

Effect of Radiotherapy on Oral Microflora of Patients with Head and Neck Malignancies

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Abstract: Globally cancer is a big threat to life of human beings and scientists are researching mechanisms to get rid from this deadly disease. After Human Genome Project now scientists are moving towards the microbiome studies of individuals. The head and neck cancers for which radiotherapy is an effective treatment has resulted in harmful effects of radiation on oral mucosa, dentition, salivary glands, masticatory musculature and most importantly the oral microflora. Basic and clinical data suggest that patients' oral microflora alter after radiotherapy induces diseases like candidiasis, dental or radiation caries, mucositis, hyposalivation and xerostomia. The imbalance of natural oral microflora can play an important role in the pathogenesis of head and neck malignancies after radiotherapy. Therefore this review has been written to elaborate the effects of radiation on oral microflora of patients after radiotherapy, so that timely treatment planning and preventive measures are undertaken to avoid complications.

Key words: cancer, dentition, mucositis, microflora, radiotherapy

1. Introduction

Malignancies of head and neck constitute a large percentage of cancer worldwide. Radiotherapy (RT) alone or associated with surgery or chemotherapy, has produced a significant increase in cure rates for many malignancies of the head and neck region. In comparison with surgical procedures, radiotherapy can be used as a curative, adjuvant, neoadjuvant and palliative type of treatment and is often used in conservative approaches, with protocols that preserve organs and tissues (Marta et al. 2014; de Barros da Cunha et al. 2015). Chemotherapy has also been used as complement of this treatment, because it is considered as less disfiguring and disabling, but radiation therapy is usually the more desirable modality for treatment of head and neck cancers, provided of course that there is a choice of therapy (Kamath et al. 2002). Unfortunately, apart from the target tumor, ionizing radiation also affects the healthy tissue surrounding the target, resulting in serious side effects and an overall decrease in the patients' quality of life (Baskar et al. 2014). Among all malignancies, head and neck malignancy is often associated with compromised immunity which is further compromised with radiotherapy leading to xerostomia, stomatitis, oral mucositis, dental caries, accelerated periodontal disease, fibrosis, photosensitivity, taste loss, oral infection, trismus, radiation dermatitis, soft tissue necrosis, and osteoradionecrosis (Agarwal et al. 2012). Delayed wound healing of soft tissue and bone wounds after radiation therapy can cause major clinical problems even years after radiation therapy (Baskar et al. 2014). The occurrence and severity of these reactions depend on the radiation dose, volume of irradiated tissues, fractionation scheme, type of ionizing radiation, location of the irradiated area, patient's age, systemic conditions, concomitant treatment, oral hygiene, tobacco and alcohol consumption (Al-Nawas and Grötz, 2006; Baskar et al. 2014). In patients subjected to radiotherapy, radiation has

destructive effect on the salivary glands and often leads to hyposalivation (reduced salivary flow) and xerostomia (dry mouth syndrome). This interruption of the salivary flow and associated xerostomia following radiation therapy has previously been linked with shifts in the oral Microbiome. Traditionally it has been thought that oral mucosa, tongue and pharynx harbor characteristic bacterial pathogens causing chronic inflammation and focal infections (Möller et al. 2004; Ray-Chaudhuri et al. 2013). Many of these infections derived from oral biofilms are commonly linked with the highly prevalent dental diseases (Bhatia and Ichhpujani, 1994; Robins-Sadler et al. 2003; Behl et al. 2014). For a long time, particularly periodontal disease has been emphasized in this respect (Brown et al. 1978). It is a well-known clinical fact that the patients with oral cancer often present with poor oral hygiene. In addition to bacterial organisms, oral microorganisms can include fungal, protozoal, and viral species. A variety of organisms in the microenvironment of the oral cavity adhere to the teeth, the gingival sulcus, the tongue, and the buccal mucosa and till now only 22 predominant microflora have been identified (Najjar et al. 2004). Each site has a unique way of allowing the organisms to establish their residency (Keegan, 2007). The normal flora in healthy individuals maintains similar patterns. When a local or systemic disease process or concomitant use of medications alter this overall pattern, atypical organisms begin to predominate and some normal organisms with a benign nature, such as *Candida albicans*, become pathogenic (Najjar et al. 2004). After radiotherapy oral microflora showed higher abundances of *Streptococcus mutans*, *Lactobacillus* spp., *Candida* and *Staphylococcus* spp., whereas the number of *S. sanguis*, *Neisseria* spp. and *Fusobacterium* spp. tends to decrease. These microbial changes might trigger other side effects. The overgrowth of potential harmful species such as *Candida* spp. (mainly *C. albicans*) or cariogenic species

