The Use of Photodynamic Therapy on Medication-Related Osteonecrosis of the Jaws: An Animal Study

SUMMARY

Background/ Aim: Bisphosphonate-related osteonecrosis of the jaw (BRONJ) was first introduced in 2003 and its scope was expanded by the name medication-related osteonecrosis of the jaw (MRONJ), since 2014. This study aimed to evaluate the effects of photodynamic therapy (PDT) on tissue samples by histopathological and histomorphometric examination and serum TRACP-5b (Tartrateresistant acid phosphatase-5b) measurement in rats. Material and Methods: 24 Sprague-Dawley male rats were divided into 3 groups comprising 8 animals. Zoledronic acid was administered to groups 1 and 2 and 0.9% sodium chloride was administered to group 3 intraperitoneally. After the injections were completed, dental extractions were performed. Photodynamic therapy was applied to group 2, three times a week for two weeks after the extraction. In the 16th week, sacrifice was performed. Rats were undergone histopathologic and histomorphometric evaluations. Results: Photodynamic therapy has led to a decrease in epithelial opening and inflammation and an increase in the formation of new bone. Serum TRACP-5b values were shown to decrease significantly in the presence of osteonecrosis. Conclusions: PDT was shown to be useful in reducing MRONJ risk in rats. As a serum biomarker, Serum TRACP-5b could be a valuable marker. Additional studies should confirm the findings.

Key words: Bisphosphonate, Photodynamic therapy, Osteonecrosis, MRONJ, Biomarker

Introduction

Bisphosphonates (BF) are chemotherapeutic antiresorptive drugs that affect the morphology and activity of bone cells in various ways. If a nitrogen or amino group is present in its molecular structure, it is called nitrogen-containing bisphosphonates. Nitrogen-free bisphosphonates (alkyl bisphosphonates) are etidronate, clodronate, tiludronate. Nitrogen-containing bisphosphonates (aminobisphosphonates) are alendronate, pamidronate, ibandronate, risedronate, and zoledronate. Nitrogen-containing bisphosphonates are 10 to 10,000 times stronger than nitrogen-free BF groups. The most potent nitrogen-containing BF is zoledronic acid. Depending on the route of administration, they are primarily handled orally and parenterally. Bisphosphonates administered orally are alendronate, etidronate, risedronate, and bisphosphonate, ibandronate, and clodronate administered both orally and parenterally. When they are taken orally, they are given by parenteral drugs that have very little absorption and have more side effects.

Bisphosphonates are currently used in a wide range of bone-related diseases. Commonly used diseases include osteoporosis (juvenile, postmenopausal or senile, associated with glucocorticoids or transplantation, androgen deprivation), Paget’s disease, metastatic bone diseases, multiple myeloma, osteogenesis imperfecta, malignancy-induced hypercalcemia. Oral and parenteral routes are generally used as administration routes.

Denosumab (anti-receptor activator of nuclear factor-kappa ligand [RANKL]) is an antiresorptive drug that inhibits osteoclast function, reduces bone resorption, and increases bone density. It is usually used in patients with osteoporosis or metastatic bone disease.
is a potent alternative to the unwanted side effects of bisphosphonates. However, denosumab was found to cause osteonecrosis with the use of the drug. Denosumab treatment is especially preferred in osteoporotic patients for the prevention of fractures and metastatic bone diseases due to solid tumors.

In the first published cases, there were areas characterized by exposed necrotic bone in the maxillofacial regions of patients treated with intravenous (i.v.) bisphosphonates. In an article published in 2009, the American Association of Oral and Maxillofacial Surgery (AAOMS) described the bone image of the mucosa-associated with bisphosphonate osteonecrosis (BRONJ) for more than 8 weeks in the jaws of patients who had not previously received radiotherapy from the head and neck, who had used or continued to use bisphosphonate. MRONJ (Medication-related osteonecrosis of the jaw), a definition of osteonecrosis covering all drugs in 2014, was proposed by AAOMS.

Most cases of MRONJ occur with long-term intravenous use of bisphosphonates or denosumab. The incidence of MRONJ was higher in the mandible (73%) than in the maxilla (22.5%). One of the most consistent MRONJ risk factors is invasive dental procedures, especially tooth extraction. They can be added to prosthesis irritation and periodontal diseases. AAOMS recommends a gradual approach to the treatment of the disease and conservative treatment approaches. The level of treatment with antibiotic use and conservative treatment using local mouthwashes is around 23%.

