Medication-Related Osteonecrosis Of The Jaw: A Histological Comparison With Osteoradionecrosis Of The Jaw And Osteomyelitis Of The Jaw

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Abstract

Medication-related osteonecrosis of the jaw (MRONJ) is an adverse side effect of drugs used to treat bone diseases. As the population continues to age with the aid of medications, MRONJ has been increasing in the case number and becomes an issue in dentistry. Until now, the pathophysiology of MRONJ is still unclear. A histological investigation of MRONJ specimens is a powerful tool to offer a broad view and basic knowledge of the disease. The knowledge gained from histological analysis might suggest meaningful directions for further research. In this study, 57 subjects, including patients with MRONJ, osteoradionecrosis of the jaw (ORN), osteomyelitis of the jaw (OM), and normal jaw bone, were studied. Hematoxylin and eosin-stained slides of these diagnosed cases were reviewed to confirm the diagnoses and to investigate the histologic features. Pearson's chi-square test and Fishers' exact test were performed to compare the histological features of 4 groups in pair. Logistic regression was fitted with multiple covariates to analyze the correlation. The results showed that MRONJ, ORN, and OM shared the characteristic feature of necrotic bone. The significant difference found between MRONJ and ORN was the presence of fibrous tissue ($p<0.05$), and between MRONJ and OM was the status of bacterial colonies ($p<0.05$). Although there was no significant difference in the presence of osteoclasts among groups, osteoclasts in MRONJ showed giant cells containing abnormal shaped and multi nuclei comparing with others. These findings suggest that there are differences in the histopathology of MRONJ and other necrotic diseases and these might be evidence to suggest the pathogenesis of MRONJ.

Keywords: *Medication-related osteonecrosis of the jaw, Histology, Osteoclast, Bacterial colony*

1. Introduction

Medication-related osteonecrosis of the jaw (MRONJ) is primarily a serious side effect of anti-resorptive agents used to manage skeletal events, comprising osteoporosis, multiple myeloma, and bone metastases. MRONJ was first reported by Marx (2003) as one of the most serious side effects of bisphosphonates (BPs) therapy and it was called "Bisphosphonate-related osteonecrosis of the jaw." After that, other drugs have been reported to relate to this serious disease, thus it was renamed medication-related osteonecrosis of the jaw (MRONJ). MRONJ can be diagnosed by the patient history of drug use and clinical presentations. The patient with MRONJ presents with exposed bone in the maxillofacial region that can be probed through at least one intraoral or extraoral fistula for at least 8 weeks; and has a history of received treatment with bisphosphonates or denosumab or anti-angiogenic therapy and are without being exposed to radiation for treating of head and neck cancer (Ruggiero et al., 2014). Due to the unclear underlying mechanism of MRONJ, the management is symptomatic treatment, and it is not always successful. Several hypotheses regarding the etiology of MRONJ have been proposed to involve suppression of bone remodeling, the occurrence of inflammation and infection, inhibition of angiogenesis, microtrauma, or immune cell dysfunction (Ruggiero et al., 2014), but the exact mechanism is still controversial.

Osteoradionecrosis (ORN) is a severe complication of radiotherapy used to manage head and neck cancer. The prevalence of ORN varies widely in the literature but the most frequently reported prevalence rate is 5–15%. The variability in the prevalence of ORN depends on many factors such as total radiation dose, oral hygiene, dental extractions, a property of tumor, as well as chemotherapy. According to the literature,

ORN was defined as an exposed irradiated bone that failed to heal for at least 3 months and is without evidence of persistent or recurrent tumor (Chronopoulos, Zarra, Ehrenfeld, & Otto, 2018).

Osteomyelitis (OM) is one of the oldest known inflammatory diseases. The most common cause of OM of the jaw is supposed to be induced by polymicrobial odontogenic infection. Osteomyelitis is defined as an inflammatory condition of the bone involving the medullary cavity, Haversian systems, and periosteum of the bone. In a clinical presentation, OM usually shows symptoms such as swelling, suppuration, fistula formation, and bone sequestration but not an exposed necrotic bone (Baltensperger & Eyrich, 2009).