Volume 6 Issue 12, December 2017

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resulted in higher prevalence of candidiasis and caries in patients treated with radiotherapy (De Ryck et al. 2015). Therefore, the present review discusses the researches and recent advancement on oral Microflora after radiotherapy of patients with head and neck malignancies.

2. Radiation therapy and types

Radiation therapy or Radiotherapy (RT) kills cancer cells by damaging their DNA. It can either damage DNA directly or create charged particles i.e. free radicals within the cells that can in turn damage the DNA. Radiation can damage some types of normal tissue more easily than others. For example, the reproductive organs (testicles and ovaries) are more sensitive to radiation than bones (Ray-Chaudhuri et al.

2013). Radiation doses for cancer treatment are measured in a unit called a Gray (Gy), which is a measure of the amount of radiation energy absorbed by 1 kilogram of human tissue. Different doses of radiation are needed to kill different types of cancer cells (Ray-Chaudhuri et al. 2013) (Table 1). If an area of the body has previously been treated with radiation therapy, a patient may not be able to have radiation therapy to that area a second time, depending upon how much radiation was given during the initial treatment. If one area of the body has already received the maximum safe life time dose of radiation, another area might still be treated with radiation therapy, if the distance between the two areas is large enough. The area selected for treatment usually includes the whole tumor plus a small amount of normal tissue surrounding the tumor.

Table 1: Types of Radiation Therapy for treatment of Head and Neck Malignancies

External beam radiation therapy	Internal beam radiation therapy
The radiation is delivered by a machine outside the body. This type of therapy is most often delivered in the form of photon beams i.e. either x-rays or gamma rays. One of the most common types of external-beam radiation therapy is called 3-Dimensional Conformal Radiation Therapy (3D-CRT) .	The radiation is delivered from radioactive material placed in the body near cancer cells, also called as Brachytherapy . Brachytherapy is put in place through a catheter. Once the catheter or applicator is in place, the radiation source is placed inside it. The radiation source may be kept in place for a few minutes, for many days, or for the rest of patient life.
This can be done in one of these ways: 1. Accelerated fractionation , which gives the half of the usual daily dose of radiation twice each day.	There are three types of Brachytherapy: 1. Low-Dose Rate (LDR) implants: In this type of Brachytherapy, the radiation source stays in place for 1 to 7 days. The patient is likely to be in the hospital during this time. Once the treatment is finished, the doctor will remove the radiation source and the catheter or applicator.
2. Hyperfractionation , which is a smaller than usual daily dose of radiation given twice each day.	2. High-Dose Rate (HDR) implants: In this type of Brachytherapy, the radiation source is left in place for just 10 to 20 minutes at a time and then taken out. Patients may have treatment twice a day for 2 to 5 days or once a week for 2 to 5 weeks. The schedule depends on the type of cancer.
3. Hypofractionation , which is a larger than usual daily dose of radiation given once a day for upto 3 weeks	3. Permanent implants: After the radiation source is put in place, the catheter is removed. The implants remain in the patient's body for the rest of his life, but the radiation gets weaker each day. As the time goes on, almost all the radiation will go away.

3. Development and Function of Normal Oral Microflora

The oral cavity of newborn is usually free from microorganisms or colonization begins within a few hours after birth with microorganisms from the mother, nurses and sometimes from the environment (MacFarlane and Samaranayake, 1989). The bacterial colonization started during the first months of life and the oral mucosa surfaces of babies can provide niches for bacterial colonization (Könönen, 2000). There is considerable variability in oral bacteria and both aerobes (McCarthy, 1965) and anaerobes (Brook, 2011) can be detected in the oral cavity after 8 days of birth. The *Streptococcus salivarius* a pioneer colonizer of the human oral cavity (Rotimi and Duerden, 1981; MacFarlane and Samaranayake, 1989), being detected after 8 h of birth (Rotimi and Duerden, 1981). During the first year of life, other species of *Streptococci*, *Staphylococci*, *Neisseriae*, *Vellonelliae*, *Actinomyces*, *Lactobacilli* and *Fusobacteria* also colonise. With the eruption of teeth, solid surfaces in the oral cavity are available for colonization. *Streptococcus mutans*, *Streptococcus sanguis* and *Actinomyces* establish themselves on these hard surfaces (Caufield et al. 2000).

