Association between pain severity and clinico-histopathological findings in the mandibular canal and inferior alveolar nerve of patients with advanced mandibular osteoradionecrosis

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Abstract

Objective. Pain is one of the most problematic symptoms in patients with osteoradionecrosis (ORN) of the jaws. This study investigated the associations between pain severity and morphological alterations of the mandibular canal (MC) and inferior alveolar nerve (IAN), in respective computerized tomography (CT) images and resected specimens of mandibular ORN.

Study Design. We assessed 14 lesions in 13 patients who underwent segmental mandibulectomy for surgical debridement and simultaneous reconstruction with free fibula flap (one patient exhibited bilateral lesions). The extent of the MC bone defect on preoperative coronal CT images and the number of IAN fascicles in resected specimens were evaluated. Comparisons were performed between slight and extreme pain groups. In most of the extreme pain group, MC bone defects were either absent or entire circumferential defects; IAN fascicles were either distinguishable or completely absent in resected specimens.

Results. Although there was no statistically significant association between extreme pain and CT or histopathological findings, the histopathological indistinguishability of

IAN fascicles was significantly associated with slight pain.

Conclusion. The degree of degeneration of MC and IAN may be associated with pain severity in patients with mandibular ORN.

Keywords: mandibular osteoradionecrosis; inferior alveolar nerve; mandibular canal; neuropathic pain; segmental mandibulectomy.

INTRODUCTION

Osteoradionecrosis (ORN) of the jaws is a rare but serious complication of radiation therapy (RT) for head and neck malignancies. When ORN worsens, lesions may exhibit full-thickness devitalization of bone, resorption of the inferior border of the mandible, an orocutaneous fistula, or a pathological fracture. These require surgical interventions, such as radical debridement and simultaneous reconstruction with a vascularized free flap.[1]

One of the most problematic symptoms in patients with ORN is pain, which arises from two main origins: (1) necrotic bone associated infection of the surrounding tissues, and (2) neuropathic pain (NeP).[2] Chronic drainage from superficial infection and NeP both result in physical and emotional disability in ORN patients[1]. Of note, these patients are cancer survivors who already have experienced significant stress. The International Association for the Study of Pain defines NeP as 'pain caused by a lesion or disease of the somatosensory nervous system'.[3] While there is an increasing number of studies regarding NeP, its diagnosis is still largely dependent on characteristic clinical symptoms (e.g., "pins and needles", electric shock-like sensations, burning or coldness,

and itching) and somatosensory abnormalities (e.g., hyperalgesia, hypoesthesia, allodynia).[2,4] It is not unusual for NeP in maxillofacial osteonecrosis patients to prevent their sleep or to cause awakening of the patients.[5] Antibiotic therapy may reduce, or even eliminate, pain in patients with superinfections; however, this only occurs while the antibiotic is actively administered.[5] Repeated antibiotic administration serves primarily as a palliative therapy, rather than a fundamental, curative treatment methodology. Whether conservative or aggressive, treatments for ORN should aim to both control infection and relieve pain.

Severe pain may influence the decision to surgically intervene in cases of ORN. Indeed, patients with advanced ORN, even those who exhibit pathological fractures, may choose not to undergo surgical intervention because they do not suffer from pain. Bouquot et al.[5] have proposed a model of ORN progression that is as follows: stabilized lesions without progressively increasing pain are at constant risk of inflammatory events that may exacerbate the compromised flow of marrow, thus pushing the disease across the threshold into severe pain.

The lack of understanding of the underlying mechanism of pain in ORN

patients may lead to inappropriate selection of treatment. To our knowledge, there has been no analysis of clinical and histopathological alterations of the mandibular canal (MC) or the inferior alveolar nerve (IAN) in patients with mandibular ORN. This study aimed to analyze the association of preoperative pain (prior to surgical intervention for advanced mandibular ORN) with morphological alteration of the MC, using preoperative computerized tomography (CT) images, and with histopathological degeneration of the IAN, using resected bone specimens.

Materials and Methods

Fifteen consecutive patients underwent advanced mandibular ORN treatment in our department between 2013 and 2017; this treatment consisted of segmental mandibulectomy for surgical debridement and simultaneous reconstruction with free fibula osteocutaneous flap. Two patients exhibited pathological fractures after marginal mandibulectomy for primary oral cancer and postoperative adjuvant RT. The IANs in these two patients were resected at the time of primary surgery; therefore, these patients were excluded in our study. One patient exhibited disease in both right and left

mandibular molar areas; therefore, we included a total of 14 advanced mandibular ORN lesions from 13 patients. The Medical Ethics Committee of Kobe University Hospital approved this study. All subjects provided written informed consent to release clinical information and bone samples for the study.

