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ORIGINAL ARTICLE

Histomorphometry and immunohistochemical features of grade I (WHO) oral radiomucositis

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AIMS: The aims of this study were to describe the immunohistopathological and morphometric features of oral mucositis grade I (WHO).

MATERIAL AND METHODS: Ten samples of oral mucositis were biopsied and submitted to histopathological, morphometric and immunohistochemical analyses (CD68, Ki-67 and p53). The samples were compared with the buccal mucosa of head and neck cancer patients before radiotherapy (NMCP), normal buccal mucosa (NM) and oral dysplasia (OD).

RESULTS: Epithelial thickness, area and perimeter were decreased in oral mucositis and inflammatory components, increased when compared with NMCP. CD68 immunoreactivity, near to the epithelium, was more evident in oral mucositis than in NMCP ($P = 0.01$). The Ki-67 counts were higher in oral mucositis than in NM and NMCP ($P = 0.001$ and $P = 0.043$, respectively), but without any difference with OD ($P = 0.284$). The p53 staining was present in all cases of mucositis and oral dysplasia, but negative in NMCP and NM.

CONCLUSIONS: Oral mucositis grade I (WHO) presented epithelial atypia and atrophy, increased inflammatory response, with relevant Ki-67 count and positiveness for p53.

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Keywords: radiotherapy; mucositis; CD68; p53; Ki-67; inflammation

Introduction

Head and neck carcinomas (HNCs) are usually managed by surgery and/or radiotherapy (RxT). The criteria for treatment choice include size and tumor localization, bone and muscular involvement, cervical metastasis, total or partial resection possibilities and systemic status of the patients (Sherman, 1993). Although head and neck RxT is effective, chronic and acute side-effects such as mucositis, xerostomia, hypogeusia, trismus, radiation caries, candidiasis and osteoradionecrosis are frequently reported (Rothwell, 1987; Scully and Epstein, 1996; Garg and Malo, 1997; Meraw and Reeve, 1998; Epstein *et al*, 1999; Dörr *et al*, 2001).

The most important acute head and neck RxT side-effect is oral mucositis (Sur *et al*, 1994; Spijkervet *et al*, 1989; Plevová, 1999; Dörr *et al*, 2001). Oral mucositis can obligate partial or complete interruption of RxT, decreasing treatment efficacy (Dörr *et al*, 2001). Mucositis also causes serious pain on swallowing, eating and speech impairment (Köstler *et al*, 2001). Radiation-induced mucositis arises about 2 weeks after the beginning of RxT and more than 50% of patients present severe mucositis after half the treatment is completed (Handschel *et al*, 2001b). Severity of mucositis is dependent on the type and intensity of RxT, individual response, epidermal growth factor (EGF) levels, tobacco and alcohol use (Scully and Epstein, 1996; Epstein *et al*, 2000; Handschel *et al*, 2001b).

The biology of RxT mucositis is not well understood. Interactions among mucosal cells, pro-inflammatory cytokines and local factors such as saliva and microorganisms probably play a key role (Pico *et al*, 1998; Sonis, 1998). The initial microscopic events seem to be increased submucosal vascularity and inflammatory cell infiltration, mainly macrophages (Sonis, 1998; Handschel *et al*, 2001b). Radiation causes cell lesion and liberation of cytokines from the epithelium and connective tissue, resulting in cell death and reduction of basal cell proliferation (Pico *et al*, 1998; Sonis, 1998). Clinically, radiation mucositis is characterized by erythema, followed by ulceration covered by a fibrinopurulent exudate (Scully and Epstein, 1996; Köstler *et al*, 2001). Oral mucositis has been studied experimentally (Dörr *et al*, 2001) but few reports describe the

