DETERMINATION OF ORAL MICROFLORA IN IRRADIATED OCULAR DEFORMED CHILDREN

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Abstract. Children with a retinoblastoma usually have enucleation of eye contents and undergo radiation therapy. In this group of children a slight deformation in facial growth is exhibited on the operated side. The radiation affects the oral microflora. This study was conducted to investigate the oral microflora in this group of patients compared to normal children. Nineteen children, ages 4-15 years, were tested for oral microflora by culture. A control group was comprised of 20 normal healthy children from a primary school in Bangkok, Thailand. Paraffin-stimulated whole saliva was collected and cultured for the presence of mutans streptococci, lactobacilli and *Candida* species. The isolation frequencies and abundances of lactobacilli and *Candida* species in saliva samples of irradiated children were significantly greater than healthy children. The mutans streptococci counts were significantly different from healthy controls. Radiation therapy may have had some influence on the oral ecology of these patients, so had a higher risk of caries and candidiasis than the healthy controls. Therefore, comprehensive preventive measures should be taken in these children.

INTRODUCTION

Children with retinoblastoma usually have the eye contents enucleated and undergo radiation therapy. In this group of children a slight deformation in facial growth is exhibited on the operated side. Radiation of the head and neck region has adverse effects on oral defensive mechanisms, often with severe consequences to oral health (Madeya, 1996). Radiation therapy has an indirect effect in decreasing salivary flow rates, changing microflora types, increasing the level of pathogenic oral microorganisms and inducing the formation of oral mucosal reactions (Carl, 1995). Consequently, patients are frequently affected by rapidly destructive dental caries (Lacatusu *et al*, 1996).

Oral streptococci are common microflora and localizing in the oral cavity and upper respiratory tract of humans. Mutans streptococci (MS) have long been considered the main cariogenic bacteria. Epidemiological studies have demonstrated that MS are present in substantial numbers in most active carious lesions (Kristoffersson *et al*, 1985). The other microorganism associated with dental caries is lactobacilli. Lactobacilli have been found to participate in the progression of dental lesions. The proportions and the prevalence of these bacteria are increased in advanced carious lesions (Nyvad, 1993). Patients who receive radiation therapy have been reported to have increased salivary MS and lactobacilli and to be at high risk for rampant caries (Brown *et al*, 1975).

Radiation therapy of the head and neck region has also been reported to increase the proportion of *Candida* in the oral cavity (Weerkamp *et al*, 1987). Increases in colonization and the quantity of oral *Candida* spe-

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cies also increases the risk of clinical infection (Silverman, 1990; Epstein *et al*,1993). However, the effect of radiation therapy on oral flora is mainly based on the results obtained from adult patients. Little is known about the effects in children.

The purpose of this study was to investigate the existing oral microflora in a group of irradiated children compared to normal children.

MATERIALS AND METHODS

The investigation was performed at the Maxillofacial Prosthetic Service, Mahidol University, Thailand. It was divided into two major groups: Group 1) children with irradiated ocular deformities and 2) normal children as controls. The sample was comprised of 19 children, 8 females and 11 males, with a history of retinoblastoma with a past medical history of ocular enucleation of the globe. In the irradiated group, the total amount of radiation of the upper face ranged between 4,000-5,000 cGy, with ages ranging from 4-15 years. The control group was comprised of 20 normal healthy children from a primary school in Bangkok. The aims and procedures were explained to the parents and informed written consent was obtained prior to the investigation. No eating or drinking was allowed starting 2 hours before the appointment for specimen collection. Paraffin-stimulated saliva samples were collected and transferred immediately to the laboratory where the microbiological assay was performed. The levels of MS, lactobacilli and *Candida* species were determined by culturing on Mitis-Salivarius bacitracin agar (Difco), Rogosa SL agar (Difco) and Sabouraud dextrose agar (Difco). Mitis-Salivarius bacitracin agar and Rogosa SL agar were incubated in an atmosphere of 5% CO_2 at 37ºC for 3 days. Plates with Sabouraud dextrose agar were incubated in air at 37ºC for 3 days. The identification of MS, lactobacilli and *Candida* species was based on colony morphology and Gram staining and the amount was expressed as CFU/ml. Before statistical analysis, the numbers of these microorganisms was logarithmically transformed.

Statistical analysis

The isolation frequencies of individual species were compared using the Fisher's exact test. The abundance of species in the saliva samples of irradiated children was compared with healthy controls using a *t*-test. The level of significance was set at $p<0.05$.

RESULTS

There were 19 children in the irradiated group, 8 females and 11 males, with a mean age of 6.53 ± 3.48 years and a mean radiation dose of 4,250 ± 250 cGy. Their general health was good and no anti-infective therapy was administrated during the study period. The control group was composed of 9 females and 11 males with a mean age of 6.75 ± 3.43 years, and none of the children was on medication, which might have affected detectable oral microflora.