At present, all of these three necrotic cases have become urgent issues in dental practice. Although sharing similar clinical symptoms in MRONJ, ORN, and OM, their managements are different (Lima et al., 2014; Nadella, Kodali, Guttikonda, & Jonnalagadda, 2015; Nicolatou-Galitis et al., 2019). Previous studies presented various findings on the characteristics among MRONJ, ORN, and OM disease groups (De Antoni et al., 2018; Marx & Tursun, 2012). In general, the histological features of MRONJ, ORN, and OM are quite similar, but their difference might come from different risk factors, sample selection, technique, and statistical method. Each difference found may be the premise for meaningful future research direction. Thus, the objective of this study was to evaluate the histological features of MRONJ and compare them with ORN, OM, and normal jaw bone.

2. Objectives

1) To evaluate and describe the histological features of MRONJ
2) To compare the histological characteristic of MRONJ with ORN, OM, and the control group

3. Materials and Methods

3.1 Sample selection

Prior to the study, all procedures performed in this study were approved by The Human Research Ethics Committee of Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand (060/2020). A total of 57 subjects, consisting of 17 MRONJ patients, 15 ORN patients, 15 OM patients, and 10 normal bone cases, was from the Surgical Pathology archive of the Department of Oral Pathology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand, between 2010 to 2020. The subjects were reviewed to study the patient information, including age, gender, and lesion locations. The patient history and clinical presentation information from biopsy reports of all studied cases was reviewed to whether the cases meet the diagnosis criterion. The MRONJ samples have to meet the clinical criteria as follows: clinical evidence of more than 8 weeks of the exposed jaw bone, documented therapy with antiresorptive or antiangiogenic agents, and no radiotherapy. The ORN samples have to meet the clinical criteria as follows: evidence of devitalized and exposed jaw bone in a previously irradiated field, absence of local neoplastic processes, and no therapy with bisphosphonates. The OM samples have to meet the clinical criteria as follows: evidence of chronic inflammatory processes in the jaw bone, no therapy with antiresorptive or antiangiogenic agents, and no radiotherapy. The control samples have to meet the clinical criteria as follows: never been treated with antiresorptive, antiangiogenic agents, medications significantly affecting jaw bone homeostasis, no local radiation exposure, and did not suffer from intraoral inflammation. Ten normal jaw bone specimens were retrieved as the control.

3.2 Histologic analysis

After case review, hematoxylin and eosin (H&E) slides of selected cases were retrieved for histologic analysis. All samples were analyzed under an optical microscope. To study histopathologic profile, histologic features were evaluated involving soft tissue and hard tissue as shown in Table 1.

Table 1 Histologic examination of studied parameters

Observation target
Hard tissue (Osteocyte, osteoblast, osteoclast, peripheral resorption)
Soft tissue (Granulation tissue, fibrous tissue)
Inflammatory cells (Plasma cells, lymphocytes, neutrophils)
Bacterial colony (Likert scale)

3.3 Statistical analysis

All statistical analysis was performed with SPSS software (version 26, IBM, New York, USA). Histological features of 4 groups were compared in pairs using Pearson's chi-square test and Fishers' exact test when appropriate. Logistic regression was fitted with multiple covariates to evaluate the relationship of relative factors including gender, age, location, and histologic variables. A p-value of less than 0.05 was defined as statistically significant.

4. Results and Discussion

The demographic and clinical information of 57 patients with MRONJ (17), ORN (15), OM (15), and normal jaw bone (10) was collected from biopsy reports. Females were accounted for 88% of the MRONJ patients while the majority of ORN patients were male (80%). The OM group was also female predominant. The average age of the MRONJ group was higher than the ORN and OM groups. Most lesion specimens collected in each group came from lower jawbones (> 70%). The demographic feature of the sample groups performed in this study was not similar to the previous studies (De Antoni et al., 2018; Gross et al., 2017), which might lead to different results in evaluating and analyzing histological characteristics among these disease groups. The patient data are shown in Table 2.