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microscopical aspects in humans (Pico *et al*, 1998; Sonis, 1998; Sonis *et al*, 2000; Dörr *et al*, 2001; Handschel *et al*, 2001b). A better understanding of the mechanisms involved in the initial stages of oral mucositis can be helpful for its prevention and management (Dörr *et al*, 2001; Handschel *et al*, 2001b). For example, keratinocyte growth factor (KGF) effectiveness showed in recent studies may be associated with epithelial repopulation, started 2 weeks after radiation, against atrophy and ulceration (Plevová, 1999; Köstler *et al*, 2001). Nevertheless, no previous study focused on Ki-67 epithelial labeling in oral mucositis, a reliable evaluation of proliferative reaction activity (Bruno and Darzynkiewicz, 1992). Indeed, only one study deals with macrophage subtypes during RxT and CD68 macrophage staining, which gives an overview, but including the relation with epithelium was never considered (Handschel *et al*, 2001b). The phenomenon of epithelial atypia was previously described induced by radiation, but no previous studies deal with it in oral mucositis, including p53 labeling, an important dysplastic biomarker (Kleebler and Somrak, 1993; Brien *et al*, 2001). In addition, the beginning of mucositis and the morphological and biochemical processes in humans needs more investigation. The aims of this study were to describe the microscopical aspects of oral mucositis grade I (enanthema, mild discomfort) according to World Health Organization (WHO; Handschel *et al*, 2001b) and to describe the CD68, Ki-67 and p53 immunoexpression that resulted from RxT of head and neck cancer.

Material and methods

The present study was composed from 10 patients with HNC. Patients received RxT treatment (teletherapy) exclusively or associated with surgery. All patients received oral care orientation and drugs such as pilocarpine, artificial saliva, sucralfate and fluortherapy on dentate patients. Xerostomia was classified as absent (no xerostomia complaints), moderate (xerostomia ameliorated with pilocarpine use) and severe (pilocarpine not effective to alleviate xerostomia). Biopsies with 5-mm punch, under local anesthesia, inside the radiation field, were taken from erythematous areas of the buccal mucosa without ulcerations, at least 2 weeks after the beginning of RxT when the patients presented oral mucositis grade I (WHO). Nine normal mucosal specimens from the buccal mucosa were taken, at least 3 cm away from the tumor, from patients with HNC before RxT and used as control (NMCP). Some of these samples were obtained from the study group patients before RxT. Pilocarpine was used in nine cases with subjective good results in seven.

Nine patients received teleradiotherapic treatment from Linear Accelerator (6 MeV) and one patient received Co^{60} therapy. Eight patients were males and two were females. Nine patients referred past or present tobacco use and eight referred past or present chronic alcohol use. Eight patients were treated by surgery and RxT, and two received RxT exclusively. Total doses were, on average, 6466 cGy, ranging from 4600 to 8000 cGy. Fractionated doses were, on average, 192 cGy, ranging from 180 to 200 cGy. When the biopsy was taken from mucositis grade I (WHO) area, the average dose received by each patient was 3320 cGy ranging from 2700 to 5000 cGy. Association of pilocarpine and sucralfate was recommended for eight patients and in one case, artificial saliva was indicated. The majority of patients complained of mild xerostomia. Data about gender, age, tumor localization, tumor-nodes-metastasis (TNM), doses, palliative protocols and xerostomia complaint are shown on Table 1. This study was approved by the ethical committee of the School of Dentistry of Piracicaba/UNICAMP (process 123/2001) and all the patients consented formally with the procedures.

Microscopic analysis

All specimens were fixed in 10% formalin, and embedded in parafin. Five-micron sections, stained with H&E, were studied by light microscopy and histomorphometry of the epithelium, blood vessels and inflammatory infiltrate was performed with KS 400 software (Kontrol KE 2.00, Zeiss, Germany). This software is employed to analyze staining intensity, staining area or positive cell numbers and even thickness and perimeter. In our case, we performed cell count, measures of thickness, perimeter and area. All counts were done in triplicate, under the same conditions of brightness and color. Measurements of epithelial

Table 1 Distribution of 10 irradiated patient according to gender, age, tumor localization, TNM, daily doses, at the moment of biopsies, and total doses on facial fields, drugs used and xerostomia complaints

