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Opportunistic actinomycosis in osteoradionecrosis of the jaws in patients affected by head and neck cancer: incidence and clinical significance

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Abstract

Actinomycosis is occasionally an opportunistic infection occurrence in patients with osteoradionecrosis (ORN). A retrospective study (1992–97) of 50 patients with ORN of the jaws was done to evaluate the incidence and its clinical significance in the management of ORN. Actinomycosis was diagnosed in 12% of ORN cases. In 36 of the 50 patients including five cases of actinomycosis, the ORN was considered to be resolved after treatment. The median treatment duration of ORN was significantly longer ($P < 0.007$) in patients with actinomycosis (29.7 months) than those without the disease (13.4 months). In conclusion, bone biopsy should be considered in cases of ORN with unsatisfactory response to its specific therapies, aiming to identify possible opportunistic actinomycosis infection.

Keywords: Actinomycosis incidence; Osteoradionecrosis; Jaws

1. Introduction

Osteoradionecrosis (ORN) is one of the most severe and serious oral complications of head and neck cancer treatment [1–3]. Despite of the improvements in pre-radiotherapy oral and dental care, the incidence of ORN has not significantly decreased in the last years, and has ranged from 1% to over 30% [4–6]. The pathogenesis of ORN is defined by a sequence of radiation, hypovascular–hypocellular–hypoxic tissue formation, and trauma induced or spontaneous mucosa breakdown leading to a non-healing wound [5]. This process is usually associated with signs and symptoms as intraoral/extraoral fistulas, trismus, pain, local infection, pathologic fracture and masticatory difficulties. Histologic findings include endarteritis, hyperemia, hyalinization, cellular loss, hypovascularization, thrombosis and fibrosis [7–9]. Some of these radiation effects, such as hyperemia, acute cellular loss and thrombosis occur in the early phase, and other effects like hypovascularization and fibrosis are evident 6–12 months after the end of radiation therapy [7]. Several different approaches have been used to treat ORN including conservative management, invasive surgery and hyperbaric oxygen therapy (HBO) [1–21]. Conservative measures seem ineffective and wasteful in the control of refractory ORN and are only indicated in small necrotic bone areas. Revascularization of irradiated tissues in association with invasive surgery is usually necessary for severe cases.

Actinomycosis is an infectious disease that classically presents as an acute or chronic swelling that is slowly progressive, painless, indurated, which can produce multiple abscesses and cutaneous fistulas. The acute and less frequent form is a rapidly progressive, tender and fluctuant mass, suggestive of an acute dental infection. Actinomycosis should be suspected when sulfur-like granules are observed in pus drainage. At

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microscopy, these yellow grains are composed of different species of *Actinomyces* but other bacteria can also be identified. Despite the presence of sulfur granules being almost pathognomonic of actinomycosis, the definitive diagnosis must be based on microbiological procedures or histologic examination [22–30]. Actinomycosis may behave as an opportunistic infection in patients with ORN of the jaws due to the local tissue injury by radiation therapy. However, this correlation is difficult to establish because of some clinical and microbiologic reasons: (1) clinical aspects, such as fistulas, pus drainage and tender mass, are very similar in both entities what makes low clinical suspicion of actinomycotic infection in this condition; (2) although the presence of sulfur granules is almost pathognomonic of actinomycosis, this clinical sign is not always identified in pus drainage or fistulas; and (3) microbiologic culture frequently fails to isolate *Actinomyces* [22–30].

Some studies in the literature have suggested that irradiated bone is susceptible to actinomycosis infection but only one paper has been published correlating actinomycosis and ORN [11]. The current investigation has the purpose of reporting the incidence of actinomycosis infection in ORN of the jaws and to discuss its clinical significance in the management of refractory ORN.

2. Patients and methods

The medical records of 50 patients with ORN of the jaws treated at the Hospital do Câncer A.C. Camargo, São Paulo, Brazil, between 1992 and 1997, were reviewed. Diagnosis of the ORN was based on patient's history and on clinical and radiological findings. All cases had a history of ORN of at least 3 months duration and a follow-up of at least 1 year. Histologic examination was only done for diagnosis of ORN when it was necessary to rule out local tumor recurrence or metastic disease. A total of 31 cases included in this study were submitted to histologic investigation because surgical approaches were considered an essential part for treatment of ORN. Microbiologic cultures were performed from pus drainage and fistulas during whole treatment of ORN in all these patients. Bone specimens taken from these surgical procedures were available for histologic examination (hematoxylin and eosin) in all patients. Additional staining methods (PAS, Gram, Ziehl, and Grocott's method for fungi) were performed if suspicion of actinomycosis infection was observed during this routine histologic examination.