In antimicrobial photodynamic therapy (aFDT), singlet and free radicals formed by photosensitizers that are activated by light are used to destroy microorganisms. aFDT works with a triple mechanism consisting of a photosensitizing agent, a non-toxic molecule, a light source suitable for photosensitizing (usually at a wavelength close to the infrared spectrum) and molecular oxygen. aFDT is used to inactivate various microorganisms such as bacteria, fungi, viruses, and protozoa in many different fields.

Research on the use of photodynamic therapy in MRONJ is limited to a few case reports and few animal studies in the literature. This study aimed to evaluate the effects of photodynamic therapy after histomorphometric examination and to evaluate serum TRACP-5b biomarker levels in the MRONJ rat model.

Material and Methods

In this study, 24 Sprague-Dawley male rats weighing 230 ± 50 gr and taken from Istanbul University Aziz Sancar Experimental Medicine Research Institute were used. The animal experiments under this project were approved by the Istanbul University Animal Experiments Local Ethics Committee (HADYEK-28.11.2016-File number:35980450-050.01.04). The animals were fed with standard rat feed containing 21% protein and distilled water. Three main groups of experimental animals were used. In all groups, anesthesia of experimental animals was provided with ketamine hydrochloride 100 mg/kg and xylazine hydrochloride 10mg/kg. The first right molar teeth were extracted in all groups, according to the rules of asepsis and antisepsis. Animals in group 1 (negative control) of 8 animals were administered intraperitoneally 0.2 mg/kg zoledronic acid twice a week for 6 weeks by mixing 0.9% sterile isotonic sodium chloride solution. After the teeth were taken at the end of the 6th week, the animals were followed for 10 weeks. At the end of 16 weeks, blood samples were taken under anesthesia before intracranial sacrification and sacrification was performed. Animals in group 2 (FDT group) of 8 animals were given intraperitoneally 0.2 mg/kg zoledronic acid twice a week for 6 weeks by mixing 0.9% sterile isotonic sodium chloride solution. At the end of the 6th week, the tooth extraction was carried out under the conditions indicated by applying photosensitizing agent 0.1 mg/ml toluidine blue, which is a photosensitizing agent, to the tooth extraction socket, with Fotosan 630, 3 times a week for 2 weeks, in 4 different areas for 10 seconds. Photodynamic therapy was applied for a total of 40 seconds. Experimental animals were then monitored for 8 weeks. At the end of 16 weeks, blood samples were taken under anesthesia before sacrifice and sacrifice was performed. Animals in group 3 (positive control) of 8 animals were given 0.5 ml 0.9% sterile isotonic sodium chloride solution intraperitoneally twice a week for 6 weeks. Tooth extractions were performed at the end of the 6th week. Experimental animals were followed for 10 weeks. At the end of 16 weeks, blood samples were taken under anesthesia before the sacrifice. One of the animals in this group died during the experiments, it was continued with the remaining 7 healthy animals.

Histopathological Evaluation

After decalcification, frontal sections of 3 mm thickness were taken to pass macroscopically through tooth extraction cavities. These pieces were subjected to routine tissue follow-up and paraffin blocks were prepared. For the evaluation of inflammation and new bone formation following criteria were used; If 0% (0) is absent, 5% -30% (1) is light, 30-60% (2) medium, if it covers more than 60% (3) was scored as heavy.
**Histomorphometric Evaluation**

During the examination, digital images covering the entire defect area were taken and measurements were made on these photographs. Analy Olympus AnalySIS 5 (Tokyo – Japan) "image analysis program was used for histomorphometric measurements. The area covered by the necrotic tissue was measured in millimeters square (mm²). The epithelial gap was measured in millimeters (mm) in the same program.

**Statistical Evaluation**

Statistical analyzes were performed with the SPSS version 23.0 program. The suitability of the variables to normal distribution was examined by histogram graphs and Kolmogorov-Smirnov test. Mean, standard deviation, median and minimum-maximum values were used to present descriptive analyzes. 2x2 eyes were compared with Pearson Chi-Square and Fisher’s Exact Tests. The variables with a normal distribution (parametric) were evaluated between the three groups and the ANOVA test was used for comparison between the two groups. Kruskal Wallis Test was used for comparison between non-parametric triple groups and the Mann Whitney U test was used for comparison between non-parametric binary groups. Repeated measurements were evaluated by analysis of variance. The cases where the P-value was less than 0.05 were evaluated as statistically significant results.

