Mucositis Grades and Yeast Species

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ABSTRACT

Surgically treated patients with oral, head and neck cancer commonly develop mucositis during additional irradiation therapy. Oral mucosa inflammation other than irradiation is mostly caused by Candida albicans, yeast of Candida genus. This study evaluated possible connection between grades of oral mucositis and oral yeast profile in irradiated patients before, during and after irradiation. In 25 examined patients mucositis grades »0« to »2« before irradiation with 20% positive smears and only two different species of yeasts (C. krusei 4%, C. albicans 16%) during the irradiation changed into »0« to »4« and 36% positive smears with five different species of oral yeasts (C. albicans 12%, C. glabrata 12%, C. parapsilosis 4%, C. guilliermondii 4% and Saccharomyces cerevisiae 4%). Three weeks after irradiation was finished mucositis decreased into »1« to »3« with 20% positive smears and again only two species of yeasts (C. albicans 16%, C. guilliermondii 4%). Mucositis grades was increased significantly (p=0.0037) with changes in fungi profile.

Key words: oral mucositis, oral yeasts, irradiation

Introduction

Patients that suffer from malignant mouth, head and neck diseases often develop mucositis as a result of additional radiotherapy that follows surgical removal of a tumor1–8.

Mucositis is usually defined as an inflammation of the oral (and gastrointestinal) mucosa1–3.

It is known that mucositis is a more complexed problem than inflammation of oral mucose alone. It results from the destruction of rapidly dividing epithelial cells and from vascular changes of lamina propria of the oral mucose epithelium by activating NF-κB and secondary release of inflammatory mediators such as TNF-alpha and interleukin-1 causing a variety of changes of oral mucosa, from mild atrophy to severe ulcerations9–14. Severity of mucositis is influenced by patient related factors and treatment related factors. Treatment related factors such as increased total dose of radiation, fraction size and volume of normal tissue in the irradiated field all increase the risk of mucositis15,16.

Patients related factors such as age, nutrition, smoking, alcohol consumption, dental status, prostetic dentures, overall condition of the oral mucosa prior to therapy, pre-existing oral diseases, pre-existing collagen and vascular disorders, pre-existing xerostomia and cancer status at the beginning of the therapy all influence the development and severity of mucositis2–5,7,16. The World Health Organization (WHO) defines mucositis as anatomic, symptomatic and functional manifestations which are presented in five grades of severity: »0« with no manifestation, »1« soreness and erythema, »2« erythema, ulcers, but patient can swallow solid food, »3« ulcers with extensive erythema ulcers, patient cannot swallow solid food, and grade »4« mucositis to the extent that feeding is not possible19.

In head and neck cancer therapy, all patients undergoing irradiation will develop grades »1« or »2« of mucositis10,19. More severe mucositis (grade »3« or »4«) will be developed in approximately 41% of patients receiving a combination of radiation and chemotherapy and 21% patients receiving a chemotherapy alone8,10,18.

Clinical signs of mucosal damage and cell death usually appear during the second week of radiation therapy19. Irradiation therapy also affects salivary glands causing reduced salivary flow or xerostomia. Severity of

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xerostomia depends on dosage and duration of radiation therapy\textsuperscript{20}.

Mucosal damage and xerostomia allow a possibility of microorganism superinfection\textsuperscript{21,22}. \textit{Candida albicans} is an opportunistic microorganism of oral mucosa in adult humans that causes no symptoms in healthy oral mucosa. Enlarged number of \textit{C. albicans} colonies or profile changes may cause candidiasis – an illness with acute or chronic symptoms and atrophic or hypertrophic oral mucosa changes\textsuperscript{1-3}. Oral mucosa changes caused by Candida species may have clinical manifestations similar to the mucositis caused by irradiation with mild atrophy to severe ulcerations. Those changes may be clinically described at the same way that WHO use to describe and graduate mucositis with soreness, erosions and erythema. Irradiation therapy may induce genotype and phenotype changes of oral microorganisms. Many authors describe profile changes of candida species in oral mucosa during irradiation therapy\textsuperscript{23-38}.

During irradiation therapy the predominance of \textit{C. albicans} in oral mucosa in healthy circumstances is changed to non-albicans species predominance. Candida species found and described in oral irradiation therapy are \textit{C. pseudotropicalis}, \textit{C. guilliermondii}, \textit{C. krusei}, \textit{C. glabrata}, \textit{C. tropicalis}, \textit{C. dubliniensis}, \textit{C. lusitaniae}, \textit{C. kefyr} and \textit{C. parapsilosis}\textsuperscript{23-38}. The most of changes in number of different Candida species were found after second week of irradiation therapy which correlated with more severe mucositis grades\textsuperscript{23-38}.