Table 2 Patient data

	MRONJ	ORN	OM	Control
Number	17	15	15	10
Sex	88.2% women	80% men	66.7% women	60% women
Age	74.7 $\pm$ 10.67	55.9 $\pm$ 11.78	54.7 $\pm$ 19.83	40.7 $\pm$ 17.32
Lesion location	70.6% mandible, 29.4% maxilla	86.7% mandible, 13.3% maxilla	73.3% mandible, 36.7% maxilla	80% mandible, 20% maxilla

All 17 cases of MRONJ were confirmed to have necrotic bone, characterized by empty osteocytic lacunae. 16/17 (94%) of MRONJ showed bones with peripheral resorptions indicating irregular outline. The presence of osteoblasts was identified in 7 cases (41%), and the presence of osteoclasts was identified in 11 cases (65%) of MRONJ. Meanwhile, necrotic bones were also seen in 15 cases (100%) of ORN and 13/15 (86%) of OM. 13/15 (86%) of both ORN and OM showed scalloped bone borders. The presence of osteoblasts and osteoclasts of ORN was identified in 4/15 (26%) and 9/15 (60%), respectively. In OM, the presence of osteoblasts and osteoclasts was detected in 8/15 cases (53%) and 9/15 (60%), respectively. Bone specimens of MRONJ showed similar characteristics with ORN and OM, in which the characteristic feature of necrotic bone as empty lacunae, absence of osteoblastic rimming, and border resorptions representing empty Howship's lacunae. Though border resorptions were observed in the bone specimens of ORN and OM, peripheral resorptions as abnormal bone margins appeared to be more pronounced in the MRONJ. These histological features of all 3 lesions were different from the normal jaw bone. All 10 cases of the normal bone showed vital bone with osteoblastic rimming presence and smooth bone surface without a sign of bone resorption. These findings were similar to previous studies, and it is almost impossible to distinguish the disease diagnostic based solely on the histopathological characteristics of the hard tissue (Chaisuparat & Jham, 2015; De Antoni et al., 2018; Marx & Tursun, 2012). The histological features of the hard tissue of the four groups are shown in Figure 1.