All tissue specimens were placed in 10% formalin solution and then embedded in paraffin and sectioned in slides of 4–6 microns each. These slides were deparaffinized through two changes of xylene, and ran through absolute and 95% alcohol to distilled water: (1) Gram's method was obtained using approximately 1.0 cc of 1% crystal violet solution and mixed to 5% sodium bicarbonate solution. These slides were flooded in Gram's iodine solution and rinsed in water and decolorized with mixture of ether and acetone until no more blue color ran off. These sections were finally stained with 0.1% basic fuchsin solution. Bacteria were considered positive when colored in blue and negative in red; (2) Grocott's method was obtained placing the slides in working methanamine–silver nitrate solution at 58°C until sections turned yellowish brown. Adequate silver impregnation fungi should be dark brown. Unreduced silver was removed with 2% sodium thiosulfate solution and counterstained with light green solution. Fungi are sharply delineated in black with a pale green background; and (3) Ziehl-Neelsen's method was obtained using carbol fuchsin solution and decolorized with 1% acid alcohol. Dipping in methylene blue solution counterstained these slides. Acid-fast bacilli colored in bright red.

Conservative management consisted of local irrigation (chlorexidine 0.2%), systemic antibiotics in acute episodes, oral hygiene care and gentle removal of sequestrum in the lesions. Resection/HBO therapy were performed in cases of severe pain, unsatisfactory response to conservative management and progressive clinical outcome. Treatment duration was calculated from the date of ORN diagnosis to the mucosal cover recovery.

From the medical records the following information were considered: clinical features, tumor site, tumor stage, as well as radiation dose/fractionation/delivery. The TNM system was used in staging tumors.

The following criteria were used for the diagnosis of actinomycosis: clinical presentation, pus drainage from fistula and evidence of the microorganisms in anaerobic culture or tissue specimen. Patients with a definitive diagnosis of actinomycosis were submitted to on antibiotic therapy with oral ampicilin 4 g daily, for 6–8 weeks according to clinical response.

Statistical analysis was performed with a computer program (Statistical Package for the Social Sciences, SPSS for Windows v 7.5, SPSS Inc, Chicago, IL). Descriptive analysis and non-parametric Mann–Whitney U tests evaluated the data obtained from this study. Differences were considered to be significant at $P < 0.05$.

3. Results

Of the 50 patients reviewed, 45 (90%) patients were male and five (10%) female. The age distribution ranged from 33 to 79 years old (mean: 55.3 years). Forty patients (80%) were Caucasian, seven (14%) were black and three (6%) were Japanese. These patients were treated with conservative measures (31 cases/62%) and/or surgical/HBO therapy (19 cases/38%) depending on

the clinical behavior in each case. Diagnosis of actinomycosis infection was made in six cases (12%). Histopathologic examination revealed radiation injuries of the bone specimens characterized by large areas of necrotic bone lacking osteocytes and osteoblasts in bone lacunae. The bone marrow area was replaced by very dense fibrous tissue and in other areas by granulation tissue surrounded by inflammatory cells. Bone sequestrum formation was observed in all specimens and was separated from the mandible bone by a dense fibrosis or by granulation tissue. Histologic examination also disclosed the presence of *Actinomyces* within bone tissue specimens in all six cases. These basophilic bacterial colonies of *Actinomyces* were observed deep within the bone trabeculae with an intense inflammatory response containing numerous neutrophils and lymphocytes (Figs. 1 and 2). The colonies stained positively for PAS and Gram staining methods and also by Groccot's method, filamentous branching microorganisms could be observed suggesting *Actinomyces* (Figs. 3 and 4). In these six patients, treatment of ORN included a combination of conservative management, radical surgery and HBO therapy (2 h per day; 30 sessions; 2.4 ATA, 100% oxygen). The demographic data, tumor site, tumor stage and oncologic treatment of these patients are shown in Table 1.