The aim of this study was to establish whether candida species profile changes influence and worsen the mucositis during the irradiation therapy.

Materials and Methods

We tested 25 patients (22 males – 88% and 3 females – 12%) aged 47 to 84 (average age 63) with oral, head and neck cancer. All of them were surgically treated and irradiated. The dose of irradiation was 6000 cGy in 30 separated doses of 200 cGy each. All patients had oral mucosa examined and controlled on several occasions with mucositis grades established and oral swabs on fungi taken. Oral mucositis was ranged from »0« to »4« according to the WHO recommendation. Oral swabs were distributed in agar Sabouraud dextrose/chlorphenicol and incubated at 37 degrees of Celsius for 18 hours. Emerging colonies were identified biochemically and through the germinative tube test.

Patients were examined prior the irradiation, during the second week of irradiation and three weeks after the irradiation had been finished and oral statuses (mucositis and oral swabs findings) of the same patient were compared.

Cochran’s Q and McNamara’s tests were used for comparison of oral swabs findings and mucositis grades before, during and after irradiation therapy. Correlation between mucositis and oral swabs findings were tested with \(\chi^2\)-test and the statistical significance was set at \(p<0.05\). Data were analyzed by statistical software MedCalc for Windows, version 11.5.1.0 (MedCalc Software, Mariakerke, Belgium).

Results

Prior the irradiation all patients had their oral mucosa examined and possible changes were verified according to symptoms similar to those used by WHO in mucositis grade ranging. A total of 32% (\(N=8\)) patients were without any oral symptoms with preserved integrity of normally colored and normally functional oral mucosa and 68% patients (\(N=17\)) had some clinical symptoms similar to mucositis like sorenesses, erythema, small ulcerations but with ability to eat solid food (Table 1).

In the second week of irradiation mucositis grades were higher.

Only 8% (\(N=2\)) patients were without any symptoms, 60% (\(N=15\)) patients had grades from »1« to »2«, 24% (\(N=6\)) had grade »3« with extensive erythema, deep ulcers and inability to eat solid food and 8% (\(N=2\)) had grade »4« without possibility of eating anything (Table 1).

Three weeks after irradiation had been finished most of the patients (88%, \(N=22\)) had mucositis grades from »1« to »2« and 12% (\(N=3\)) had grade »3«. No patients were without symptoms and no patients had grade »4« at this stage of observation (Table 1).

According to a rank correlation test, mucositis during the irradiation compared to the mucositis before irradiation had started, were higher and remained higher for three weeks after irradiation with statistical significance of \(p=0.012\).

Before irradiation 80% (\(N=20\)) of all patients had negative swabs. Among 20% (\(N=5\)) positive oral swabs two different species of yeast were found: \textit{C. albicans} in 16% (\(N=4\)) patients and \textit{C. krusei} in 4% (\(N=1\)) patients (Table 2).

| TABLE 1 |

MUCOSITIS GRADES BEFORE, DURING AND AFTER IRRADIATION

<table>
<thead>
<tr>
<th>Mucositis grade (according WHO)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before irradiation</td>
<td>8* (32%)</td>
<td>9 (36%)</td>
<td>8 (32%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second week of irradiation</td>
<td>2 (8%)</td>
<td>5 (20%)</td>
<td>10 (40%)</td>
<td>6 (24%)</td>
<td>2 (8%)</td>
</tr>
<tr>
<td>Three weeks after irradiation finished</td>
<td>17 (68%)</td>
<td>5 (20%)</td>
<td>3 (12%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* results are presented as N (%)
In the second week of irradiation 64% (N=16) of all patients had negative oral swabs. Among 36% (N=9) positive oral swabs there were five different species of yeast: *C. albicans*, *C. glabrata*, *C. parapsilosis*, *Saccharomyces cerevisiae* and *C. guilliermondii*. A percentage of all found yeasts compared to *C. albicans* percentage were two times higher (Table 2).

Three weeks after irradiation had been finished, 80% (N=20) of oral swabs were found negative and among 20% (N=5) of positive swabs, two different species of yeast were found, 16% (N=4) of *C. albicans* and 4% (N=1) of *C. krusei*. The distribution of oral yeast species was the same before and after the irradiation with prevalence of *C. albicans* (Table 2). Mucositis appearance was more frequent during therapy and three weeks after with statistical significance of p<0.001.