**Results**

In our study, 8 of 8 animals (100%) in 1st group and 6 of 8 animals (75%) in 2nd group who were treated with zoledronic acid and underwent tooth extraction were clinically diagnosed after 8 weeks. In the third group, the tooth extraction sockets were completely healed.

In this study, as a result of the histomorphometric examination, necrosis and epithelial gap were compared between the groups; The necrosis median value of the first group (4.26 mm²) was significantly higher than the third group (1.41 mm²) (p<0.05) (p: 0.004). The necrosis median value (2.51 mm²) of the second group was lower than the first group (4.26 mm²), although this difference was not statistically significant.

The median value of the epithelial gap of group 1 (1.77 mm) was significantly higher than group 2 (0.33 mm) and group 3 (0.11 mm). The median value of the epithelial gap of the third group (0.11 mm) was significantly lower than the other two groups (p<0.001). The epithelial gap of the FDT group was significantly lower than the negative control (p<0.001) (Table 1).

| Group 1 (Negative Control) | Group 2 (FDT) | Group 3 (Positive control) | p* |
|----------------------------|---------------|-----------------------------|----|
| Necrosis                   | Median        | Min. | Max. | Median | Min. | Max. | Median | Min. | Max. | 0.004 |
| 4.26                      | 2.96          | 4.77 | 2.51 | 0.19   | 5.50 | 1.41 | 1.22   | 1.52 |
| Epithelial gap            | 1.77          | 1.10 | 2.90 | 0.33   | 0.18 | 0.61 | 0.18   | 0.11 | 0.55 | <0.001 |

*Kruskal Wallis Test

| Z.A. (Group 1 and 2) | Positive control (Group 3) | p* |
|----------------------|----------------------------|----|
| Necrosis             | Median | Min. | Max. | Median | Min. | Max. | 0.004 |
| 3.30                 | 1.91   | 5.50 | 1.41 | 1.22   | 1.52 |
| Epithelial gap       | 0.86   | 0.18 | 2.90 | 0.18   | 0.11 | 0.55 | 0.001 |

*Mann Whitney U Test

When necrosis and epithelial gap were compared between the study and control groups; necrosis median value of the study group (3.30 mm²) was found to be higher than the control group (1.41 mm²) (p: 0.004). Similarly, the median value (0.86) of the study group was higher than the control group (0.18) (p: 0.001) (Table 2).

When the levels of inflammation and new bone formation were compared between the groups, a significant difference was observed between the groups. According to this, all of the first group had a severe degree of inflammation, while the second group had a mild inflammation rate (37.50%) than the third group (85.71%) (p: 0.002). In group 3, the majority (85.71%) had mild inflammation, while the rate of severe inflammation in group 3 was 0%.

Although there was no statistically significant difference in the PDT group, moderate (25%) and severe (37.5%) inflammation rates were lower than the 1st group and higher than the 3rd group.

Slightly new bone formation rate in group 1 (25%) was lower than in group 2 (62.50%) and group 3 (28.57%) (p: 0.010). The rate of mild new bone formation in the FDT group was significantly higher than the negative control (p: 0.010) (Table 3).
When the degree of inflammation and new bone formation were compared between the study and control groups, a significant difference was observed between the groups. Accordingly, the rate of severe inflammation in the study group (68.75%) was higher than in the control group (0.00%), while the rate of mild inflammation was lower in the study group (18.75%) than in the control group (85.71%) (p: 0.006). The rate of mild new bone formation was higher in the study group (43.75%) than in the control group (28.57%) (p: 0.007) (Table 4, Figures 1-5).