The developing intestinal and oral microbiota plays an important role in the health and well being of the host (Marcotte and Lavoie, 1998). The normal microflora of the oral cavity contains 75 to 100 different microbial species. The normal oral flora occupies available colonization sites which makes it more difficult for other non-indigenous microbial species to become established (Dewhirst et al. 2010). In addition, the oral bacteria exert microbial antagonism against non-indigenous species by production of antimicrobial compounds, which can be classified as low-molecular-mass (LMM) compounds such as hydrogen peroxide (H₂O₂), carbon dioxide (CO₂), diacetyl (2,3-butanedione), uncharacterized compounds, and high-molecular-mass (HMM) compounds like bacteriocins (Aas et al. 2005). Also, the oral flora contributes to host nutrition through the synthesis of vitamins and immunity by inducing low levels of circulating and secretory antibodies that may cross react with pathogens. On the other hand, the normal oral flora can also be the usual cause of various oral diseases in humans - including abscesses (infections), dental caries (cavities), gingivitis (inflammation of the gums) and periodontal disease (gum infection resulting in tooth loss) (Aas et al 2005; Dewhirst et al. 2010). Though the oral cavity is teaming with a variety of microbial species, the more common species which can be both helpful as well as harmful to the host are in the table 2:

Table 2: Human normal oral microflora and its functions

Sr. No.	Oral Microorganisms	Functions
1.	<i>Actinomyces naeslundii</i>	Forms dental plaque by adhering to the surface of the teeth.
2.	<i>Actinobacillus actinomycetemcomitans</i>	Severe infection of the periodontium, although it is also associated with non oral infections
3.	<i>Bacteroides melaninogenicus</i>	causes periodontal disease
4.	<i>Bifidobacterium lactis BB-12</i>	Modified the protein composition of the salivary pellicle and specifically prevent adhesion of other bacteria, such as <i>S. mutans</i> .
5.	<i>Candida albicans</i>	Opportunistic potential pathogen and an agent of oral infections such as thrush.
6.	<i>Capnocytophaga</i>	Part of the oral commensal flora but opportunistic pathogen
7.	<i>Corynebacteria</i> species	Predominate organism on the skin, but also found in the mouth.
8.	<i>Fusobacterium</i>	Normal inhabitants of the oral cavity, but can also cause periodontal disease
9.	<i>Lactobacillus</i> species	Considered as a "friendly bacteria" and part of a large group of lactic-acid producing bacteria. However, the acid formation can contribute to the formation of dental caries.
10.	<i>Porphyromonas gingivalis</i>	Can cause periodontal disease
11.	<i>Prevotella</i> spp.	Predominate in periodontal disease and periodontal abscesses
12.	<i>Staphylococci epidermidis</i>	Predominate organism on the skin, but also found in the mouth
13.	<i>Streptococcus salivarius</i>	Commensal bacterium of the oral cavity and it is the first bacterium which colonizes the dental plaque, before being joined by numerous other species of various genera.
14.	<i>Streptococci mutans</i>	Involved in plaque formation and initiation of dental caries
15.	<i>Treponema denticola</i>	Found in the periodontal pockets of adults suffering from periodontitis
16.	<i>Treponema orale</i> and <i>T. Vincent</i>	Common inhabitants of gingival crevices and subgingival areas
17.	<i>Veillonella</i>	Another bacteria involved in plaque formation