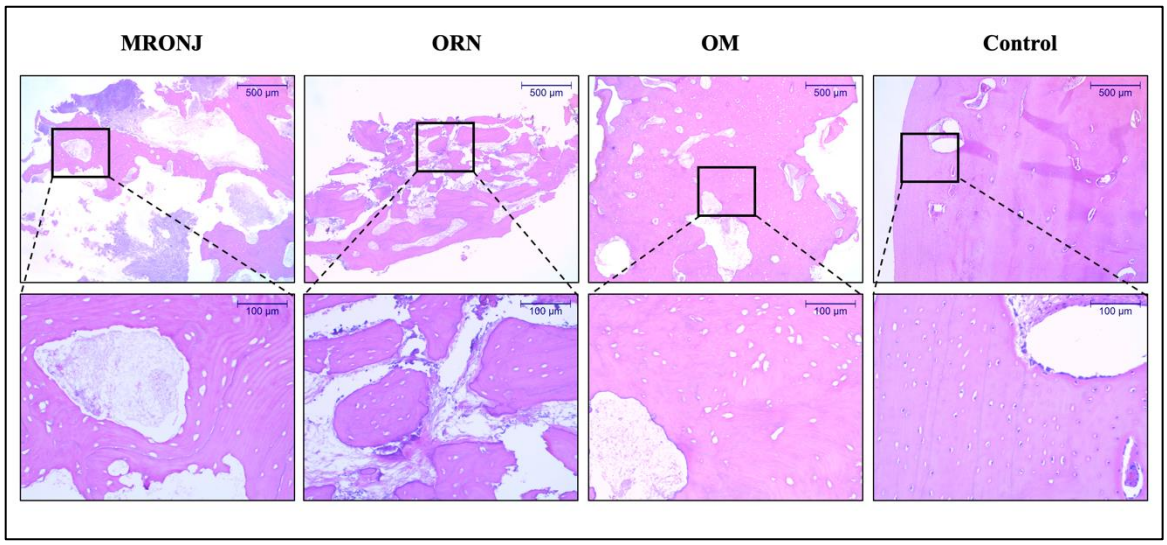

Figure 1 Histological features of necrotic bone comparing with the normal jaw bone. Representative images are shown at 2 magnifications.

Soft tissue observations in MRONJ showed that 9/17 cases (52%) identified the presence of granulation tissue and 3/17 cases (17%) identified the presence of fibrous tissue. Inflammatory cell infiltration was observed in 15/17 cases (88%) with mainly mixed inflammatory cells. Meanwhile, the presence of granulation tissue and fibrous tissue observed in ORN were 7/15 cases (46%) and 9/15 cases (60%), respectively. Soft tissue evaluation in OM indicated 5/15 cases (33%) to have granulation tissue and 3/15 cases (20%) to have fibrous tissue. Inflammation was identified in 13/15 cases (86%) of ORN and 14/15 cases (93%) of OM. Normal jaw bone showed granulation tissue in 1 case (10%), fibrous tissue in 1 case (10%), and inflammation in 2 cases (20%). As shown in Figure 2B, the analyzed result indicated a significant difference in the presence of granulation tissue between MRONJ and the control group ($p<0.05$). ORN showed a significant difference in marrow fibrosis compared with others ($p<0.05$). This finding was consistent with a newly accepted theory about radiation-induced fibrosis damages to normal tissue (Lyons & Ghazali, 2008). Inflammatory cell infiltration increased in the necrotic group diseases compared with the normal jaw bone ($p<0.01$), however, the inflammation was equally found in all three necrotic bone groups when compare in pair ($p>0.05$). The presence of neutrophils was observed in all three necrotic bone groups, and there was no difference between them ($p> 0.05$), unlike the previous study which showed that the MRONJ significantly lack leukocytes (De Antoni et al., 2018; Marx & Tursun, 2012). The role of inflammation is one of the proposed hypotheses of MRONJ pathology. The occurrence of inflammation and the presence of inflammatory cytokines have been shown their role in the process that drives MRONJ to occur (Morita et al., 2017). The histological features of the soft tissue of four groups are shown in Figure 2.