*Chi-Square Test

**Table 3. Comparison of inflammation and new bone formation between groups**

| Inflammation | Group 1 (Negative control) | Group 2 (FDT) | Group 3 (Positive control) | p* |
|--------------|-----------------------------|---------------|----------------------------|----|
|              | n  | %  | n  | %  | n  | %  |     |
| Inflammation |    |    |    |    |    |    |     |
| No           | 0  | (0.00) | 0  | (0.00) | 0  | (0.00) |      |
| Mild         | 0  | (0.00) | 3  | (35.50) | 6  | (85.71) | 0.002|
| Moderate     | 0  | (0.00) | 2  | (25.00) | 1  | (14.29) |      |
| Severe       | 8  | (100.00) | 3  | (37.50) | 0  | (0.00) |      |
| New bone formation |    |    |    |    |    |    |     |
| No           | 6  | (75.00) | 2  | (25.00) | 0  | (0.00) |      |
| Mild         | 2  | (25.00) | 5  | (62.50) | 2  | (28.57) | 0.010|
| Moderate     | 0  | (0.00) | 1  | (12.50) | 3  | (42.86) |      |
| Severe       | 0  | (0.00) | 0  | (0.00) | 2  | (28.57) |      |

* Chi-Square Test

**Table 4. Comparison of inflammation and new bone formation levels between zoledronic acid groups and positive control group**

| Z.A. (Group 1 and 2) | Positive Control (Group 3) | p* |
|----------------------|-----------------------------|----|
| Inflammation | n  | %  | n  | %  |     |
| No           | 0  | (0.00) | 0  | (0.00) |      |
| Mild         | 3  | (18.75) | 6  | (85.71) | 0.006|
| Moderate     | 2  | (12.50) | 1  | (14.29) |      |
| Severe       | 11 | (68.75) | 0  | (0.00) |      |
| New bone formation | n  | %  | n  | %  |     |
| No           | 8  | (50.00) | 0  | (0.00) |      |
| Mild         | 7  | (43.75) | 2  | (28.57) | 0.007|
| Moderate     | 1  | (6.35) | 3  | (42.86) |      |
| Severe       | 0  | (0.00) | 2  | (28.57) |      |

* Chi-Square Test

**Figure 1.** In the first group, the multilayer squamous epithelium over the tooth extraction socket could not cover the wound surface. Debris and necrotic bone fragments are seen on the surface.

**Figure 2.** In the second group, the surface epithelium partially covers the wound surface. Necrotic bone tissues are observed in the connective tissue containing lymphoplasmocytic cell infiltration (H&E X40).
There was no statistically significant weight change between groups throughout the study (p>0.05).

When serum TRACP-5b results were compared between the groups, a significant difference was observed between the groups. Post-hoc analysis revealed that this significance was caused by the significant difference between the 2nd and 3rd groups. According to this, serum TRACP-5b average of the second group (6.04 ± 1.59 U/I) was lower than the 3rd group (8.47 ± 1.32 U/I) (p: 0.013) (Table 5).

When serum TRACP-5b results were compared between study and control groups, the mean serum TRACP-5b (6.51 ± 1.49 U/I) of the study group was observed significantly lower than the control group (8.47 ± 1.32 U/I) (p: 0.007) (Table 6).

| Table 5. Comparison of serum TRACP-5b results between groups |
|---------------------------------------------------------------|
| Group 1 | Group 2 (FDT) | Group 3 |
| Mean   | s.d. | Med. | Mean   | s.d. | Med. | Mean   | s.d. | Med. | p*       |
|--------|------|------|--------|------|------|--------|------|------|----------|
| TRACP-5b (U/I) | 6.97 | ±1.33 | 7.20   | 6.04 | ±1.59 | 5.58   | 8.47 | ±1.32 | 8.30 | 0.013 |

*ANOVA Test

| Table 6. Comparison of serum TRACP-5b results between zoledronic acid groups and positive control groups |
|-------------------------------------------------------------------------------------------------------|
| Z.A. (Group 1 and 2) | Positive control (Group 3) |
| Mean | s.d. | Med. | Mean | s.d. | Med. | p* |
|------|------|------|------|------|------|----|
| Serum ELISA (U/I) | 6.51 | ±1.49 | 6.33 | 8.47 | ±1.32 | 8.30 | 0.007 |

* Independent T Test
Discussion

Although the first MRONJ cases have been reported more than 15 years ago, the pathophysiology of the disease has not been clarified yet. Controversy continues between clinicians and researchers about the potential mechanisms underlying MRONJ. Factors such as impaired bone remodeling, inhibition of bone resorption, microtrauma, congenital or acquired immunosuppression, vitamin D deficiency, accumulation, and toxicity of antiresorptive drugs in soft tissue, inflammation, and infection continue to be investigated in scientific studies. Zoledronic acid, a nitrogen-containing bisphosphonate, is one of the most commonly used drugs in MRONJ studies.