4. Changes in Oral Cavity Microflora after Radiotherapy

Radiotherapy-induced damage is not only in the oral mucosa, salivary glands, bone, dentition, and masticatory musculature, but also has more deleterious effects on the oral microflora (Table 3). Radiotherapy-induced microflora based complications in patients are complex and difficult to handle. Dynamic pathobiological processes lower the quality of life and predispose patients to serious clinical disorders (Stephen et al. 2003). The changes in oral microbial population of the patients leads to severe pain in the oral cavity and these patients are unable to eat solids (Takeshita et al. 2011). Diet and more specifically, the protein and starch content of food influenced the oral microbial abundance of *Lactobacilli* and *Streptococci*. After radiotherapy the tube-fed patients showed disruption of indigenous oral microbiota, allowing other opportunistic pathogens like *Corynebacterium striatum* and *Streptococcus agalacticae* to grow in large numbers and become predominant species in oral cavity (Takeshita et al. 2011). The *Candida albicans* is commensal inhabitant of the oral cavity in a large proportion of individuals. Under normal conditions, these fungal organisms co-exist with other microorganisms of the normal oral flora and do not cause any disease. However, cancer patients receiving radiation therapy are prone to higher risk for oral fungal infection, because of imbalance in the oral flora, hyposalivation (secondary to radiotherapy) and local tissue damage (mucositis secondary to radiotherapy) (Lalla et al. 2014). For all cancer treatments, the weighted prevalence of oral colonization of *Candida* species was 48.2% before treatment, 72.2% during treatment and 70.1% after the treatment. The prevalence of oral fungal colonization during chemotherapy (72.8%) was similar to that during radiation therapy (74.5%). Different studies reported that *Candida albicans* prevalence was highest (46%) during cancer therapy, whereas prevalence rates of *Candida tropicalis* was

16.6%, 5.5% for *Candida glabrata*, and 3% for *Candida krusei* (Lalla et al. 2014). Therefore, the low immunity may allow the establishment of opportunist infections, such as candidiasis, a common fungal infection caused by *Candida albicans*. Gaetti-Jardim et al. 2011 examined saliva, mucosa and biofilm from fifty cancer patients, before radiotherapy, during radiotherapy and 30 days after radiotherapy. Most prevalent microorganisms after radiotherapy based on PCR detection were *Candida albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. parapsilosis* followed by *Citrobacter*, *Enterobacter*, *Enterococcus*, *Klebsiella*, *Proteus*, and *Pseudomonas*. Whereas before radiotherapy, targeted bacteria were cultivated from 22.2% of edentulous patients and 16.6% of dentate patients; 30 days after radiotherapy, these microorganisms were recovered from 77.8% edentulous and 46.8% dentate patients. Modifications in the oral environment due to radiotherapy treatment seems to facilitate the colonization of oral cavity by members of family *Enterobacteriaceae*, *Enterococcus* spp. and *Candida* spp. Xerostomia or reduced salivation is one of the most frequent effects of radiotherapy in the head and neck region (Emidio et al. 2010). Salivary glands are expected to be relatively radio-resistant due to slow turnover rates of their cells. Yet, quantitative as well as qualitative changes in the saliva may be seen shortly after antineoplastic therapy. Since there is an increase in viscosity and proportion of organic material, the color of the saliva may change from transparent to opaque white or opaque yellow. The pH and buffering capacity of saliva decreases, and there is alteration in electrolyte levels. The radiation therapy causes fibrosis, degeneration of salivary acinar cells, and necrosis of salivary glands that lead to these changes (Emidio et al. 2010). Due to low pH, the oral flora shows a shift from Gram-positive to Gram-negative bacteria (Mathur et al. 2012). Further, Sonalika (2012) reported that in addition to radiation therapy tobacco chewing habit and presence of squamous cell carcinoma in oral cavity also causes alterations in healthy oral microflora in patients. Abnormal flora developed due to radiotherapy in oral squamous cell carcinoma patients can