					RxT(cGy)				
Patient	*Gender*	*Age (years)*	*Site of tumor*	*TNM*	*Daily*	*Biopsy*	*Total*	*Drugs*	*Xerostomia*
1	M	48	Conjunctive	IV	200	3000	6000	Pilocarpine, sucralfate	Absent
2	F	54	Tonsil	IV	200	3000	4600	Pilocarpine, sucralfate	Severe
3	M	55	Larynx	III	180	3200	6900	Pilocarpine, sucralfate	Moderate
4	M	64	Cervical metastasis	IV	180	3000	6480	Artificial saliva, sucralfate	Absent
5	M	60	Pyriform sinus	III	180	2700	5040	Pilocarpine, sucralfate	Moderate
6	M	50	Retromolar area	IV	200	3400	8000	Pilocarpine, sucralfate	Moderate
7	M	54	Floor of the mouth	IV	180	3400	7040	Pilocarpine, sucralfate	Moderate
8	F	43	Palate	IV	200	4600	6000	Pilocarpine, sucralfate	Severe
9	M	42	Tonsilar area	I	200	5000	7000	Pilocarpine	Absent
10	M	58	Floor of the mouth	IV	200	2400	7600	Pilocarpine, sucralfate	Moderate

M, male; F, female; RxT, radiotherapy; cGy, centigrays.

thickness (performed using the mean length of three lines measuring the basal to superficial layer), perimeter (measuring the total contour of epithelium using an adjustable line) and area (measuring the epithelial area inside the square) were performed on ×40 magnification, inside a square of 16 606 610.95 μm^2. Vessels and cellular quantification, using a direct count, were performed at ×400 magnification.

Immunohistochemistry

Immunostaining was performed using 3 μm sections of paraffin-embedded tissue of oral mucositis and control cases, fixed in 10% buffered formalin. All reactions followed standard protocols. Sections were deparaffinized and rinsed for 5 min under running water. Endogenous peroxidase activity was blocked by incubating the slides in 3% H_2O_2. Antigen retrieval was obtained by 10 mM citric acid digestion, pH 6.0, using two cycles of 12 min in a microwave. After cooling for 15 min, the slides were transferred to phosphate-buffered saline, incubated overnight with primary antibody for CD68 (PG-M1, Dako, Carpenteria, CA, USA, 1:400), Ki-67 (Clone MIB1, Dako, Carpenteria, CA, USA, 1:200) and p53 (Clone DO-7, Dako, Glostrup, Denmark, 1:200) followed by streptavidin–biotin peroxidase complex (StrepABC Complex/HRP Duet kit, Dako, Glostrup, Denmark). Reactions were developed with 3,3-diaminobenzidine tetrahydrochloride (DAB, Sigma, St Louis, USA) containing 0.01% H_2O_2 and counterstained with hematoxylin. Cytoplasmic and membranous labeling were considered positive. For CD68 quantification, both the positively stained and all negatively stained cells were counted in five sampled high-power fields (magnification ×400), immediately below the epithelial basal stratum including the parabasal layer, using the KS 400 software (Kontrol KE 2.00, Zeiss, Germany). The percentage of positive cells was calculated based on the total number of counted cells. For Ki-67 and p53 evaluation, seven samples of buccal normal mucosa (NM) (mean age: 19.5 ± 0.5 years, non-smokers and alcohol users), 13 cases of oral mild to moderate dysplasia (OD) (mean age: 54.4 ± 15.2 years, alcohol and tobacco use reported; Table 2), according to pathologist criteria, and NMCP was used as control. Ki-67 counts were performed using the software KS 400, using ×400 magnification, considering on average, 1000 cells per slide (positive/negative) sampled in different fields. Then p53 analysis was done observing positive cells across the epithelium, including suprabasal layers.

Statistical analysis

Statistical analysis was performed using the non-parametrical Mann–Whitney test (SPPS 13.0, Software, Chicago, IL, USA).