The following epidemiological data were gathered retrospectively from patients' medical charts: age, sex, type of RT, radiation dose, chemotherapy, existence of pathological fractures, type of analgesia, and hypoesthesia of areas innervated by the IAN (i.e., lip and chin). IAN sensory impairment was assessed using pin-prick and light touch tests.[6]

Subject grouping

Although all patients that were included in this study had experienced pain, the degree of pain varied widely among patients. NeP was diagnosed according to the clinical assessment of patients' symptoms (specifically, the presence or absence of intermittent and sudden tingling, or of lightning pain that occasionally caused sleep deprivation)[4,5]. For patients with suspected NeP, an oral anticonvulsant was administered. Narcotic

analgesics, including tramadol, were used for patients who had severe intractable pain that was not affected by anti-inflammatory analgesics. Pain that required the administration of anticonvulsants (e.g., pregabalin) or opioids was defined as severe pain. Pain that required long-term administration of non-steroid anti-inflammatory drugs (NSAIDs), typically consisting of propionic acid, was defined as moderate pain. Pain that could be controlled with occasional administration of acetaminophen, or that did not need analgesics, was defined as mild pain. Antibiotic therapy (oral amoxicillin in most cases) was administered only during bouts of acute inflammation that were caused by worsening infection.

Histopathological analysis of inferior alveolar nerve

All bone specimens were decalcified after surgery and formalin-fixed without freezing. Thin sections were cut from paraffin blocks, then stained with hematoxylin and eosin for evaluation by microscopy.

In a previous study, Svane et al.[7] reported that the mean number of IAN fascicles harvested from 10 human cadavers was 12.19 at the mental foramen, 18.29 at

the first molar, 21.6 at the second molar, and 21.14 at the third molar, respectively (Table 1).

As an initial step to confirm the validity of our calculations of the number of IAN fascicles harvested in our study, 15 bone specimens without neural invasion and history of RT were randomly and blindly selected from patients who underwent segmental mandibulectomy to treat oral cancer. These randomly selected specimens were regarded as the non-irradiated group. All non-irradiated bone specimens were taken from molar regions. Figure 1 shows bone specimens from two patients in the non-irradiated group. Two types of IAN fascicles were identified: in one type, designated as “distinguishable fascicles”, each fascicle can be distinguished (Figure 1A); in the other type, designated as “indistinguishable fascicles”, each fascicle cannot be distinguished (Figure 1B). Of randomly selected 15 non-irradiated bone specimens, five exhibited indistinguishable fascicles. The remaining 10 non-irradiated bone specimens were taken from seven men and three women. In these 10 bone specimens with distinguishable fascicles, the number of fascicles within the epineurium was counted (Figure 1C; in this representative section, the number of fascicles was 14,

outlined in white). Subsequently, the cross-sectional area of IAN in these 10 bone specimens with distinguishable fascicles was calculated using ImageJ software. As shown in Figure 1D, the cross-sectional area of the epineurium was regarded as the cross-sectional area of the IAN (white circle).

We evaluated the IAN near the center of the lesions in ORN patients as described above for non-irradiated specimens. Figure 2 shows the clinico-histopathological findings in a patient who exhibited bilateral ORN in the mandibular molar region. Each fascicle of IAN could not be distinguished in the right lesion (Figure 2C'); nevertheless, fascicles could clearly be distinguished in the left lesion (Figure 2D') and in the right posterior margin (Figure 2E'). Therefore, the right IAN fascicles in this patient were categorized as "indistinguishable fascicles" (Figure 2C'), and the IAN fascicles in the left lesion and the right posterior margin were categorized as "distinguishable fascicles" (Figures 2D' and E'). The number of IAN fascicles near the center of the ORN lesions was counted only in "distinguishable fascicles" (e.g., the calculated numbers of IAN fascicles in the representative section in Figure 2D' was 10). The cross-sectional area of the IAN near the ORN lesion was

calculated in the same manner as in non-irradiated specimens. Image acquisition of the bone specimens was performed with a BZ-X 700 (Keyence, Osaka, Japan).

Computerized tomographic analysis of mandibular canal in osteoradionecrosis

As shown in Figure 3, the bone defect of MC near the deepest area of osteolysis in ORN patients was classified into three types (no defect [Figures 3A and A'], partial defect [Figures 3B and B'], and entire circumferential defect [Figures 3C and C']) by assessing coronal planes on preoperative CT. The fascicles in Figure 3A'' were distinguishable, and numbered seven. The fascicles in Figure 3B'' were indistinguishable; therefore, the number of fascicles was not counted. The IAN was not found in the bone sample in Figure 3C'', probably due to neurotmesis.