exacerbate mucositis and can cause systemic infections. The frequency of isolation of total aerobes and anaerobes, coliforms and gram negative anaerobic bacteria was significantly high in oral squamous cell carcinoma patients compared to healthy controls, whereas *Candida* sp. was isolated most frequently during radiation period. Similarly, Pushalkar et al. 2012 investigated the association of oral bacteria in oral squamous cell carcinoma tissues and compared it with adjacent non tumour mucosa sampled 5 cm distant from the same patient. By using culture independent 16S rDNA approaches, Denaturing Gradient Gel Electrophoresis, cloning and sequencing, assessed the total bacterial diversity in these clinical samples. Denaturing Gradient Gel Electrophoresis fingerprint showed variations in the band intensity profiles within non-tumour and tumour tissues of the same patient and also among the two groups. The clonal analysis indicated that from a total of 1200 sequences characterised, 80 bacterial species were detected representing 6 phyla-Firmicutes, Bacterioidetes, Proteobacteria, Fusobacteria, Actinobacteria and uncultivated TM7 in non-tumour and tumour libraries. Bacterial species, *Streptococcus* sp. Oral taxon 058, *Peptostreptococcus stomatis*, *Streptococcus gordonii*, *Gamella haemolysins*, *Gamella morbillorum*, *Johnsonella ignava* and *Streptococcus parasanguinis* were highly associated with tumour site, whereas *Granulicatella adiacens* was prevalent at non-tumour site. *Streptococcus intermedius* was present in 70% of both non-tumour and tumour sites. De Frietas et al. (2013) identified and quantified *Candida* on head and neck irradiated patients with two comparative elderly populations. Among the 92 patients surveyed, 51 (55.4%) had scores classified as positive for *Candida*. Gender was not associated with *Candida* portability. *Candida* portability was significantly associated with the presence of candidiasis and xerostomic state. The isolates and definitive confirmed colonies were *Candida albicans*, *C. dubliniensis*, *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. parapsilosis*, *C. guilliermondii*, *C. lusitaniae*, and *C. kefyr*. Among the species identified, *C. albicans* was the most frequent, followed by *C. tropicalis*, *C. parapsilosis* and *C. glabrata*. On multiple statistical models, only radiotherapy treatment was associated with positiveness to *Candida*. On the Contrary, Hu et al. 2013 used a high-throughput sequencing technique (pyrosequencing) to estimate the detailed diversity of plaque microbiota of irradiated patients. *Streptococcus* and other caries-related bacteria such as *Veillonella* and *Actinomyces* fluctuated significantly and accounted for a large proportion of the bacterial communities. These three genera interact with each other in the oral cavity, and they may play an important role in the development of dental caries. The *Granulicatella* was dramatically reduced in dental plaque after radiotherapy. Therefore, authors tempting to speculate that *Granulicatella* is unlikely to contribute to the pathogenesis of post-radiation diseases such as radiation caries. *Derxia*, which has also been found in tropical soils and *Luteococcus*, which were also isolated from human blood and the peritoneum (Collins et al. 2003) fluctuated differently, but little information about its pathogenicity in oral cavity is available so far. Other predominant genera such as *Rothia*, *Prevotella*, *Capnocytophaga* and *Neisseria*, might be involved in the susceptibility of an individual to periodontal disease (Ling et

al. 2010). In contrast Schmidt et al. (2014) reported that in the cancer tissue samples *Firmicutes* (especially *Streptococcus*) and *Actinobacteria* (especially *Rothia*) was significantly decreased relative to contralateral normal tissue samples from the same patient. In another study Devi and Singh (2014), reported that after standard radiotherapy, there was a profound shift in the oral microflora to predominance of acidogenic microbes, primarily *Streptococcus mutans* and *Lactobacilli* coincident with a decrease in salivary flora and an increase in caries risk. Dental caries in irradiated patients may develop rapidly as early as 3 months after radiotherapy. Henceforth, De Ryck et al. (2015) reported the potential role of the oral microbiota in radiotherapy-induced side effects like mucositis and performed band wise cluster analysis after Denaturing Gradient Gel Electrophoresis to gain insights into the microbial shifts that occurred during radiation therapy. Samples of the first weeks of irradiation clustered together and towards the end of the therapy the richness decreased (-14%) and the oral microbial community became dominated by a small fraction of species. These studies reported here suggest that by monitoring the microbial shifts during radiation therapy, the role of the microbiota in disease initiation and development can be elucidated and treatment regimes can be adapted.