The highest mucositis grade found during the irradiation was »4», while prior the irradiation the highest grades were »2« and »3«, the same as after irradiation therapy. Oral mucositis grades had increased during irradiation period with statistical significance of p=0.012.

Mucositis and positive oral swabs before the irradiation and three weeks after the irradiation were the same (16%). We have not found statistically significant correlation between the presence of Candida and mucositis before irradiation or three weeks after (p>0.05).

Oral mucositis increased simultaneously with increased number of oral yeast species in positive oral swabs findings with statistical significance of p=0.0037 during the irradiation therapy.

**Discussion**

In most studies patients surgically treated and irradiated after suffering from malignant mouth, head and neck diseases were examined during the therapy for various factors such as clinical and microbiological changes and for possibilities of oral functions.

Oral mucositis has been identified as the most debilitating side effect of cancer therapy by patients. Various authors describe mucositis as oral mucosa disease caused by destruction of rapidly dividing epithelial cells, exclusively caused by irradiation without any bacterial infection\(^2\). Some other authors defined mucositis as oral and gastrointestinal acute mucosa disease directly caused by irradiation, chemotherapy, mechanical causes (solid food, brushing), or thermal exposure (hot food), tumor topic (direct) influence, tumor indirect influence causing immunity disorders, xerostomia, due to the poor oral hygiene and current chronic diseases such as candidiasis\(^6,10\). The terms stomatitis and mucositis have been used interchangeably when describing inflammatory oral conditions. Assessment scales must precisely describe mucosal damage, reproducibly measure severity and objectively classify changes. The WHO is ranging mucositis by five grades from »0« to »4« according to clinical changes and functional possibilities.

Patients are usually examined for mucositis during irradiation therapy, mostly after second week of irradiation and three or four weeks after irradiation has been finished\(^29,40\). In our study we also assessed mucositis before irradiation had even started, using the same criteria as the WHO has recommended.

We found that 32% (N=8) of our patients had no mucositis (grade »0«) and that 68% (N=17) had mild mucositis before irradiation. None of our patients had severe mucositis (grades »3« or »4«) prior to radiotherapy.

Other studies had not assessed oral mucositis prior the irradiation therapy. The reason was that mucositis is exclusively caused by irradiation without any bacterial infection, so other authors found no reason to examine mucosal inflammation. We found a number of positive oral smears on yeast species among our patients prior a therapy. We found that 80% (N=20) of our patients had negative oral smears before irradiation had started and 20% (N=5) were positive on Candida species.

*C. albicans* was found in 16% (N=4) of patients and *C. krusei* in 4% (N=1) of them. These results didn’t correlate with findings in other similar studies. Brasilians had 42.9% positive patients colonized exclusively with *C. albicans* before irradiation started, Mexicans 43% and Jordanian 72.6%\(^24,28,30\). Although those numbers were not similar to our findings, the distribution of Candida species was. In all studies *C. albicans* prevailed. Beside

<table>
<thead>
<tr>
<th>Yeast-like fungi species</th>
<th>Negative smears</th>
<th>Positive smears</th>
<th>Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before irradiation</td>
<td>N=20 (80%)</td>
<td>N=4 (16%)</td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N=1 (4%)</td>
<td><em>C. krusei</em></td>
</tr>
<tr>
<td>Second week of irradiation</td>
<td>N=16 (64%)</td>
<td>N=3 (12%)</td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N=3 (12%)</td>
<td><em>C. glabrata</em></td>
</tr>
<tr>
<td></td>
<td>N=1 (4%)</td>
<td><em>C. parapsilosis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=1 (4%)</td>
<td><em>S. cerevisiae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N=1 (4%)</td>
<td><em>C. guilliermondii</em></td>
<td></td>
</tr>
<tr>
<td>Three weeks after irradiation finished</td>
<td>N=20 (80%)</td>
<td>N=4 (16%)</td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N=1 (4%)</td>
<td><em>C. krusei</em></td>
</tr>
</tbody>
</table>
C. albicans we found C. krusei, Brazilians found C. dubliniensis and Jordanians found C. glabrata.

Mucositis and positive oral smears at the same time had 16% (N=4) of our patients. We didn’t find statistically significant correlation between the presence of candida in oral mucosa and mucositis before irradiation (p>0.05). So, our conclusion was that mucositis in those cases was caused by presence of bacteria of some kind or due to mechanical or chemical factors.