Statistical analysis

Statistical analyses were performed using R software (R Development Core Team, 2011). Mann Whitney U test and Fisher's exact test were performed. A threshold of $P < 0.05$ was used to define statistical significance.

Patients with mild pain, or without pain, were classified into the “slight pain group”. Patients with severe or moderate pain were classified into the “extreme pain group”. Comparisons were performed between slight and extreme pain groups; further, comparisons of the number of cross-sectional IAN fascicles were performed between ORN and non-irradiated bone samples.

Results

We analyzed a total of 14 advanced mandibular ORN lesions from 13 patients (12 men and 1 woman) who underwent advanced mandibular ORN treatment in our department. Twelve patients received conventional RT and one patient received intensity-modulated RT. The median time interval between the end of RT and surgery for ORN was 81 months (range, 6–195 months). The median radiation dose was 66 Gy (range, 60–81 Gy). Of 13 patients, nine received concomitant intravenous chemotherapy (69.2%), and the chemotherapeutic regimens were cisplatin and 5-fluorouracil in 4 (30.8%), cisplatin alone 3 (30.8%), and cisplatin and nedaplatin 1 (7.7%). The details of pain management regimen before surgery are shown in Table 2. One bilateral ORN patient had moderate

pain that required sustained administration of NSAIDs only for the left side (Figure 2D); in this patient, pain in the left ORN lesion was regarded as moderate, while pain in the right lesion was regarded as mild (Figure 2C). Therefore, in 14 ORN lesions of 13 patients, six was regarded as mild pain (42.9%), six moderate pain (42.9%), and two severe pain (14.2%). In coronal CT images of 14 ORN lesions, we found no MC bone defect in three lesions (21.4%), a partial MC bone defect in five lesions (35.7%), and an entire circumferential MC bone defect in six lesions (42.9%).

Table 1 shows the results of comparison of IAN fascicles between ORN and non-irradiated cancer bone samples. In 14 ORN bone samples, "distinguishable fascicles" and "indistinguishable fascicles" were each found in six respective samples (42.9% each). Both MC and IAN were completely absent in two ORN bone samples (14.2%), as exemplified in Figure 3C. In comparisons of both age and number of IAN fascicles, between six patients with "distinguishable fascicles" and the 10 non-irradiated patients, we found no significant difference ($P = 0.96$ and 0.51, respectively). In contrast, we found that the cross-sectional area of IAN was significantly larger in non-irradiated samples than in ORN samples ($P = 0.02$).

Table 3 shows the association between pain severity (slight and extreme) and clinico-histopathological findings. There were no significant differences in age, radiation dose, or chemotherapy between slight and extreme pain groups (P = 0.66, 0.52, and 1, respectively). Although we found no significant association between extreme pain and a variety of clinical variables (hypoesthesia, pathological fracture, and defect extent of MC on coronal CT images), there was a statistically significant association of "slight pain" with histopathological indistinguishability of IAN fascicles (P = 0.03).

Discussion

The current study revealed several novel clinico-histopathological findings in patients with advanced mandibular ORN. First, although the MC was absent in some CT images, the complete absence of IAN was uncommon because the degenerated IAN was found in resected histopathological specimens. Second, in the "extreme pain" group, most coronal CT images exhibited either complete absence of MC bone defects or an entire circumferential defect. Notably, most fascicles of IAN were either histopathologically distinguishable or disappeared in specimens of the "extreme pain" group. Third, the

histopathological indistinguishability of IAN fascicles was significantly associated with the "slight pain" grouping in patients. Fourth, there was a significant difference in the cross-sectional area of IAN, but not the fascicular number, between ORN and non-irradiated bone specimens.

Degeneration of inferior alveolar nerve

The fascicular numbers of the peripheral nerve vary widely from nerve to nerve, and there is a tendency for fascicular numbers of IAN to increase distally.[8,9] There have been no significant differences between nerves specimens from dentulous, partially edentulous, and completely edentulous subjects, raising the possibility that the majority of axons in the mandibular nerve, irrespective of the presence of teeth, are dedicated to distal orofacial soft-tissue innervation.[8] In contrast, there have been reports that both the number of fascicles and the cross-sectional area for the trigeminal nerve branches decrease in a proximal-to-distal manner.[7,10] Table 1 shows the fascicular characteristics of human IAN in the previous reports.[7,8,11] The interfascicular pattern of the peripheral nerve from proximal to distal locations changes as follows: it is