Further, Almståhl et al. (2015) investigated post radiotherapy effects after 3 years of irradiation and reported higher numbers and proportions of *Lactobacilli*, *Candida albicans* in the supragingival plaque. On the contrary higher numbers of enterococci in the vestibulum in the molar region and on the tongue, a lower total count and lower numbers of *Streptococci*, *Streptococcus salivarius* and *Fusobacterium nucleatum* on the tongue were observed. Although both stimulated and unstimulated salivary secretion rates increased over time, the proportion of microorganisms associated with oral health decreased, and microorganisms associated with oral disorders increased. Increased *Lactobacilli* and *C. albicans* 2 yrs post radiotherapy may reflect not only a reduced whole saliva flow rate, but also reduced buccal gland saliva and an acidic environment. In another study Ligtenberg and Almståhl (2015) reported that hyposalivation leads to changes in the oral microflora. In combination with a lower defense, this leads to a higher susceptibility to oral infections such as caries and mucosal infections. Gao et al. (2015) studied the variation in oral microflora of the subgingival plaque during and after radiotherapy using 16S rDNA. A total of 120 genera were found; five genera (*Actinomyces*, *Veillonella*, *Prevotella*, *Streptococcus*, *Campylobacter*) were found in all the studied patients before and after radiotherapy. The richness and diversity of oral ecology decreased with increased radiation dose which was gradually restored with time. Zhang et al. (2015) isolated eleven genera of microbes (*Streptococcus*, *Neisseria*, *Scardovia*, *Porphyromonas*, *Fusobacteria*, *Lautropia*, *Veillonella*, *Capnocytophaga*, *Rithia*, *Leptotrichia* and *Prevotella*) and reported *Streptococcus* spp. and *Neisseria* spp. in more than 80% of samples. However, no clear relationship between the characteristics of patient salivary microbiota and radiation caries one year after intensity modulated radiation therapy (IMRT) was established. The study clearly suggested that

salivary function in irradiated patients does not recover fully after 12–36 months, although the pH value and buffering capacity of saliva returns to normal after one year or more following intensity modulated radiation therapy. Further, it was concluded that population-based long term studies are required to reveal the factors for the absence of radiation caries. In a recent study, Schuurhuis et al. (2016) reported that different radiation treatments with intensity modulated radiation treatment to head and neck cancer patients result in different changes in the oral microflora with opportunistic pathogens such as *Staphylococci*, enteric rods and *Candida* sp. increasing in prevalence with or without chemotherapy, but not after surgical intervention. In another study Vanhoecke et al. (2016) reported the effect of irradiation on behavioral characteristics of oral microbial species in the context of mucositis particularly on growth and biofilm formation in different culture conditions. Biofilm formation of *Klebsiella oxytoca* and *Candida glabrata* was affected by irradiation and depended on the culturing conditions. Furthermore, irradiated *Klebsiella oxytoca* microbes were found to be more virulent on *Glabrata melonella* larvae as compared to the non irradiated ones. Authors reported that low-dose irradiation could have an impact on functional characteristics of microbial species.

5. Dental/Radiation caries

It has always been a matter of debate whether radiation caries is due to a direct or indirect effect of irradiation on teeth, or due to oral microflora. The most threatening complication for the dentition, however, is radiation-related caries. Radiation caries is a highly destructive form of dental caries which has a rapid onset and progression (Karmiol and Walsh, 1975; Vissink et al., 2003). Dental caries may become evident as early as three months following the initiation of radiotherapy. In severe cases, a previously healthy dentition can be completely lost within a year (Dreizen et al., 1977). Early studies from the seventies and eighties emphasize on a correlation between the reduction of the amount of saliva, lower pH and growth of acidophilic organisms, which can indirectly lead to radiation caries (Al-Nawas and Grötz 2006). Both high consumption of short chain carbohydrates and reduced oral hygiene are known as promoting factors for radiation caries (Grötz et al. 2001). Similarly, Marcotte and Lavoie (1998) reported caries and periodontal diseases are associated with indigenous bacteria and identified the role of secretory immunoglobulin A (SIgA) in the control of the oral indigenous microbiota. In contrast Epstein et al. (1998) reported that patients, who had not experienced tooth decay for some time, might develop radiation caries when submitted to radiotherapy. The main factor for the development of such injuries is the decrease of saliva amount and its qualitative alterations. Besides, radiation has a direct effect on teeth, making them more susceptible to decalcification (Silverman, 1999). In another study reported (Otmami, 2007) changes in the chemical composition of saliva and increased amounts of cariogenic oral bacteria result in rapid decalcification of dental enamel.