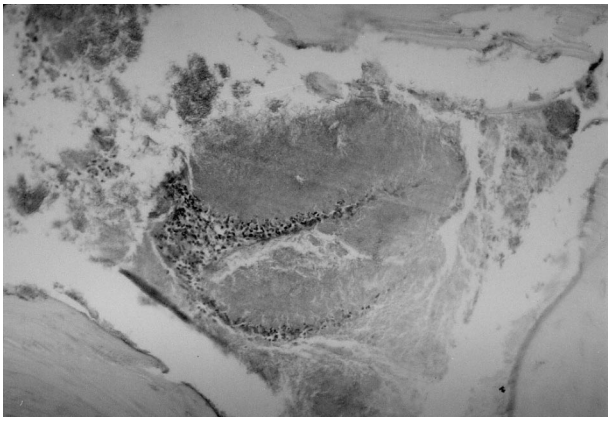

Fig. 1. Histologic section of bone specimen showing basophilic bacterial colony of *Actinomyces* surrounded by an intense inflammatory response in bone marrow space (hematoxylin and eosin stain. Magnification, ×100).

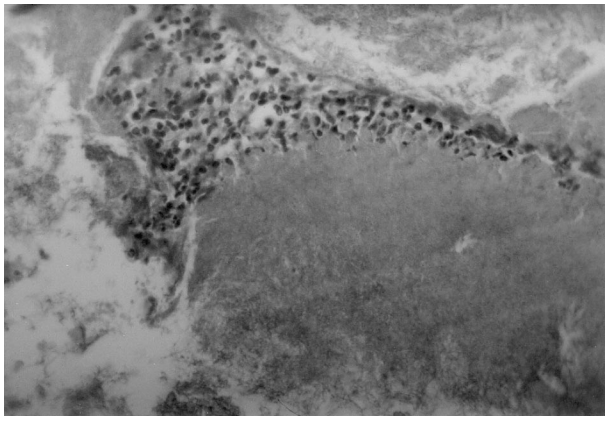

Fig. 2. Basophilic colony of *Actinomyces* surrounded by numerous neutrophils and lymphocytes (hematoxylin and eosin stain. Magnification, ×400).

Fig. 3. Positive staining of colony of microorganisms for gram's method (Gram stain. Magnification, ×400).

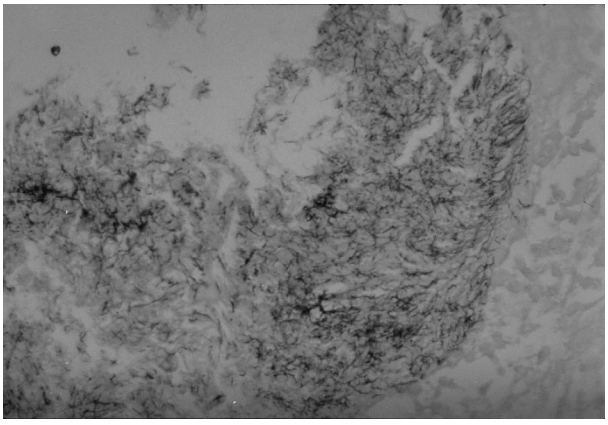

Fig. 4. Colony of filamentous branching microorganisms stained positively for Groccott's method (Groccott stain. Magnification, ×400).

Demographics and clinicopathologic characteristics of the 50 patients with ORN including age, sex, gender, tumor TNM stage, total radiation dose, HBO therapy, ORN site, stage, duration of treatment and results are presented in Table 2. There were no statistical differences between patients who had actinomycosis and those who had not in demographic and clinicopathologic characteristics with the exception of ORN stage, for which the patients with actinomycosis had more aggressive ORN ($P < 0.021$) than those patients without actinomycosis. Patients with actinomycosis (five cases/83.3%) were more frequently treated with HBO therapy ($P < 0.011$) than those without actinomycosis (14 cases/31.8%). Patients with or without actinomycosis had no differences in healing of ORN ($P < 0.122$);

Table 1
Summary of the six cases of osteoradionecrosis (ORN) associated to actinomycosis[a]