monofascicular at proximal locations, then oligofascicular between proximal and distal locations, and finally polyfascicular at distal locations.[12,13] Fibrosis resulting from peripheral nerve injury is classified into three types: fibrosis of the epineurium, in which the surrounding layer of the epineurium is involved such that scar strangulation compresses the entire nerve trunk; interfascicular fibrosis, in which the connective tissue between fascicle groups is involved; intrafascicular fibrosis, in which the connective tissue of the endoneurium of each fascicle is involved.[12] Thus, the morphology and number of nerve fascicles is diverse and likely reflects the degree of tissue damage, fibrosis, and degeneration. Our study showed that there is a significant difference in the cross-sectional area of IAN between ORN and non-irradiated bone samples. We also found crosswise and mesiodistal differences in the bilateral ORN patient (Figures 2C, 2D, and 2E). **The** morphological alterations of the IAN are probably **due to radiation damage** combined **with pathogenic conditions** associated with ORN, such as chronic infection and sustained inflammation. However, we must note that indistinguishable IAN fascicles were found in some ORN bone specimens as well as non-irradiated cancer bone specimens. As shown in Table 1, all previous reports

that likely used mandibular bone specimens without pathologies revealed that IAN fascicles were clearly distinguishable even at low magnification inspection and the number of fascicles was relatively large (range, 9.4–21.14). Although we could not find the reports analyzing the influence of aging on nerve fascicles in human, there was no statistically significant change in any histometric parameter (i.e., diameter and fiber density) of peripheral nerve after maturity in dog.[14] We could not also find the reports about the pathological significance of indistinguishability of IAN in oral and maxillofacial regions. However, a review by Sakakura et al.[15] studied perineuronal fibrosis and indistinguishability of renal nerve. The authors aim to establish criteria for histopathological evaluation after renal sympathetic denervation that is a catheter based procedure using radiofrequency ablation aimed at treating resistant hypertension. Nerve changes due to injury directly with the radiofrequency ablation in the plane of section (i.e., nerves that lie directly in the path of ablation) or along its length outside the plane of section (i.e., upstreatm or downstream injury with ablation) may be classified as degenerative, necrotic, and chronic, among others.[15] Chronic changes include peri- and endoneural fibrosis, axonal atrophy, and loss.[15] Nerve changes due to injury are

classified into five grades (grade 0 to 4), and severe injury (i.e., grade 4) changes are typically overwhelming and may consist of marked perineuronal inflammation and/or fibrosis.[15] In their figure of hematoxylin and eosin staining to show the histologic grading scheme for nerve changes, the nerve fascicles in grade 0 or 1 (i.e., no or minimal injury) are distinguishable, nevertheless, the nerve fascicles in grade 2, 3, or 4 (i.e., mild, moderate, or severe injury) are indistinguishable.[15] Indistinguishability of nerve fascicles is probably caused by nerve injury. The indistinguishability of IAN fascicles observed in the ORN and non-irradiated oral cancer specimens in this study may reflect the chronic nerve changes caused by lesions (i.e., ORN or cancer-related inflammation).

Association between pain severity and nerve degeneration

Even though ORN pain exhibits a neuralgic character, it primarily results from intraosseous fluid dynamics and inflammatory mediators, rather than direct nerve damage.[5] Damage to sensory nerves is not central to the etiology of pain in ischemic osteonecrosis; elevated intramedullary pressures and ischemia are most often the

dominant causes of pain.[5] Although ischemia is one of the etiologies of ORN, large portions of ORN lesions may not experience ischemia. Necrotic changes (i.e., the lack of blood vessels within the Haversian canals) are more prevalent in cortices than in cancellous bones in mandibular osteoradionecrosis, probably due to a decrease of periosteal blood supply caused by radiotherapy.[16] To understand the underlying mechanism of pain in ORN patients, viability within bone (e.g., residual blood flow within cancellous bones) should be considered. For example, in diabetic neuropathy with an ischemic etiology, patients exhibit positive symptoms including pain, paresthesia, and hypoesthesia from early to intermediate stages; in later stages of disease progression, patients experience negative symptoms that include sensory loss increase.[17-19] An additional example is pulpitis, which causes intense pain enough to disturb sleep; unless the infected pulp is properly removed, it becomes necrotic and progresses to apical periodontitis. Until acute infection-related inflammation occurs, patients with apical periodontitis often feel no pain. Classification according to pain severity and IAN degeneration places patients in this study into four types: (1) “extreme pain” patients with remaining distinguishable fascicular IAN; (2) “slight pain” patients

with indistinguishable fascicular IAN; (3) “extreme pain” patients with neurotmesis (complete histopathological disappearance of IAN); (4) others (e.g., slight pain with distinguishable fascicles or extreme pain with indistinguishable fascicles). As with diabetic neuropathy (i.e., sensory loss means that patients have late-stage diabetic neuropathy), the disease stage of mandibular ORN patients classified into the “slight pain” group in this study is mostly more advanced than the “extreme pain” group according to the severity of IAN degeneration. Oral and maxillofacial surgeons should appreciate the association between pain severity and nerve degeneration to provide proper disease stage information to patients, and pay attention to the possibility that patients with only slight pain have more advanced lesions