Results

The samples of oral mucositis were obtained from reddish areas of irradiated buccal mucosa, which probably represented more inflammatory events and atrophy. Microscopically most of the cases of mucositis grade I (WHO) showed epithelial hyperparakeratosis (eight cases), ectasic and numerous blood vessels (all cases) and mild inflammatory infiltrate (seven cases). Seven cases presented evident epithelial atypia, characterized by pleomorphism, hyperchromastism, eosinophilia, increased nuclear–cytoplasmic ratio and enlarged nuclei while six cases showed evident vacuolization of epithelial cells. Histopathological, morphometric and immunohistochemical findings are shown in Table 3. Epithelial thickness in mucositis was smaller than NMCP ($P = 0.002$), as well as epithelial area and perimeter ($P = 0.041$ and $P = 0.003$, respectively). Increased vascularity and inflammatory infiltration were also significantly higher in mucositis than in NMCP ($P < 0.001$ and $P = 0.002$, respectively). CD68 positive cells in oral mucositis (29.78% ± 6.889%) were found mainly in the connective tissue and sporadically in the mucosal epithelium, while a few CD68 positive cells were found in the NMCP (18.557% ± 5.333%). CD68 positive cells were more common in the oral mucositis cases than in the NMCP ($P = 0.01$). The CD68 positive cells pattern in both groups is illustrated in Figure 1. Statistical differences of Ki-67 counts were shown in oral mucositis versus NM, and oral mucositis versus NMCP (oral mucositis – 41.66%, NM – 17.05%; $P = 0.001$ and $P = 0.043$, respectively), but no differences were found in oral mucositis versus OD (OD – 51.72%, $P = 0.284$). OD showed significant increased counts of Ki-67 positive cells compared with NMCP and NM samples (NMCP – 29.82%; $P < 0.001$, both). The Ki-67 NMCP count was higher than the NM ($P = 0.003$). Suprabasal staining (over two layers up the basal) was evident in OD, but was also presented in oral mucositis. Basal and parabasal (up to two layers up the basal) staining had been found in NM, NMCP and oral mucositis being basal labeling more evident in the last. The Ki-67 immunohistochemical pattern is illustrated in Figure 2. All samples of OD and oral mucositis were positive for p53 different to NM and NMCP which were negative.

Table 2 Clinical data regarding the 13 cases of oral epithelial dysplasia

Patient	*Gender*	*Age (years)*	*Site*
1	F	56	Buccal mucosa
2	M	72	Lower lip
3	M	43	Palate
4	F	60	Palate
5	F	71	Buccal mucosa
6	M	38	Buccal mucosa
7	M	79	Lower lip
8	M	60	Lower lip
9	M	33	Buccal mucosa
10	M	39	Buccal mucosa
11	M	41	Buccal mucosa
12	M	69	Buccal mucosa
13	M	47	Buccal mucosa

M, male; F, female.

Discussion

Mucositis is a limiting acute side-effect of radiotherapy that can even cause interruption of RxT treatment

Table 3 Histomorphometrical findings in mucositis samples ($n = 10$) compared with control groups

Group/finding	*NM*	*NMCP*	*Mucositis*	*OD*
Hyperparakeratosis	Negative	Negative	Positive (eight cases)	Positive
Pyknosis (associated with cell death)	Negative	Negative	Positive (four cases)	Negative
Cellular atypia	Negative	Negative	Positive (seven cases)	Positive
Apoptotic bodies	Negative	Negative	Positive (two cases)	Negative
Epithelial thickness (μm)	ne	427.18 ± 203.35	353.7 ± 161.01	ne
Epithelial perimeter (μm)	ne	5084.57 ± 724.48	4536.09 ± 763.03	ne
Epithelial area (μm^2/per field)	ne	592 922.5 ± 212 552.59	476 279.5 ± 155 754.87	ne
Blood vessels (vessels per field)	ne	7.22 ± 2.87	10.85 ± 3.66	ne
Inflammatory infiltrate (cells per field)	ne	14.63 ± 29.56	28.85 ± 11,86	ne
CD68 (percentage of positive cells) (%)	ne	18.557 ± 5.333	29.78 ± 6.889	ne
Ki-67 (percentage of positive cells) (%)	17.05 ± 4.74	29.82 ± 10.13	41.66 ± 15.47	51.72 ± 10.339
p53 (positiveness)	Negative	Negative	Positive (all cases)	Positive