Case	Age/sex	Tumor site (Stage)	Tumor treatment (Dosage)	Treatment of ORN	Time RT-ORN (months)
1	70/M	Palate (T2N0MO)	Radiation, exclusive (46 Gy)	Marginal resection + HBO	28
2	58/M	Tonsillar (T3N1M0)	Surgery + postoperative radiation (40 Gy)	Sequestrectomy	4
3	39/M	Tongue (T4N2M0)	Surgery + postoperative radiation (72 Gy)	Marginal resection + HBO	2
4	56/M	Larynx (T2N0M0)	Radiation (45 Gy) + salvage surgery	Sequestrectomy	2
5	62/M	Tongue (T1N0M0)	Radiation, exclusive (70 Gy)	Marginal resection + HBO	4
6	59/M	FloorMouth (T4N2M0)	Radiation (72 Gy) + salvage surgery	Radical resection + HBO	72

[a] RT, radiotherapy; M, male; HBO, hyperbaric oxygen therapy.

Table 2
Univariate analysis for clinicopathologic variables

Variable	Patients without actinomycosis/%	Patients with actinomycosis/%	*P*
Age			0.279
< 55	25/56.8	2/33.3	
> 55	19/43.2	4/66.7	
Sex			0.384
Male	39/88.6	6/100.0	
Female	5/11.4	–	
Gender			0.384
Caucasian	36/81.8	4/66.7	
Black	8/18.2	2/33.3	
Clinical stage			0.706
I–II	18/41.9	3/50.0	
III–IV	25/58.1	3/50.0	
Site of ORN			
Mandible	40/90.9	6/100.0	0.441
Maxila	4/9.1	–	
Stage of ORN			
Acute/progressive	15/34.1	5/83.3	0.021
Chronic/stable	29/65.9	1/16.7	
Total dose			
< 62 Gy	22/50.0	3/50.0	1.00
> 62 Gy	22/50.0	3/50.0	
HBO			
Yes	14/31.8	5/83.3	0.011
No	30/68.2	1/16.7	
Healing of ORN			
Yes	31/70.5	5/83.3	0.122
No	13/29.5	1/16.7	
Treatment of ORN mean (months)	13.4	29.7	0.007

however, the median treatment time of ORN was significantly longer ($P < 0.007$) in patients with actinomycosis than those without disease (29.7 and 13.4 months, respectively) (Table 2).

4. Discussion

This study presents a retrospective investigation of six cases of actinomycosis observed in patients with ORN of the jaws diagnosed during a 5-year period. Their distribution according to age and gender agreed with the population profile of patients with head and neck cancer. In five patients, the initiating factor for the development of ORN were the surgical oncological procedures performed prior to and/or after radiation therapy with insufficient time for wound healing. In Case 6, the initiating factor was not related to trauma but there was a spontaneous mucosa breakdown.

Actinomyces belong to the family Actinomycetales and were first described almost 200 years ago. Currently, *Actinomyces* are considered to be bacteria, but they were previously reported as fungi or some forms in between these two species [23,27–29]. *Actinomyces* are microaerophilic, gram-positive, filamentous, branching, non-acid, nonspore-forming bacteria. Some different species of *Actinomyces* have been identified in the last decades. The most commonly observed species is *Actinomyces israelii* that is responsible for infection in man. Other species of *Actinomyces* can also produce infection in man such as *A. naeslundii, A. viscosus,* and *A. odontolyticus*. *Actinomyces bovis* has not been isolated in man but has been attributed to this species the responsibility for 'lumpy jaw' in cattle. Other different species can be classified within the family of *Actinomyces* (*Nocardia* and *Arachnia propionica*) that are microorganism morphologically identical and can only be distinguished by their end products and aerobic growth. *Actinomyces* is a commensal mucosal inhabitant of the oropharyngeal and gastro-intestinal region. It has been isolated from tonsillar crypts, mucosal surfaces, caries lesions and saliva [23–26,27,29].

Actinomycosis has been divided according to the anatomic site affected by the lesion in cervicofacial, abdominal and pulmonary forms. The disease affects the head and neck region in approximately 55%, abdominal 20%, and pulmonary 15% [22,23,28,29]. The remaining are observed in other locations and account for 10%. Cervicofacial actinomycosis mainly occurs in

young adults, and is more common in males than females (ratio 3:1 male/female) [28–30].