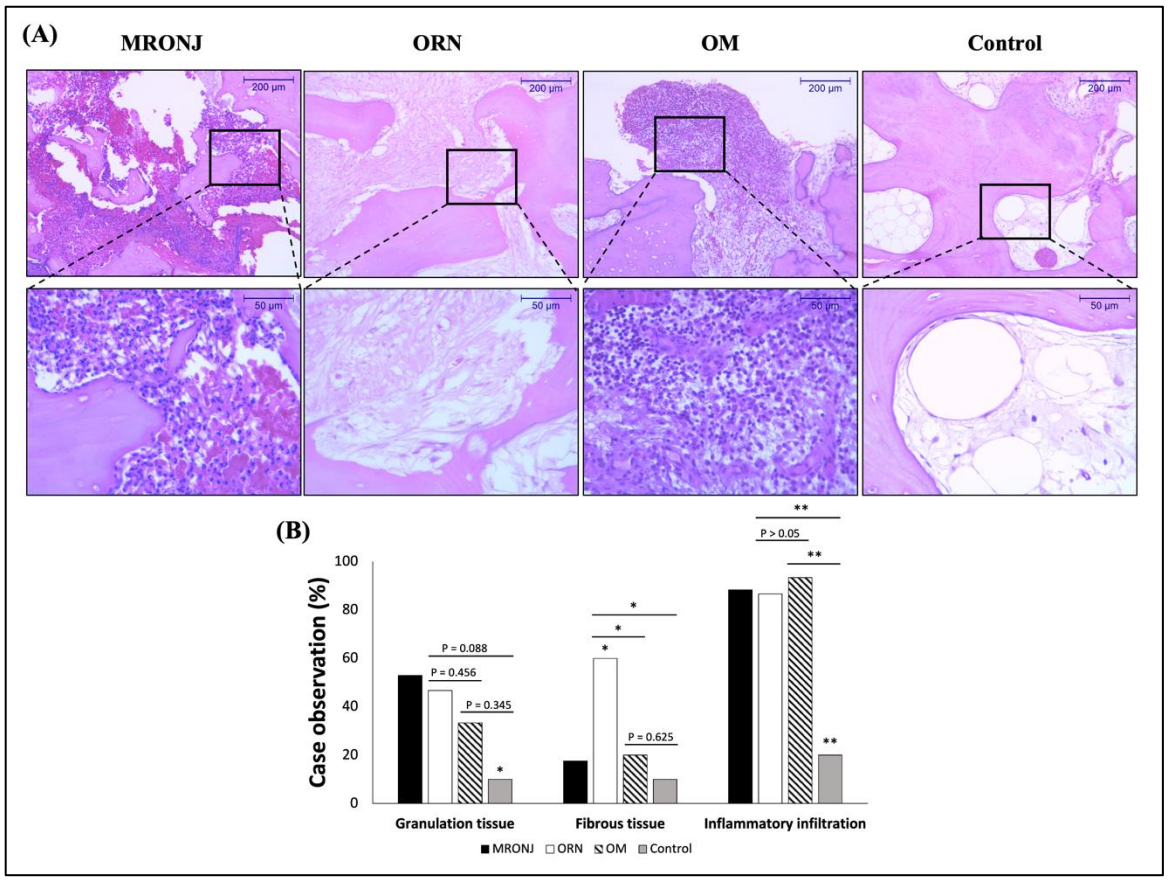

Figure 2 Histological feature of soft tissue and inflammation in necrotic bone groups and normal jaw bone. (A) Representative images of soft tissue are shown at 2 magnifications. (B) Histological analysis of soft tissue.

Histological evaluation of the bone specimens showed the presence of bacterial colonies in 15/17 cases (88%) of MRONJ. Similarly, ORN showed the presence of bacterial colonies in 13/15 cases (86%), and this percentage in OM was 9/15 cases (60%). The normal jaw bone had no sign of infection. The analyzed result indicated that there was a significant difference in the status of microorganisms between MRONJ and OM ($p<0.05$), but not between MRONJ and ORN or ORN and OM (Figure 3E). Intriguingly, the statistical analysis pointed out the significant association between the bacterial density and the presence of osteoblasts ($p<0.01$) in the necrotic specimens. However, a significant association was only found between the status of microorganisms and the presence of osteoblasts in MRONJ ($p=0.026$) and OM ($p=0.007$) when analyzing individual groups. A logistic regression model fitted with other factors as covariates also showed no significant correlation between the bacterial density with the presence of osteoblasts ($p=0.243$) (Table 3). The characteristic of bacterial colonies was also quite different among groups. As shown in Figure 3A-D, the MRONJ and ORN specimens showed a lot of dense bacterial clusters found in the peripheral area of bone whereas sparse bacterial colonies located within the marrow bone space of OM specimens, in agreement with previous studies (Chaisuparat & Jham, 2015; De Antoni et al., 2018; Marx & Tursun, 2012). A bacterial infection is considered a component in the pathogenesis of MRONJ when necrosis occurs only in the jaw, where it is easily damaged and penetrates by microorganisms. The high prevalence of bacteria, especially *Actinomyces spp*. in MRONJ, has been reported and is receiving increasing attention (Russmueller et al., 2016).