ne, not evaluated; NM, normal mucosa; NMCP, normal mucosa cancer patient; OD, oral dysplasia.

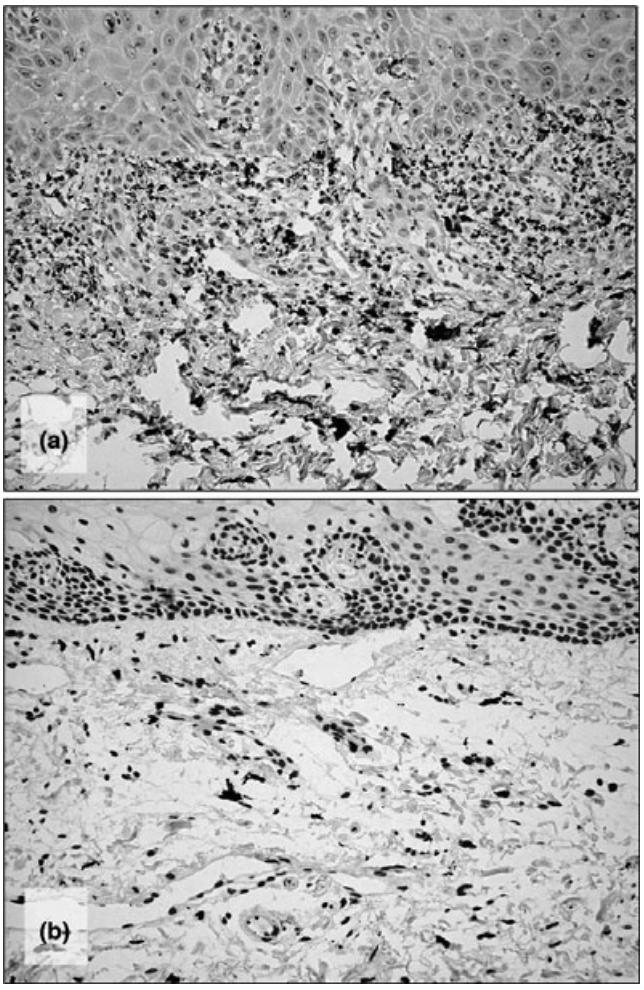

Figure 1 CD68 positive cells (immunohistochemical) (×200). (**a**) Oral mucositis showing many CD68 positive cells close to the epithelium. (**b**) Normal mucosa showing sporadic CD68 positive cells

(Sur *et al*, 1994; Spijkervet *et al*, 1989; Dörr *et al*, 2001; Köstler *et al*, 2001). Mucositis usually arises after 2 weeks of RxT treatment and after half the radiation course, almost all the patients presented severe ulcerative mucositis (Handschel *et al*, 2001b; Köstler *et al*, 2001). In our study, patients were biopsied when they received 3370 cGy on an average. All the patients presented clinically oral mucositis grade I (WHO), although they were submitted to pilocarpine and sucralfate therapies. Pilocarpine is helpful to minimize dry mouth sensation (Epstein and Schubert, 1987; Greenspan and Daniels, 1987) and sucralfate is a useful adjunctive therapy for mucositis (Epstein and Wong, 1994; Sur *et al*, 1994; Lievens *et al*, 1998; Cengiz *et al*, 1999; Etiz *et al*, 2000). Although some studies showed that sucralfate does not prevent or treat mucositis (Epstein and Wong, 1994; Lievens *et al*, 1998) a histopathological study demonstrated a reduction of submucosal inflammatory infiltrate when it was used (Etiz *et al*, 2000).