In our study, zoledronic acid (Z.A.) MRONJ model was preferred as in most other studies. The dose of Z.A. used in our study was determined to be 2.4 mg/kg for each rat in accordance with similar studies. In our study, zoleodronic acid was given 0.2 mg/kg twice a week for 6 weeks to reach a total dose of 2.4 mg/kg. As in the literature, intraperitoneal injections have been performed effectively.

As a result of the literature review, most animal models with MRONJ were evaluated by using bisphosphonate followed by tooth extraction. In addition to bisphosphonate use, different models such as corticosteroid administration, surgical expansion of the sockets with drills, and vitamin D deficiency have been tried.

Another parameter investigated in our study was serum TRACP-5b biomarker levels. Various biochemical markers can contribute to clinicians by carefully examining areas such as monitoring antiresorptive therapy, assessing fracture risk, and assessing the risk of skeletal events in bone metastases. No biomarker has yet been agreed upon and recommended for clinical use.

In our study, FDT device with a LED light source producing red light at 630 nm wavelength and a toluidine blue photosensitizer was used. Toluidine blue is a non-toxic, non-irritating, photosensitizing agent effective against microorganisms. Toluidine blue acts by interacting with lipopolysaccharides in gram-negative cell membranes in the presence of the light of appropriate wavelength. It exhibits maximum absorption and optimum photodynamic properties with light at a wavelength of 630 nm.

In our study, the animals were divided into three groups. 8/8 (100%) of animals in group 1 and 6/8 of animals (75%) in group 2, was diagnosed as MRONJ clinically after 8 weeks.

In the study of Ervolino et al., no weight change was observed in the rats in the experimental and control groups, including Z.A. and FDT administration, similar to our study. MRONJ-like lesions were observed in 100% of the group who received only zoledronic acid and tooth extraction. The first MRONJ animal studies included Allen et al., and Sonis et al. used 10 female Wistar rats in their study. Sacrification was performed 7 weeks after tooth extraction and histopathological examination, computed tomography, and bone scintigraphy were evaluated. MRONJ was seen in 100% of the study group.

In the study of Ersan et al., osteonecrosis was observed in all groups treated with zoledronic acid and zoledronic acid+teriparatide. Osteonecrosis was not seen in the control group.

Kim et al. used 48 Sprague-Dawley rats in their study. After sacrificed, 75% (27/36) of the rats in the study group had MRONJ. In the study of Ali-Erdem et al., 80 Wistar rats were found to have a 60% incidence of osteonecrosis in zoledronic acid and dexamethasone-treated rats. In this study, rats received medication only 3 times and sacrificed were performed only 28 days after tooth extraction. Howie et al. reported that in all rats (100%) in the group receiving zoledronic acid, MRONJ was diagnosed after eight weeks. In this study, no liver or kidney toxicity was observed in animals due to zoledronic acid use. Dayisoglu et al. showed that 0.1 mg/kg zoledronic acid was administered intraperitoneally 3 times a week for 8 weeks and it was observed that 66% MRONJ developed in the zoledronic acid and tooth extraction group. In the same study, inflammation is not seen only in the control group who received serum injections, but 100% severe inflammation is measured in the tooth extraction group with zoledronic acid administration. In the study of Vidal-Gutierrez et al., osteonecrosis was observed clinically in 100% of the animals in the study group. On histological examination, inflammatory infiltration was found in the whole study group (100%) without any classification. In Yang et al. study, Sprague-Dawley rats were administered zoledronic acid intravenously once a week for 3 weeks and tooth extractions were performed. Inflammatory infiltration was found in the study group and osteonecrosis was found in all (8/8). Tissue healing was uneventful in the control group.

In our study, serum TRACP-5b measurements were compared between groups before the sacrifice. Serum TRACP-5b levels are lower when the osteonecrosis occurs. This result is following the literature. This result should be confirmed with a large number of groups and more than one-time TRACP-5b measurements. Kim et al. used 48
Sprague-Dawley rats in their study. After sacrifice, 75% (27/36) of the rats in the zoledronic acid-applied group had MRONJ. CTx, Glu-OC, TRACP 5b, RANKL, OPG, and TRACP-5b biomarkers were investigated in serum samples. In this study, it was stated that serum TRACP-5b and RANKL/OPG could be used as biomarkers in MRON and this should be confirmed by human studies33,36.