Aggressive and extensive caries, commonly known as radiation caries, tends to spread to all dental surfaces, changing their translucency and colour. Radiation caries is not caused directly by irradiation, but results from the sequelae of xerostomia and a cariogenic shift in microflora. Other than xerostomia and hyposalivation which were among the most common treatment side effects on patients during and following radiotherapy treatment, the carious process causes increased friability and the breakdown of teeth (Tolentino et al. 2011). They are the result of radiation-induced damage to the salivary glands. Patients with chronic hyposalivation are at risk for demineralization and dental cavitation (dental caries), often presenting as a severe form of rapidly developing decay that results in loss of dentition. Usual post-radiation oral care which includes the use of fluoride, may decrease, but does not eliminate dental caries associated with radiation-induced hyposalivation (Deng et al., 2015). In a review article, Kaul et al. 2015 reported that changes in the chemical composition of saliva and increased amounts of cariogenic oral bacteria result in rapid decalcification of dental enamel. On the contrary Zhang et al. (2015) had reported previously that there was no clear relationship between high risk of developing radiation caries and the changes in the oral microbiota within the first year following radiotherapy. The salivary function and the salivary microbiota are correlated to the absence of radiation caries after one year of radiotherapy. Notably, authors did not detect any significant decrease in pH value and buffering capacity of the stimulated saliva when compared with normal values. Zhang et al. (2015) reported the quantity of *Streptococcus mutans* and *Lactobacilli* were same in both the radiation caries free patients and radiation caries patients. The results were in contradiction with previously published reports possibly because all the participants in their studies had not been treated by radiotherapy and had healthy salivary function and their data showed only a weak correlation between oral microbial diversity with the absence of radiation caries. It was concluded that population-based a long term study is required to reveal the factors for the absence of radiation caries. As mentioned earlier, radiation may permanently alter the quality and quantity of salivary flow. Saliva plays an integral role in the prevention of dental caries because with its protective action the cariogenic oral bacteria cannot colonize on the teeth. In the absence of a strict and meticulous preventive hygiene regimen, rampant caries typically results. After radiotherapy within three months carious lesions begin to appear and proceed rapidly to devastate the dentition. The key to managing this problem in patients likely to undergo radiotherapy is prevention. A thorough dental clinical examination including full mouth radiographs, diagnosis, and the treatment should be done before the start of radiotherapy. Additionally, a complete examination of the mucosa, dentition, and periodontium should also be done. After the radiotherapy special care of patients for dental hygiene, daily fluoride application, carbohydrate restriction and frequent dental follow up are essential.

Table 3: Summary of studies on Oral Microbiota modification after radiotherapy of patients with head and neck malignancies

Study	No. of subjects	Techniques used	Material analyzed	Microorganisms reported/ Commentary
Martin et al.	31 patients were studied.	Microbiological	Saliva	<i>Candida albicans</i> and <i>C. tropicalis</i> were the principal yeasts