Epithelial hyperparakeratosis was found in eight cases and this probably results in a previous whitening of the oral mucosa after 2 weeks of radiotherapy (Scully and Epstein, 1996). A study with samples taken from 22 patients before and during irradiation for squamous cell carcinomas in the head and neck region showed microscopically, keratosis on irradiated mucosa (Dörr *et al*, 2001). Mature and well-differentiated cells, which promote epithelial keratinization, were also detected on desquamation of irradiated epithelium after 2 weeks of RxT (Stokman *et al*, 2002). Nevertheless, thinning of the epithelium and denuded areas was also observed at the end of the second week of the RxT (Scully and Epstein, 1996; Dörr *et al*, 2001). In our study, the epithelial perimeter and area of irradiated mucosa were lower than NMCP, with a reduction on epithelium thickness and rete pegs. Reduction of germinal and functional cell layers occurs progressively during RxT (Dörr *et al*, 2001). This explains why the mucosa turns erythematous and then ulcerates (Scully and Epstein, 1996). A study that evaluated two different mice strains submitted to RxT and showed reduced ulcer duration in a strain with a higher proliferative rate of oral mucosal cells (Dörr *et al*, 2002).

Epithelial atypia is evident on repair-like cells affected by radiotherapy with some being misinterpreted as malignancies (Kleebler and Somrak, 1993). Cellular pleomorphism, hyperchromatism, eosinophilia,

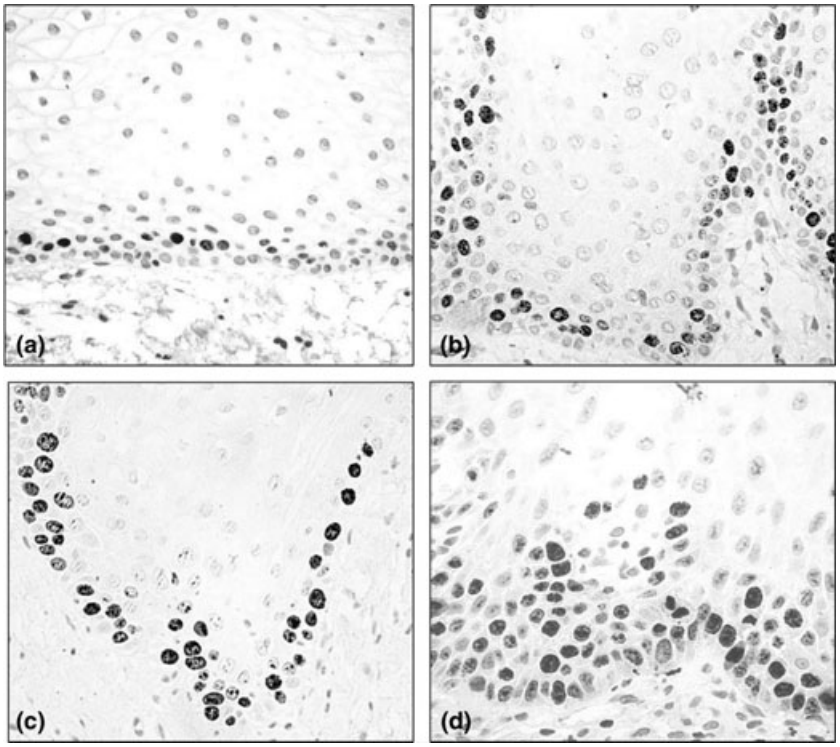

Figure 2 Expression of Ki-67-positive cells, Immunoperoxidase (200×). (**a**) Normal mucosa (NM) showing a few positive cells in parabasal layer. (**b**) Normal mucosa of cancer patients (NMCP) showing parabasal positive cells more commonly than NM. (**c**) Radiation-induced oral mucositis presenting basal and parabasal positive cells. (**d**) Oral dysplasia presenting basal, parabasal and suprabasal positive cells