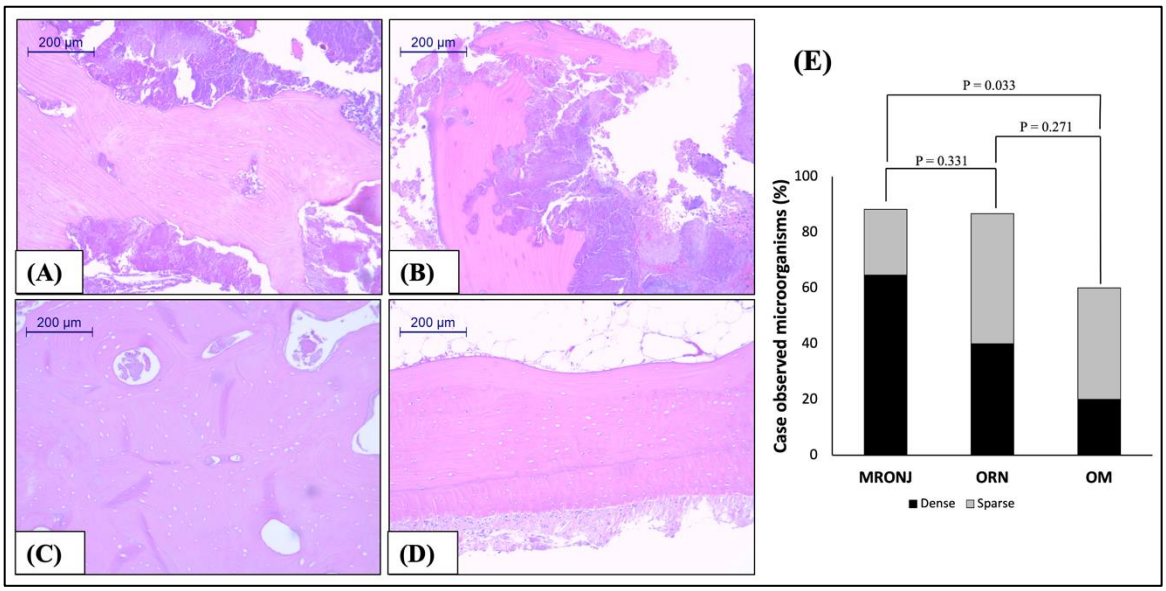

Figure 3 The presence of bacterial colonies observed in necrotic bone groups. Massive bacterial colonies were found in the bone surface of (A) MRONJ specimens and (B) ORN specimens. (C) Sparse bacterial colonies were observed to locate within the marrow bone space of OM specimens. (D) Normal jaw bone had no sign of infection. (E) Analysis of bacterial colonies.

Table 3 Association between the bacterial density and the bone cells with other factors as covariates

	Odd ratio	95% confidence interval	p-value
Osteoblast	0.267	0.029 – 2.451	0.243
Osteoclast	3.561	0.388 – 32.667	0.261
Gender	8.258	0.851 – 80.119	0.069
Age	0.939	0.870 – 1.013	0.104
Lesion location	2.853	0.391 – 20.831	0.301
Diagnosis	4.725	1.164 – 19.177	0.030*
Inflammation	11.319	0.646 – 198.269	0.097