In Çapar’s study, MRONJ developed in rats clinically. Serum CTx, ALP (Alkaline phosphatase), and TRACP-5b biomarker levels were not statistically different between MRONJ and control groups (p>0.05)38. In our study, unlike this study, serum TRACP-5b value was found to be lower in the study group developing MRONJ than the control group. This may be due to the small number of subjects in this study.

Jang et al.38 displayed that serum CTx was not found to be useful whereas TRACP-5b values were significantly lower in the animals diagnosed with MRONJ in all measurements compared to the control group (p<0.05). Similar to our study, this study found that serum TRACP-5b levels were lower in the osteonecrosis group.

FDT devices are systems that can have different wavelengths and different photosensitizers. In most of the studies in the literature, diode laser or low-level laser is preferred as a light source. It is considered that laser light sources positively affect tissue healing in addition to the known biostimulation effect and antimicrobial FDT effect. In our study, unlike other MRONJ studies in the literature, the light device (Fotosan 630, CMS Dental, Copenhagen, Denmark) LED (Light Emitting Diode) light source was used. The effects of similarities and differences between laser light and LED light source on tissue healing after antiresorptive drug use should be evaluated with additional controlled studies.

In our study, there was a significant difference between FDT group and negative control in terms of a decrease in the epithelial gap, mild new bone formation, and a decrease in severe inflammation. The area of necrosis decreased in the PDT group compared to the negative control group but did not make a significant difference.

In the case series consisting of 12 patients made by Astrid et al., a methylene blue photosensitizing agent with a concentration of 50 mg/5 ml was used with a red light diode laser at 680 nm wavelength. In this study, it has been reported that PDT gives very successful results especially in the early stages and it can be evaluated as a supportive treatment in the advanced stages. Also, it has been evaluated that the use of laser as a light device can positively affect the healing with the effect of biostimulation.

In the study of Israeel et al., an 83-year-old male patient who had used denosumab to prevent bone metastases from prostate cancer was diagnosed with MRONJ in a region of the lower jaw alveolar bone. It was reported that after the PDT, findings of infection disappeared and the healing was observed as a result of radiological and clinical follow-up.

In a case report of Poli et al., a female patient who had been using intramuscular clodronate (a nitrogen-free bisphosphonate) was diagnosed with MRONJ. The treatment plan was surgical debridement of the flap, and photodynamic therapy was applied to the surgical field. Photodynamic therapy was performed using a diode laser light device (HELBO® TheraLite Laser, Bredent Medical, Senden, Germany) using a 660 nm wavelength photosensitizing agent based on methylene blue. At the end of 6 months follow-up, the treatment was found to be successful clinically and radiologically.

Sarkanat et al. performed the MRONJ tooth extraction model. The rats were divided into two groups as experimental and control groups, each consisting of 10 animals. In the experimental group, FDT was applied with an 808 nm wavelength low-level laser device and 1 mg/ ml Indocyanine green photosensitizing agent for the following 7 weeks. A significant decrease in MRONJ stage (transition from stage 1 to stage 0), a decrease in inflammation, an increase in live bone rate and necrosis bone rate, an increase in neovascularization and an increase in osteoclast number were measured in the experimental group compared to the control group. Although different PDT devices and photosensitizing agents were used, similar results to our study were obtained in this study.

In the studies of Ervoli et al. and Sarkanat et al., they created a model of FDT with MRONJ tooth extraction, similar to our study in terms of general principles but with differences in experimental procedures and different parameters. These two studies are important in terms of being pioneering studies. Common to these two studies is the application of FDT with different low-level lasers of different wavelengths and different photosensitizers. Both of them showed that FDT supports healing after tooth extraction. Our study supports these two studies with the results of a decreased epithelial gap, increased new bone formation, and decreased severe inflammation in the FDT group compared to the negative control group. Many variables such as light source, photosensitizer, application dose, and duration of the ideal FDT application that can be used in the prevention of MRONJ should be investigated and compared with the next studies.

The application of FDT together with surgical intervention may reduce the risk of impaired wound healing and increases the probability of success. However, further controlled studies are needed to evaluate better the use of FDT in MRONJ.

**Conclusions**

According to the findings of this study, it is considered that FDT can be used as a useful and
supportive treatment option in the prevention and treatment of MRONJ after additional controlled animal and human studies are performed by using more subjects and experimental groups. As a serum biomarker, TRACP-5b can be a valuable marker showing osteonecrosis but there should be further studies to support this result.

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