma: Mexico City General Hospital experience. Med Oral Patol Oral Cir Bucal. 2013;18(2): e306–e311.
29. Isaac JS, Qureshi NR, Isaac U. Report on oral cancers patients at atomic energy medical center, Jamshoro during the year 2002: A pilot study. J Pak Dent Assoc. 2003;12(3):176-8.

30. Jamal S, Moghal S, Mamoon N, Mushtaq S, Luqman M, Anwar M. The pattern of malignant tumours: Tumour registry data analysis, AFIP, Rawalpindi, Pakistan (1992-2001). J Pak Med Assoc. 2006; 56(8):359-62.
31. Haq MEU, Abid H, Hanif MK, Warraich RA, Mahmood HS, Saddique K. Frequency and Pattern of Oral and Maxillo-facial Carcinomas. ANNALS. 2009;15(4):171-175.
32. Ali M, Bhatti AH, Tariq M, Khan SA, et al. An Epidemiological Study of 202 Cases of Oral Cavity Cancer (OCC) in Pakistani Subjects. Biomedica. 1998;14:27-31.
33. Shiu MN, Chen TH. Impact of betel quid, tobacco and alcohol on three stage disease natural history or leukoplakia and cancer: Implication for prevention of oral cancer. Eur J Cancer Prev. 2004;13(1): 39-45.
34. Rosenquist K. Risk factors in oral and oropharyngeal squamous cell carcinoma: A population –based case – control study in southern Sweden. Swed Dent J Suppl. 2005;179:1-66.
35. Khan MA, Saleem S, Shahid SM, et al. 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.
36. Shenoi R, Devrukhkar V, Chaudhuri, et al. Demographic and clinical profile of oral squamous cell carcinoma patients: A retrospective study. Head and Neck. 2012;49(1):21-26.
37. Fan KH, Lin CY, Kang CJ, et al. Postoperative concomitant chemoradio-therapy improved treatment outcomes of patients with oral cavity cancer with multiple-node metastases but no other major risk factors. PLoS One. 2014;9(2): e86922.
38. Brosky ME. The Role of Saliva in Oral Health: Strategies for Prevention and Management of Xerostomia. J Support Oncol. 2007;5:215–225.
39. Taylor SG, Murthy AK, Vannetzel JM, et al. Randomized comparison of neoadjuvant cisplatin and fluorouracil infusion followed by radiation versus concomitant treatment in advanced head and neck cancer. J Clin Oncol. 1994; 12(2):385-95.
40. Zidan J, Kuten A, Rosenblatt E, Robinson E. Intensive chemotherapy using cisplatin and fluorouracil followed by radiotherapy in advanced head and neck cancer. Oral Oncology. 1997;33(2): 129–135
41. Volpato LE, Silva TC, Oliveira TM, et al. Radiation therapy and chemotherapy-induced oral mucositis. Braz J Otorhinolaryngol. 2007;73(4):562-8
42. Wygoda A, Maciejewski B, Skladowski K, et al. Pattern analysis of acute mucosal reactions in patients with head and neck cancer treated with conventional and accelerated irradiation. Int J Radiat Oncol Biol Phy. 2009;73(2):384-90.
43. Ohrn KE, Wahlin YB, Sjoden PO. Oral status during radiotherapy and chemotherapy: A descriptive study of patient experiences and the occurrence of oral complications. Support Care Cancer. 2001;9(4):247–257.
44. Wijers OB, Levendag PC, Braaksma MM, et al. Patients with head and neck cancer cured by radiation therapy: A survey of the dry mouth syndrome in long-term survivors. Head Neck. 2002;4(8): 737-47.
45. Xu L, Zhang H, Liu J, Chen X. Investigation of the oral infections and manifestations seen in patients with advanced cancer. Pak J Med Sci. 2013; 29(5):1112-5.
46. Panghal M, Kaushal V, Kadayan S, Yadav JP. Incidence and risk factors for infection in oral cancer patients undergoing different treatments protocols. BMC Oral Health. 2012;20(12):22.
47. Dahiya MC, Redding SW, Dahiya RS, et al. Oropharyngeal candidiasis caused by non-albicans yeast in patients receiving external beam radiotherapy for head-and-neck cancer. Int J Radiat Oncol Biol Phys. 2003;57(1):79-83.
48. Jham BC, Reis PM, Miranda EL, et al. Oral health status of 207 head and neck cancer patients before, during and after radiotherapy. Clin Oral Investig. 2008;12(1):19-24.
49. Redding SW, Zellars RC, Kirkpatrick WR, et al. Epidemiology of oropharyngeal Candida colonization and infection in patients receiving radiation for head and neck cancer. J Clin Microbiol. 1999; 37(12):3896-900.
50. Deng Z, Kiyuna A, Hasegawa M, et al. Oral candidiasis in patients receiving radiation therapy for head and neck cancer.

Otolaryngol Head Neck Surg. 2010; 143(2):242-7.
51. Lalla RV, Latortue MC, Hong CH, et al. A systematic review of oral fungal infections in patients receiving cancer therapy. Support Care Cancer. 2010;18(8):985-92.
52. Tanida T, Okamoto T, Okamoto A, et al. Decreased excretion of antimicrobial proteins and peptides in saliva of patients with oral candidiasis. J Oral Pathol Med. 2003;32(10):586-94.
53. Azizi A, Rezaei M. Prevalence of Candida species in the oral cavity of patients undergoing head and neck radiotherapy. J Dent Res Dent Clin Dent Prospects. 2009;3(3):78-81.
54. Chen TY, Webster JH. Oral monilia study on patients with head and neck cancer during radiotherapy. Cancer. 1974;34(2): 246-9.
55. Suryawanshi H, Ganvir SM, Hazarey VK, Wanjare VS. Oropharyngeal candidosis relative frequency in radiotherapy patient for head and neck cancer. Journal of Oral and Maxillofacial Pathology. 2012;16(1): 31-37.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11830