Osteoclasts are worth noting despite no significant difference in their presence when compare groups in pair ($p>0.05$). Empty Howship's lacunae observed in most specimens of MRONJ suggests that many osteoclasts have disappeared due to drug effects. Image of dispersed nuclei into the cytoplasm and loss of ruffle border indicative of apoptosis cell was also observed in osteoclast of MRONJ as shown in Figure 4. However, the frequency of osteoclast encounters does not change significantly, suggesting that osteoclast might against drug-induced apoptosis and somehow persisted. Interestingly, many osteoclasts of MRONJ were noted with giant shapes comparing with small osteoclasts found in other groups (Figure 5). The giant, hyper-nucleated osteoclasts and the increase in the number of osteoclasts were also reported (Gross et al., 2017; Weinstein, Roberson, & Manolagas, 2009). The response of osteoclast in MRONJ remains unclear now. The analytical result showed that there was a significant relationship between the presence of osteoblast and osteoclast ($p<0.05$) in MRONJ and OM, but not in ORN ($p = 0.103$). These results support findings that osteoblasts, osteoclasts, and osteocytes respond mutually in MRONJ (Kim et al., 2019). Adjusting for the patient demographic data, diagnosis group, peripheral resorption, inflammatory cell infiltration, and level of bacterial colonies as covariates, a logistic regression model showed the presence of osteoblast (OR = 64.374, one-sided $p = 0.007$) still be significantly associated with the presence of osteoclast (Table 4). Therefore osteoblast might play a role in osteoclast's response in MRONJ. Further studies are needed to evaluate osteoclast features and function to confirm the proposed mechanism.

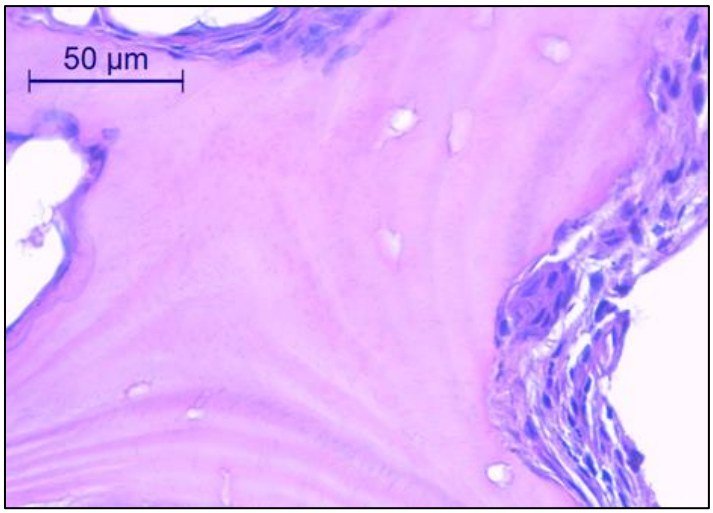

Figure 4 Osteoclast in MRONJ showed an image of dispersed nuclei into the cytoplasm and loss of ruffle border