vacuolization, enlarged nuclei and an increased nuclear/cytoplasmic ratio were observed in atypical cells in our study. Evaluation of the esophageal cells after chemoradiotherapy revealed 7.5% of cases with dysplasia-like epithelial atypia, with similar histomorphology like our atypical cells (Brien *et al*, 2001). In this report, dysplasia-like cases were less positive for p53 and MIB1 than true dysplasia. In several instances, the dysplasia-like changes were misinterpreted as neoplastic, which in retrospect led to unnecessary treatment. Similar diagnosis difficulties can be found between dysplasia-like induced by radiation and true dysplasia in the esophagus (Brien *et al*, 2001).

Inflammatory mononuclear infiltrate found in our cases was similar to that described by Etiz *et al* (2000) and Handschel *et al* (2001b). The latter study showed that the infiltrate was composed of CD4+ and 8+ lymphocytes and macrophages, mainly RM3/1 subtype macrophages. Granulocytes were rarely found. The CD68 positive cells in mucositis cases were found intensely and close to the epithelium may be down-regulating T lymphocytes, may be involved in suppressor functions (Handschel *et al*, 2001a). Nevertheless, further investigations are necessary to establish the role of the macrophages in oral mucositis. In short, during radiotherapy, macrophages seem to be the most prevalent inflammatory cell in oral mucositis (Handschel *et al*, 2001b). Our data are also in accordance with Handschel *et al* (2001b), which described a few macrophages scattered in the connective tissue in NMCP cases. Etiz *et al* (2000), compared sucralfate versus placebo use in head and neck radiotherapy and described statistical differences of the inflammatory infiltrate between irradiated tissues with and without previous sucralfate use. We used sucralfate in nine of 10 patients; therefore, we cannot evaluate its interference on the inflammatory infiltrate. Nevertheless, the inflammatory infiltrate was more intense in the oral mucositis group than found in NMCP.

Our study showed a significantly increased number of blood vessels compared with non-irradiated mucosa. This corroborates the findings of Etiz *et al* (2001) and Handschel *et al* (2001a), who reported an increased number of blood vessels during RxT and increased vascular permeability. A previous paper commented that ICAM-1 and E-selectin molecules and beta-2-integrin expression were increased in irradiated tissue and VCAM-1 expression was lower, indicating increased mononuclear leukocyte transendothelial migration and vascular preservation during RxT (Handschel *et al*, 1999). Although vascular alterations are better known on chemotherapy (Sonis, 1998), vascular proliferation possibly is associated with inflammation, but this awaits further evidence.

Ki-67 is widely used as a marker of cell proliferation (Gerdes *et al*, 1991; Bruno and Darzynkiewicz, 1992). Cells in G1, S, G2 and mitosis are positive for Ki-67, but cells in G0 and early G1 do not express Ki-67 (Gerdes *et al*, 1984). Direct correlation was observed between increased Ki-67 counts with dysplastic lesions (Kovesi and Szende, 2003). This finding is in accordance with increased positiveness for Ki-67 found in OD compared with normal tissue. It was previously observed that at the end of the first week of RxT, the proliferative activity of epithelial cells decreased. Epithelial cellular turnover was partially restored after the end of the first week and the continuing rate of declining cellular counts during the remainder of the therapy reduced