There are some limitations in this study. First, we have reported on a small patient sample. Second, additional **tests** to analyze etiologies that may affect nerve degeneration (e.g., fibrosis, hypoxia, or loss of vascularity) **were** not performed **and are needed**. Further immunohistopathological investigations should be needed. Third, all resected bone specimens of ORN could not be evaluated; thus, there might be a neurotmesis at a site distant from the prepared histological section. This study

investigated the association between clinico-histopathological findings and preoperative pain severity and revealed a significant association between the histopathological indistinguishability of IAN fascicles and "slight pain." The histopathological indistinguishability of IAN fascicles may reflect degenerative, necrotic, and chronic changes in nerve tissue associated with sensory loss. However, in one "extreme pain" patient, the IAN fascicles were indistinguishable. In this study, the intensity of pain was retrospectively classified into three categories according to the type of analgesic medication that was administered to each patient. Currently, various screening tools are used to assess NeP.[4,20,21] To accurately evaluate pain severity, it may be necessary to undertake a prospective study with a large sample size that uses current NeP screening tools.

Conclusions

Although the current study showed that there was no statistically significant association between severe pain and CT or histopathological findings, the histopathological indistinguishability of IAN fascicles was significantly associated with slight pain in

patients with mandibular ORN.

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Figure captions

Fig. 1. Histopathological images of the inferior alveolar nerve (IAN) of non-irradiated bone samples. (A) Distinguishable IAN fascicles. (B) Indistinguishable IAN fascicles. (C) A representative image of the process to count the fascicular number of IAN. White circles indicate each fascicle. The fascicular number in this specimen is 14. (D) A representative image of the process used to calculate the cross-sectional area of IAN. The area of epineurium indicated by white circle was calculated with ImageJ software. White lines indicate the long and short axis of epineurium. Scale bar: 200 μm.

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Fig. 2. Coronal images of computerized tomography (CT) and histopathological results in a patient with bilateral mandibular osteoradionecrosis. (A) A resected bone specimen. (B) Coronal CT image. Enlarged views of the white dotted box indicating right (C) and left (D) mandibular canals within the center of the lesions, and mandibular canal near

the right posterior margins (E). Histopathological images of the inferior alveolar nerve (IAN). (C'–E'). Although the IAN fascicles were distinguishable in the left lesion (D') and right posterior resected margin (E'), IAN fascicles in the right lesion were indistinguishable (C'). This patient exhibited moderate pain in the left lesion preoperatively; osteolysis was more severe in the right lesion. Scale bar: 200 μm.

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Fig. 3. Bone defects of the mandibular canal in coronal computerized tomography images are classified into three types: no defect (A); partial defect (B); entire circumferential defect (C). Enlarged views of the white dotted box indicating mandibular canals (A'–C'). Histopathological results show three types of morphological alteration of the inferior alveolar nerve (IAN): distinguishable fascicles (A'');

indistinguishable fascicles (B'); disappearance of the IAN (C'). The patients represented in (A and C) were included in the "extreme pain" group, and the patient represented in (B) was in the "slight pain" group. Scale bar: 200 μm.