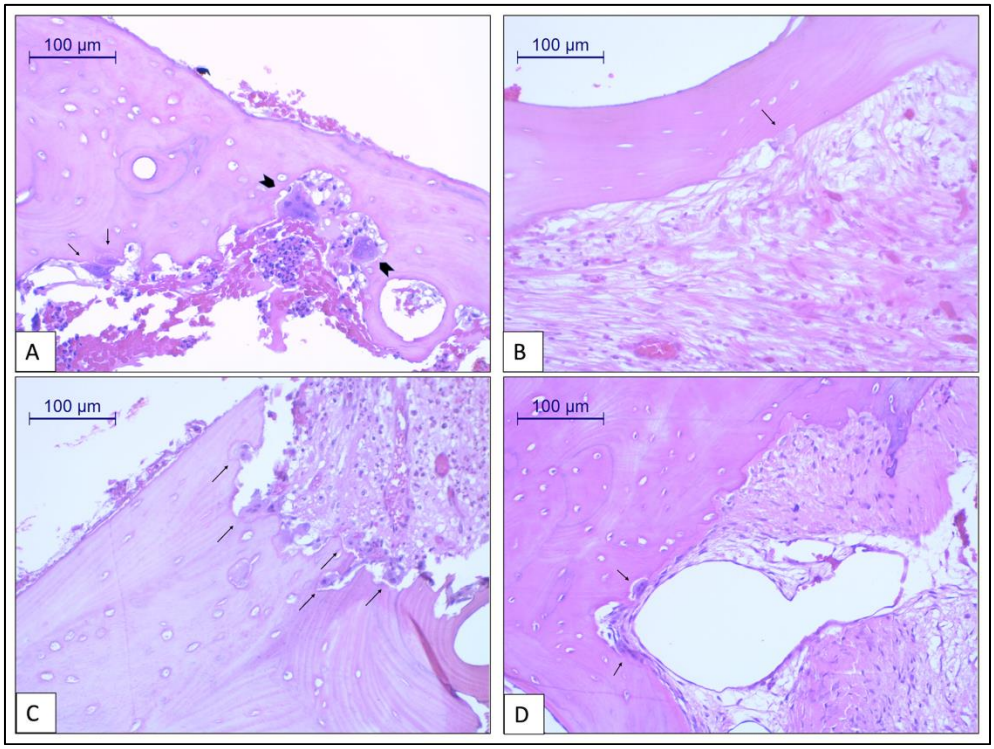

Figure 5 Osteoclasts (black arrow) were seen to digest bone. (A) Giant osteoclasts (black chevron) and small osteoclasts of MRONJ were digesting necrotic bone, surrounding area showed blood clots and cellular debris. (B) Necrotic bone with prominent marrow fibrosis was resorbed by the osteoclast. (C) Many small osteoclasts with 2-4 nuclei were digesting necrotic bone in OM specimens, inflammatory cell infiltration was also observed. (D) Bone resorption by osteoclasts in the normal jaw bone.

Table 4 Association between the presence of osteoclast and osteoblast with other factors as covariates

	Odd ratio	95% confidence interval	p-value
Osteoblast	64.374	3.073 –1348.612	0.007*
Gender	0.821	0.175 – 3.852	0.803
Age	1.058	0.994 – 1.126	0.076
Lesion location	5.874	0.640 – 53.889	0.117
Diagnosis	0.693	0.213 – 2.251	0.541
Peripheral resorption	0.860	0.062 – 11.947	0.911
Inflammation infiltration	1.166	0.100 – 13.618	0.902
Bacterial colony	0.561	0.094 – 3.366	0.527

MRONJ is a skeletal disease with a complex mechanism. There are many risk factors in which their roles have to be seen in the overall picture. As the cells are directly affected by antiresorptive agents, the role of osteoclasts is highlighted. Histopathological evidence of empty Howship's lacunae in bone border and dispersed nuclei into the cytoplasm in osteoclasts suggested the apoptosis process of these giant bone-eating cells. This finding is consistent with widely accepted knowledge about the mechanism of action of antiresorptive agents. On the other hand, the giant shape and same frequency of osteoclast encounter when comparing MRONJ specimens with other bone groups suggest a transformation to survive (Weinstein et al., 2009). This conflicting finding showed the complicated response of osteoclast in an oral environment with multi influencing factors. No relationship was founded between osteoclast and other factors such as inflammation and microorganism due to a lack of details. Observing and evaluating the presence of features is not enough to conclude. It is necessary to investigate more about the number and morphology of osteoclasts to find out the results. However, other studies have shown that osteoclast, inflammation, and bacteria are closely related (Morita et al., 2017; Williams et al., 2020).

5. Conclusion

The histopathologic observation from this study showed the similarities in necrotic bone and inflamed soft tissue characteristics among MRONJ, ORN, and OM. The significant difference noted between MRONJ and ORN was fibrosis, which is evidence to support the proposed pathogenesis about radiation-induced fibrosis of ORN. Bacterial colony status between MRONJ and OM also showed a significant difference. Furthermore, bacterial density on the necrotic bone groups was found to be related to the presence of osteoblasts and osteoclasts. However, the actual relationship between them was not clear on further analysis. Other highlighted points in the MRONJ are the peripheral resorption that showed irregular shape, high prevalence of dense bacterial clusters on bone surfaces, and giant osteoclasts. Although the presence of these characteristics is not enough to permit a conclusive diagnosis of MRONJ, this histopathological evidence supports the view of the role of osteoclasts and microorganisms in the pathogenesis of MRONJ. Further studies are needed to investigate the real connection between the osteoclasts and bacteria with the underlying mechanism.

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