During the second week of irradiation, mucositis percentage among our patients increased to 92% (N=23). A total of 60% (N=16) had grades from »1« to »2«, 24% (N=6) had grade »3« and 8% (N=2) had grade »4«. It is known that mucositis grows with advanced radiotherapy and in the second week of radiotherapy mucositis is supposed to be the worst. The mean overall incidence of mucositis was 80% among patients with conventional irradiation therapy in 33 studies18. The frequency of mucositis was the highest in patients treated with altered fractionation of radiotherapy affecting 100% patients overall.

Grades »3« and »4« of mucositis in those studies experienced 34% of patients with conventional irradiation treatment which correlates with our findings (32%)18. Mucositis occured in the second week of irradiation among our patients in 92% of cases which was significantly more (p=0.012) then prior the therapy.

In the second week of irradiation 64% (N=16) of our patients had negative oral swabs. Among 36% positive oral swabs C. albicans was found in 12% (N=3) cases, C. glabrata also in 12% (N=3), C. parapsilosis in 4% (N=1) of cases, C. guillermondii in 4% (N=1), and Saccharomyces cerevisiae, yeast of non-candida origine, in 4% (N=1) of cases. Although there were more positive oral swabs in the second week of irradiation compared to the findings before, there was no statistically significant difference (p>0.05). Mucositis and positive oral swabs on yeast species during irradiation therapy at the same time were found in 40% (N=10) of our patients.

Oral mucositis increased to the grade »3« and »4« simultaneously with increased number of yeast species found in oral mucosa with statistical significance (p=0.0037).

Three weeks after irradiation therapy had been finished, mucositis rates were »1« and »2« in 88% (N=22) and »3« in 12% (N=3) of patients. None of the patients were without any symptoms. As irradiation therapy has lasted for four to six weeks and its effects were cumulative we expected to find such results. It is similar to the literature reports and incidence of mucositis appearance of 80–100%41,42.

Oral swabs findings 3 weeks after irradiation had been finished were negative in 80% (N=20) of our patients and were positive in 20 (N=5). C. albicans was found in 16% (N=4) and C. krusei in 4% (N=1) of patients. These findings didn’t correlate with the literature findings where incidence of positive oral swabs was 70.1%37,38. Our findings regarding distribution of yeast species at this point were the same as before irradiation had started with prevalence of C. albicans over C. krusei. The percentage of patients with positive oral swabs was similar as in literature43. Number of patients that had mucositis and positive oral swabs at the same time three weeks after irradiation had been finished were 16%. Mucositis appeared more frequent three weeks after irradiation then before with statistical significance (p=0.001) and more frequent after (100%) then during (92%) the therapy but without statistical significance (p=0.07).

We didn’t find statistically significant difference among distribution of candida before and after irradiation (p>0.05) which was not in agreement with the current literature30–38. During irradiation therapy we had more positive oral swabs then before the therapy had started and after the therapy had finished but without statistical significance. Also, we didn’t find any statistically significant correlation between distribution of yeast species and a grade of mucositis (p>0.05) three weeks after the irradiation therapy had been finished.

Conclusion
We found the same percentage of patients with positive oral swabs before and after the irradiation therapy with the same distribution of fungi – two species of Candida: C. albicans and C. krusei. We also found the incidence of mucositis significantly higher during the therapy and three weeks after the therapy had been finished. Findings of positive oral swabs during irradiation were also higher then before and after irradiation therapy but without statistical significance. Oral mucositis increased (to »3« and »4«) simultaneously with increased number of oral yeast species in positive oral swabs findings. Mucositis appeared more frequently during irradiation therapy, with increased positive oral swabs findings.

REFERENCES


SAŽETAK

Bolesnici operirani od tumora usta, glave i vrsta često obojevaju od mucositis za vrijeme dodatnog liječenja zračenja. Upala sluznice usta osim radijacijom može biti izazvana C. albicans, gljivicom koja kao i druge vrste kandida spada u rod kvasnica. Cilj ovog rada bio je utvrditi moguću povezanost stupnja mukozitisa i prisustva više vrsta kvasnica.

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STUPANJ MUZOSITISA I VRSTE KVASNICA

C. albicans, C. krusei, C. parapsilosis, C. guilliermondii, C. guilliermondii

16% i C. albicans, C. krusei, C. parapsilosis, C. guilliermondii, C. guilliermondii

Stupanj mucositis kod bolesnika prije, za vrijeme i nakon zračenja. Od 25 ispitanika prije zračenja (p>0,05). Stupanj mukozitisa povećava se statistički značajno (p=0,0037) s povećanjem broja vrsta Findsnu. Nije pronađena statistički značajna povezanost pozitivnog nalaza kandide na oralnoj sluznici i mukozitisa.