The isolation frequencies and abundances of MS, lactobacilli and *Candida* species in saliva samples from the irradiated children were compared with those of the control group (Table 1). MS were found in all children in the irradiated group, and in 17 children of the control group. The difference between groups was not statistically significant. Lactobacilli and *Candida* species were found in only a few subjects of the control group. The isolation frequencies of lactobacilli and *Candida* species in irradiated children were significantly greater than those of the control group. The numbers of MS, lactobacilli and *Candida* species detected in the saliva samples, were significantly greater in the irradiated children than in those of the control group.

Table 1
Isolation frequencies and abundance (log_{10} CFU/ml, means ± SD) of mutans streptococci, lactobacilli and *Candida* species.

Microorganisms	Control group (n = 20)		Irradiated group (n= 19)	
	Abundance	Frequency	Abundance	Frequency
Mutans streptococci	4.58 ± 1.33	17/20	6.15 ± 0.79[a]	19/19
Lactobacilli	0.85 ± 0.70	5/20	4.34 ± 1.50[b]	14/19[c]
Candida species	0.25 ± 0.27	4/20	3.60 ± 1.38[b]	15/19[c]

Significantly different from control [a]p< 0.05,[b]p< 0.01; [c]significantly different from control by Fisher's exact test (p<0.05)

DISCUSSION

The radiation therapeutic regimen of retinoblastoma usually involves the field located on the upper face region, covering part of the parotid gland and maxillary region with the minor salivary gland. Irradiation often causes degeneration and necrosis of the salivary glands, resulting in atrophy of the salivary glands and further xerostomic effects The risk of infection increases when there is a reduction in saliva, because saliva has the function of protecting the oral tissue against pathogens and helps oral tissues to heal (Squier, 1990). Ionizing radiation also causes a pronounced reduction in salivary flow rates and the onset of clinical xerostomia (Schubert and Izutsu, 1987). Then, a pronounced shift in specific microbial components of the oral microflora can easily occur (Keene *et al*, 1994). As a result, a higher chance in developing dental caries appears more frequently, and caries lesions may be detected clinically within a few months.

This study assessed the presence of potential oral pathogens, MS, lactobacilli and *Candida* species in a group of children with retinoblastoma who received radiotherapy. The results are in agreement with the findings of Epstein *et al* (1993) which reported an increase in lactobacilli levels in head and neck cancer patients after radiation therapy. The results showed that the isolation frequencies and abundances of lactobacilli and *Candida* species in saliva samples of irradiated children were significant greater than in healthy children. However, the levels returned to pretreatment levels after 1 month. On the basis of other previous studies, it can be noted that lactobacilli are associated with caries and are aciduric bacteria found after cellular products from MS have altered the environment (Hadley, 1933). In our irradiated group of children, although the isolation frequency of MS was similar to that of healthy controls, their counts were significantly different. MS are the main cariogenic bacteria in the oral cavity. It is thought that MS are relatively stable colonizers in the oral cavity, but when a physiological or ecological disturbance, such as xerostomia occurs in the oral cavity, changes in the oral microflora can often be found. Many studies have shown that after radiation therapy, MS and lactobacilli increased (Brown *et al*, 1975; Keene and Fleming, 1987; Lacatusu *et al*, 1996). Thus, through the elevation of MS levels found in this group of irradiated children, the environment was acidified to allow for lactobacilli development.

Alteration of the salivary pool affected by radiation is a major problem that produces xerostomia and in turn affects saliva content. Compositional changes in saliva after radiation therapy also contributes to altered microflora. Thus, the oral environment changes the microorganisms cultured, such as MS, *Candida albicans*, *Lactobacillus* and others.

Candida species are symbiotic organisms that reside normally on the oral mucosa and in the lumen of the GI tract. They can, not only cause local infection of the oral mucosa, but also result in esophageal candidosis or in systemic dissemination, which is difficult to recognize and responds poorly to treatment (Wingard *et al*, 1979). In patients who receive radiation therapy, candidiasis is the most common infection of the oropharynx (Redding *et al*, 1999). It is thought that radiation-induced histological changes leading to oral mucositis, quantitative and qualitative changes in saliva and salivary flow, and facilitate yeast infection (Epstein *et al*, 1993). In a recent study which monitored oral yeast carriage in patients with head and neck cancers undergoing radiation therapy, Redding *et al* (1999) noted oral *Candida* carriage in 73% of patients. This is in accordance with our results that *Candida* species were detected in 79% of irradiated patients compared to 20% of controls. However, other researchers have been unable to note any differences in oral yeast colonization between controls and test subjects after radiation therapy (Spijervert, 1991). Additional studies assessing salivary flow rates, buffering capacity, pH and non-specific antimicrobials of whole saliva in irradiated children must be conducted to learn more about the changes that occur throughout the course of radiotherapy, the long-term effects, and the effectiveness of a prophylaxis program in these patients.

In conclusion, our study confirmed the finding of previous studies regarding the changes in oral microflora after radiation. Findings suggest radiation therapy in this group of irradiated ocular deformed children might have some influence on oral ecology in this type of patient, placing them at higher risk for caries and candidiasis than healthy controls. Therefore, comprehensive preventive measures are recommended for these children. Recommended measures have included the application of topical fluoride, rigorous oral hygiene, limitation of cariogenic food intake, and special chewing gums designed to stimulate remaining salivary function.

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