substantially (Dörr *et al*, 2001). Nevertheless, this proliferative reaction remains increased after the second week because of breaking of the normal restriction to asymmetric stem cell division and in addition, cells normally destined to exfoliate without division, regain their capacity to divide (Denham and Hauer-Jensen, 1996; Dörr, 1997; Dörr *et al*, 2001). In accordance with this fact, it is possible to justify the increased oral mucositis count compared with NM and NMCP. The samples were obtained mainly just after the second week of RxT when the reactional proliferation is evident (Dörr *et al*, 2001). To fortify this hypothesis, strong basal staining was only shown in oral mucositis and even remarkable proliferative activity was observed in the parabasal and suprabasal layers. Although oral mucositis and OD presented higher counts of Ki-67 positive cells, no statistical differences were observed when both were compared in our cases. This probably happened because of the dysplastic alterations observed in mucositis, which presented similar dysplastic features, in the majority of cases, than true oral dysplasia (Gonzalez-Moles *et al*, 2000). In addition, basal proliferation was evident in oral mucositis. It can be explained due to the alteration of the balanced replacement of proliferative parabasal cells and the break of normal restriction to asymmetric basal cell division (Kotelnikov *et al*, 1996; Dörr, 1997).

The NMCP samples showed statistical significant higher Ki-67 positive cells than NM. This probably happened because of the chronic use of tobacco and alcohol in NMCP that contributed toward mitogenesis and proliferation. Increased Ki-67 expression after cessation of smoking could indicate permanent epithelial alteration (Strong *et al*, 1984). In addition, Kotelnikov *et al* (1996) studied NMCP without dysplasia near to malignant tumors and observed high counts of positive cells for IdUrd and BrdUrd proliferation markers, probably resulting from the cancerization field. Although our samples were taken at least 3 cm away from the tumor, they were also exposed to carcinogens (Strong *et al*, 1984).

The expression of p53 in the various forms of dysplastic lesions points to the increasing instability of the genome, parallel with the severity. Even in relative incipient dysplastic lesions p53 positiveness was noted in accordance with our findings (Kovesi and Szende, 2003). The p53 protein induces apoptosis through transactivation of its target genes and is a major determinant of cellular responses to radiation (Burns and El-Deiry, 2003; Coates *et al*, 2003). Ionizing radiation of epithelial cells conducts to up-regulation of wild-type p53 and the subsequent induction of p21(waf1) (Marijnen *et al*, 2002). In normal rectal mucosa, after radiation, p53 and p21(waf1) were strongly up-regulated compared with the expression in unirradiated normal tissue ($P < 0.001$; Marijnen *et al*, 2002). A previous study described that ionizing radiation induced apoptosis in normal proliferating retinal epithelial cells through p53 activation associated with its increased level in the tissue (Jiang *et al*, 2004). Our data confirm an increased level of p53 in the irradiated tissue and its possible association with apoptotic death across the epithelium during the RxT sequence (Sonis, 1998; Jee *et al*, 2005). The p53 staining was not a differential between dysplastic-like and true dysplasia demonstrated by Brien *et al* (2001), maybe because of not similar expression behavior in the mucosal sites and the therapeutic approachment, which included chemotherapy.

The p53 activation down-regulating the hyperproliferation resulted in apoptosis, leading to epithelial atrophy in inflammatory processes (Zamolo *et al*, 2005). In the inflammatory processes, the apoptosis phenomenon is associated with Fas (CD95), a cell surface molecule that mediates the receptor-triggered, including auto-reactive T-cells, found in chronic, nonspecific inflammation (Baris *et al*, 2005). In addition, the radiation may break the DNA chain and hydrolyze the plasma membrane inducing cell apoptosis (Sonis, 2004). Further analysis may be realized to determine if resultant atrophy is also associated with p53 leading to apoptosis because of radiation, mainly or also associated with inflammatory process.

The resultant atrophy, reduced perimeter and area, apoptosis, p53 alteration and increased cellular turnover observed in our study could be suggesting the usefulness of cellular protectors, like amifostine agents and growth factors, like KGF and GM-CSF to prevent and treat oral mucositis induced by radiation (Plevová, 1999; Köstler *et al*, 2001; Sprinzl *et al*, 2001).

Conclusions

Grade I (WHO) oral mucositis caused by radiotherapy presented distinctive epithelial alterations, increased number of blood vessels, infiltration of mononuclear cells with increased number of macrophages, increased count of Ki-67 compared with normal tissue controls and positiveness for p53.

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