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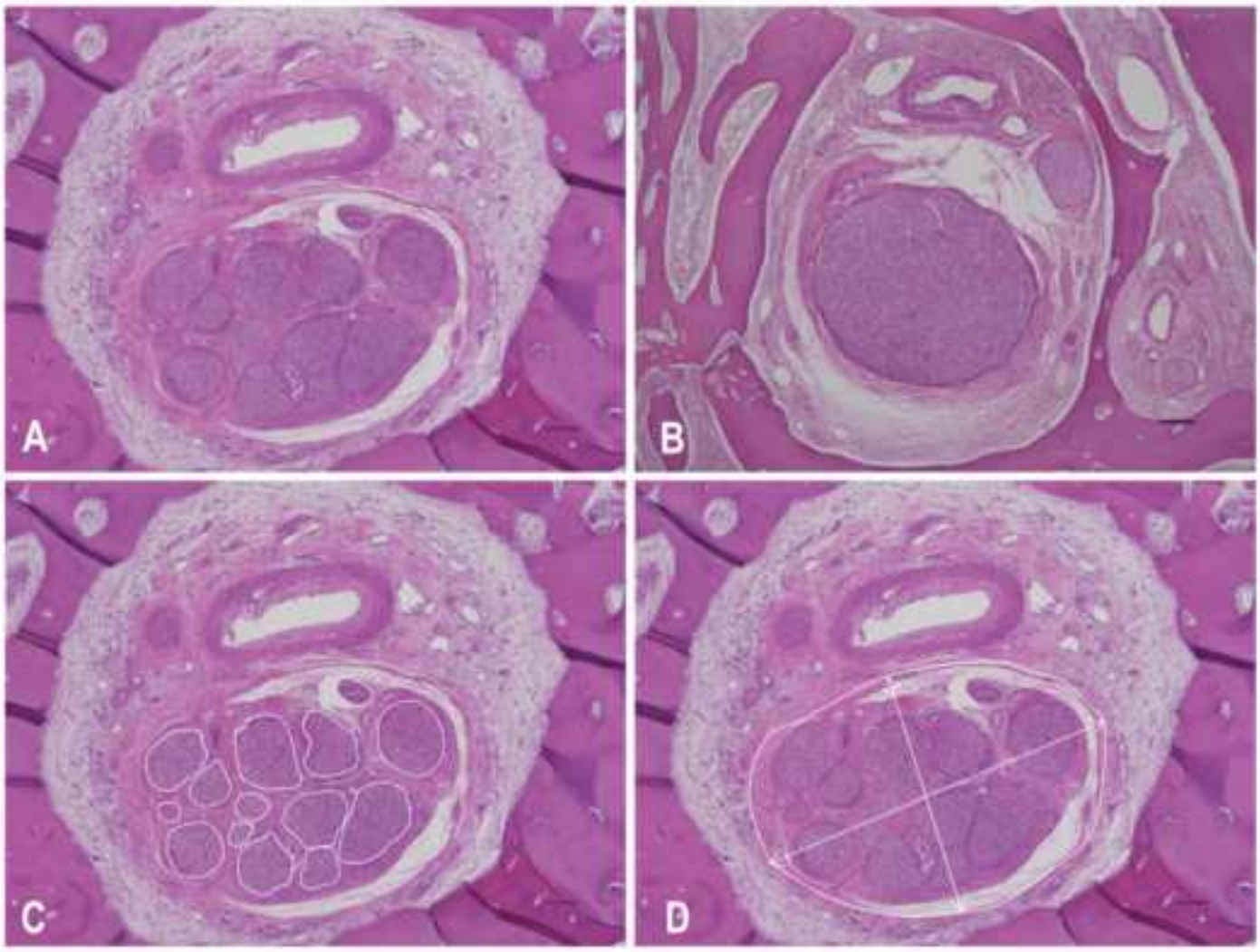