1981	22 with oral neoplasms and 9 with laryngeal neoplasms	Methods		isolated through out the period along with <i>T glabrata</i> and <i>Rhodotorula rubra</i> . The number and types of fungi remain high in both the groups of patients and persisted at higher levels for 4-6 months after the treatment.
Brown et al. 1978	42 head and neck cancer patients 36 Post radiation therapy patients	Biochemical tests	Saliva	Post irradiation caries was associated with increased plaque <i>Lactobacillus</i> sp, <i>Candida</i> sp, and <i>Streptococcus</i> sp. Conversely, plaque <i>Staphylococcus</i> sp, <i>Streptococcus salivarius</i> , and <i>Veillonella</i> sp were significantly higher in the caries-inactive than in the caries-active group.
Kuten et al. 1986	36 patients	Microbiological analysis	Saliva	Decrease in salivary secretion is accompanied by rise in salivary sodium concentration and in oral yeast flora.
Keene and Fleming, 1987	68 adult xerostomic patients with head and neck cancer	Fluoride gels analysis	Saliva	The levels of <i>Lactobacilli</i> and <i>Streptococcus mutans</i> were significantly elevated in all post radiotherapy patients.
Epstein et al. 1998	22 cancer patients with head and neck radiation	Microbiological Analysis	Saliva	No significant changes in cariogenic oral flora were seen during and early after radiation therapy, despite xerostomia. However, colonization by <i>Candida albicans</i> increased during radiation therapy.
Kamath et al. 2002	35 patients with oropharyngeal cancer and fifteen controls were included.	Microbiological analysis	Swabs from the tonsillar fossae.	<i>Streptococcus pneumoniae</i> was significantly decreased at end of irradiation, while there was an increase in <i>Staphylococcus aureus</i> , <i>Pseudomonas</i> , <i>Bacteroides</i> and <i>Candida</i> species. There was no change in culture and sensitivity pattern prior to and after radiotherapy.
Tong et al. 2003	12 Nasopharyngeal cancer patients with equal number of controls	Biochemical tests along with 16S rDNA sequencing homology analysis	Saliva	<i>S. mitis</i> and <i>S. salivarius</i> were the predominant non-mutans <i>Streptococci</i> in the high-caries-risk oral flora following radiotherapy.
Stokman et al. 2003	65 patients with malignant tumour in head and neck region out of which 58 were evaluable	Microbiological methods	Saliva	The presence of <i>Candida</i> species and aerobic Gram-negative bacilli has no effect on the development and severity of radiation-induced mucositis.
Sharma 2005	45 patients with OSCC and 229 healthy controls	Microbiological methods	Saliva	<i>P. melaninogenica</i> , <i>Leptotrichia buccalis</i> , <i>Capnocytophaga ochracea</i> , <i>C. gingivalis</i> , <i>Eubacterium saburreum</i> , and <i>S. mitis</i> were significantly higher in radiotherapy patients than in controls.
Eliassonn et al. 2006	10 patients after 3-5 years of RT with 10 control participants	Microbiological analysis with pH measurement using microelectrodes	Saliva	Compared with their controls, the irradiated group displayed increased numbers of lactobacilli and <i>Candida</i> species and reduced buffering capacity of saliva.
Srithavaj and Thaweboon 2006	19 children with radiotherapy for retinoblastoma and control group of 20 healthy children from a primary school in Bangkok, Thailand	Microbiological Assay	Saliva	The irradiated children showed abundance of <i>Lactobacilli</i> and <i>candida</i> sp. as compared to healthy children.
Al Nawas and Grotz 2006	22 patients with squamous cell carcinoma of head and neck	Molecular biology using DNA probes	Saliva	The <i>Lactobacilli</i> counts varied from mean value of 575 cfu/ml at first month to 910 cfu/ml at 12 months whereas <i>Streptococci</i> counts varied from 680 cfu/ml to 1000 cfu/ml at 12 months with borderline statistical significance.
Gaetti-Jardim et al. 2011	50 patients receiving Radiotherapy	Microbiological growth on specific media along with PCR assays using specific primer pairs	Saliva, mucosa and Biofilm samples	The most prevalent yeasts in patients submitted to radiotherapy were <i>Candida albicans</i> , <i>C. tropicalis</i> , <i>C. krusei</i> , <i>C. glabrata</i> and <i>C. parapsilosis</i> and bacteria were <i>Citrobacter</i> , <i>Enterobacter</i> , <i>Enterococcus</i> , <i>Klebsiella</i> , <i>Proteus</i> , and <i>Pseudomonas</i> .

6. Conclusion

Oral microbial flora beyond doubt, have a very important role to play in patients suffering with head and neck malignancies. A thorough understanding of the normal and altered microflora due to radiotherapy and the mechanism

behind such population is of great significance it ameliorate the agony of patients with head and neck malignancies. The investigations of microbiota gene functions are required to better understand the impact of microbiota transformation during treatment of cancers. Continued surveillance of the oral cavity and early management of complications arising at later stages are of utmost importance in the long term care of

the patients receiving radiation treatment. A combination of enhancing radiotherapy safeguards, oral health instructions (clinicians should be aware of important microbial shifts and monitor the presence of pathogens to avoid more undesirable side effects), effective oral care and timely dental intervention may be essential to decrease oral sequelae and preventing microflora induced complications and thus increasing patients' quality of life.

7. Conflicts of interest

There are no conflicts of interest.

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