It is believed that clinical manifestation is caused by an endogenous infection with a history of trauma, either accidental (bone fracture) or from dental treatment such as root canal therapy, extraction, pericoronitis and caries with periapical lesions, that permits anaerobic growth into adjacent soft and/or hard tissue. Other risk factors found are diabetes, immunosuppression and local tissue injury by radiation therapy. Although irradiated patients have been pointed to be susceptible for actinomycosis infection, only one study was found in the literature correlating actinomycosis and ORN [11]. In a 5-year retrospective study of 50 cases of ORN of the jaws, six cases (12%) of *Actinomyces* infection were found. This incidence can be even higher because of difficulties to establish the definitive diagnosis of actinomycosis. Actinomycosis infection is established based on clinical aspects, positive culture of the microorganism, or its observation in tissue sections [22–30]. However, the clinical aspects of cervicofacial actinomycosis are very similar to ORN, except for the observation of sulfur-like granules in actinomycosis what makes very difficult clinical suspicion of actinomycosis infection in these patients. Another major difficulty in the establishment of an actinomycotic infection diagnosis is to obtain a positive culture. The major problems related to microbiologic examination are concomitant aerobic and anaerobic bacterial overgrowth. These microorganisms are extremely delicate especially if the species are exposed to air for a prolonged period because of the slow growth pattern and the necessity of a careful anaerobic culturing atmosphere [22–30]. Histologic examination is probably the most common technique to obtain the definitive diagnosis of actinomycosis and it was used in our study. In 1983, Happonen et al. [11] reported a histopathologic study of five cases of actinomycosis in ORN of the jaws in which the difficulty to reach a final diagnosis of actinomycosis was also shown. In that study, culture was negative for four out of five cases and definitive diagnosis was possible through by immunocytochemical methods using specific antisera against the suspected pathogenic microorganism.

Treatment of cervicofacial actinomycosis usually involves long-term and high-dosage antibiotic therapy associated with surgical removal and drainage of the infection foci [22–30]. Several antibiotics have been used for actinomycosis such as tetracycline, clindamycin, erythromycin, chloramphenicol and penicillin, which is the preferred one. In our cases treatment of actinomycosis consisted of surgery in association with antibiotic therapy (ampicilin 4 g daily for 6–8 weeks) until complete clinical healing of actinomycosis infection (no evidence of pus drainage or fistula). However, there are controversies about the dosage and duration of antibiotic therapy [22–30].

Radiation therapy creates one of the predisposing factors for the occurrence of an actinomycosis infection; that is a favorable environment for the microorganism to flourish due to bone tissue alterations. For a long time ORN was considered an osteomyelitis in irradiated bone as a result of a triad of radiation, trauma and infection [5–7]. The pathogenesis of ORN is understood as a sequence of radiation, formation of hypoxic, hypocellular and hypovascular tissue, followed by tissue breakdown and non-healing wound [5]. Microorganisms do not play a role in the pathogenesis of ORN, but may secondarily infect the wound turning its diagnosis and treatment more difficult. The current study shows that patients with actinomycosis infection had significantly longer ($P < 0.007$) treatment period than those without actinomycosis.

Treatment of ORN cannot be universally applied in the same manner for all patients. Some authors [6,20] suggest a more conservative therapy consisting of surgical debridement and resection of nonviable necrotic tissue, and other authors [7–9,12,15–18,21] report that it should include revascularization of the tissue with HBO. Clinical and experimental studies have confirmed the benefits of revascularization of irradiated tissues in association with conservative procedures or sequestrectomy and resection [7–9,12,15–18,21]. However, some studies have also reported that wound healing can have a reduced potential or take a longer time but may still lead to final repair of tissue [6,20]. Our previous study [19] with 104 patients with ORN of the jaws showed that complete healing of ORN occurred in 44 (42.3%) cases with a mean healing time of 24 months. The same observation was reported by Wong et al. [20] in 1997. The current study shows that opportunistic actinomycosis in ORN of the jaws can affect the results of treatment in a number of patients.

ORN is a distinctive entity resulting from a number of radiation injuries in hard and soft tissues and, consequently, a spectrum of treatment modalities is indicated. Small lesions and/or those that produce sequestrum usually have a good prognosis that may heal after conservative management. Refractory ORN requires surgery and HBO therapy. The refractory ORN due to association of actinomycosis infection is responsible for longer period of treatment because of the nonspecific therapy applied in this situation. Bone biopsy should be considered in cases of refractory ORN that are not responsive to either conservative or surgery/HBO therapy.

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