Figure 1

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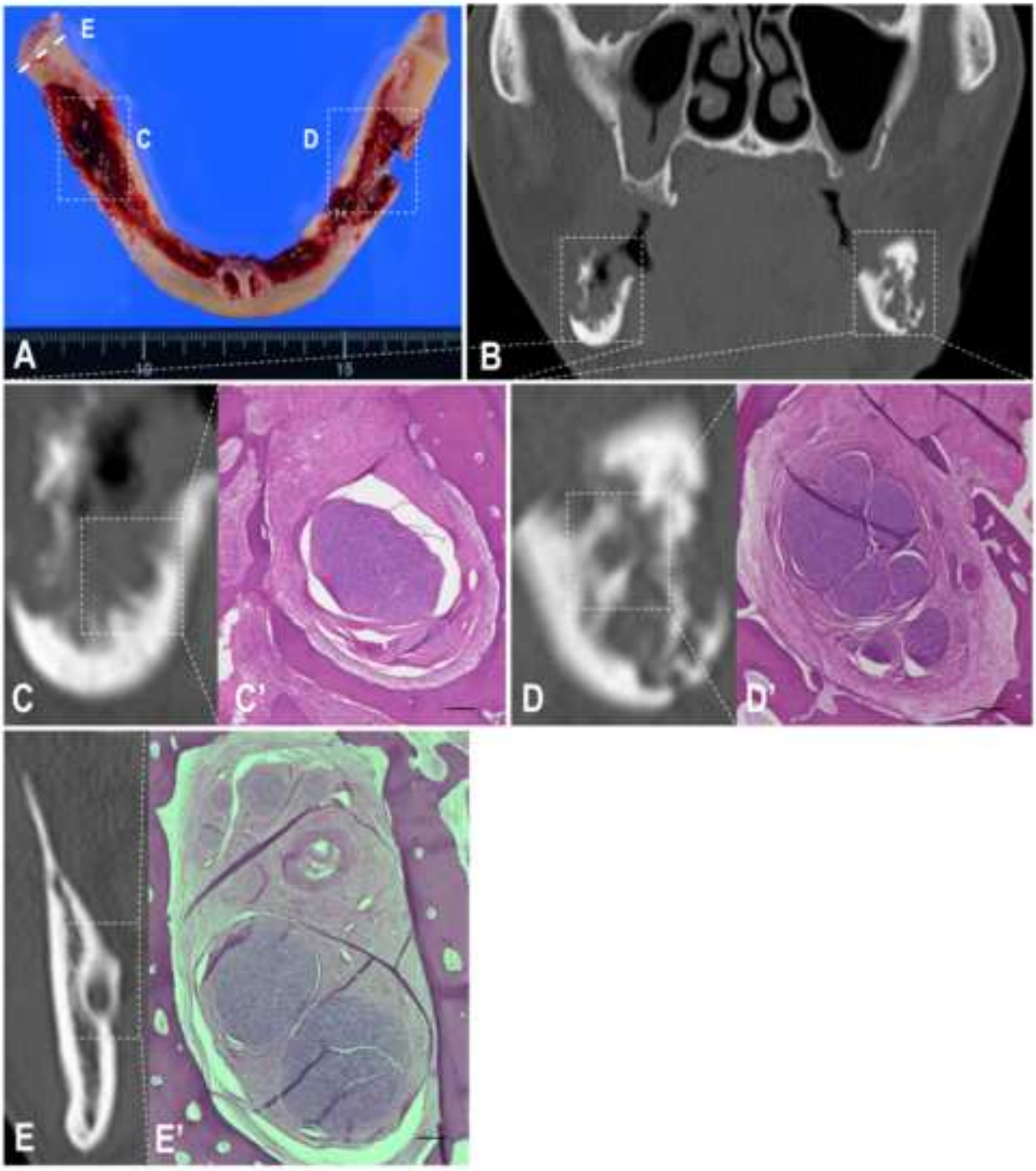

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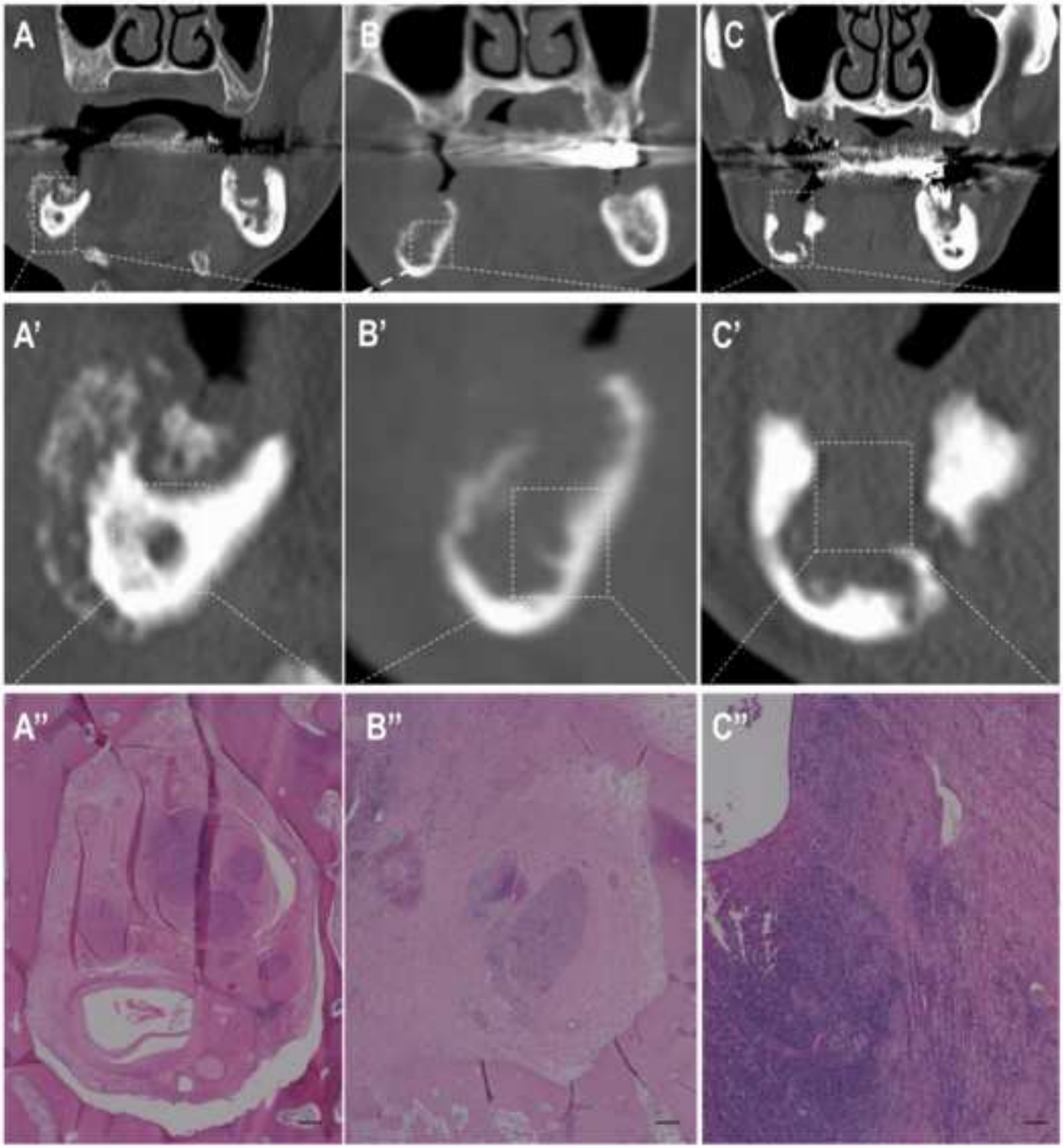

Figure 3

Table I. Comparison of fascicular characteristics of human inferior alveolar nerve with previous reports

	n	Age	No. of fascicles	Cross sectional area (mm^2)
Our data				
Osteoradionecrosis	14 (6)[c]	65 (58–80)	9.5 (3–12)	1.31 (0.98–1.96)
Non-irradiated cancer	15 (5)[c]	68 (57–81)	10 (5–21)	2.01 (1.01–2.60)
Svane et al.[7a]	10	36.2 ± 16.4		
Mental foramen			12.19 ± 3.58	1.18 ± 0.27
1st molar			18.29 ± 7.46	1.29 ± 0.18
2nd molar			21.60 ± 8.04	1.49 ± 0.23
3rd molar			21.14 ± 7.05	1.64 ± 0.27
Eppley et al.[8a]	18	60 ≤		
Mandibular nerve				
Lingual			8.5 ± 3.2	1.55 ± 0.38
Molar			9.4 ± 3.3	1.62 ± 0.22
Mental			10.9 ± 2.3	1.52 ± 0.52
Pennisi et al.[11a]	3	55 ± 6.6		
Mandibular nerve[b]				
Total			21.7 ± 9.4	8.0 ± 0.52
Sensory			19.3 ± 9.7	6.3 ± 0.49
Motor			2.3 ± 0.5	1.7 ± 0.05

Our data were expressed as median (range). Data in previous reports were expressed as mean ± standard deviation.

[a]All were cadaveric studies.

[b]Mandibular nerves cut 5 mm distal to their origin from the ganglion were analyzed.

[c]Number of lesions (number of lesions with histopathologically indistinguishable fascicles).

Table II. Details of pain management regimen before surgery and pain classification

Pain classification	Pain management regimen before surgery	Patients (%)
Mild	No analgesics administrated	3 (23)
	Occasional administration of acetaminophen	2 (15)
Moderate	Long-term administration of NSAIDs[a]	6 (46)
Severe	NSAIDs/opioids/anticonvulsants	2 (15)

[a]Non-steroid anti-inflammatory drugs

Table III. Association between pain severity and clinico-histopathological findings in mandibular osteoradionecrosis

	Slight pain group (n=6)	Extreme pain group (n=8)	*P* value[b]
Hypoesthesia	3 (50)	7 (87.5)	0.2448
Pathological fracture	2 (33.3)	5 (62.5)	0.5921
Defect extent of mandibular canal on coronal CT image			
No defect	0 (0)	3 (37.5)	0.2088
Partial defect	4 (66.7)	1 (12.5)	0.0909
Entire circumferential defect	2 (33.3)	4 (50)	0.6207
Fascicles of inferior alveolar nerve near the center of lesions			
Distinguishable	1 (16.7)	5 (62.5)	0.1375
Indistinguishable	5 (83.3)	1 (12.5)	0.0256
Disappear	0 (0)	2 (25)	0.4725
Number of distinguishable fascicles	12	9 (3–11)[a]	

Unless otherwise noted, data are reported as number (percentage) of lesions

[a]Median (range)

[b]Fisher's exact test