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Influence of a Proposed Micro-Osteoperforation Approach on the Root Resorption During Orthodontic Tooth Movement: An Immuno-Histochemical Study in a Rabbit Model

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Abstract

THE PURPOSE of the current study was to investigate the impact of a new proposed micro-osteoperforation (MOP) with 2 other MOP approaches on orthodontically-induced root resorption (OIRR) that accompanies orthodontic tooth movement (OTM) in rabbits. Our study included forty-five (of age 24 weeks) albino adult male rabbits which were divided into 3 groups equally regarding the tested 3 MOP approaches; the first new approach (OTM+MOP/2H&1V), the second approach (OTM+MOP/1V), and the third approach (OTM+MOP/3H), which were performed for the first group by 2 horizontal (2H) MOP plus 1 vertical (1V) MOP plus OTM, for the second group by 1 vertical (1V) MOP plus OTM, and for the third group by 3 horizontal (3H) MOP plus OTM, respectively. All approaches were performed in front of the first premolar of mandibular right side of rabbit (RMP1), while the positive (+ve) control (C) group was the same tooth, but on left (LMP1) receiving OTM only (OTM/+veC). Each premolar was protracted by a closed coil spring delivering a (50g) force. After 1, 2, and 3 weeks of OTM, certain interleukines (ILs) biomarkers, named the IL-1 alpha (IL-1α) and IL-10 were detected the OIRR through the immunohistochemistry (IHC) of the compression mesial side of both premolars root regions (cervical, middle and apical thirds). The results revealed a significant increase in the expression of IL-1α and IL-10 which were confirmed in the compression side of the MOP groups ($P<0.001$ to $= 0.041$). Also, the results indicated that the middle and cervical third regions were significantly higher ($P < 0.05$) than the apical third in all groups. Besides, the results depicted that the new approach, the (OTM+MOP/2H&1V) group showed a moderate ILs expression between the higher, the (OTM+MOP/1V) group and the lower, the (OTM+MOP/3H) group. The study concluded that all MOP approaches could induce OIRR at the compression side of PDL during OTM, but with a different range. The current study concludes that the new approach, the (OTM+MOP/2H&1V) group might be the preferred approach for stimulating faster OTM because it causes a moderate OIRR after the less inducing group, the (OTM+MOP/3H) when examined in rabbits.

Keywords: Biomarkers; immunohistochemistry; micro-osteoperforation; orthodontic tooth movement; root resorption.

Introduction

Long-term comprehensive orthodontic treatments (OT) (periodontal phenomenon) [1], particularly of extracted cases [2] which may take about (2.5 years) of treatment [3], can bring many risks to patients [4, 5], such as root resorption (RR) [3, 6], periodontal disease [7], discomfort [8], and low patient compliance. Thus, the efforts to increase the rate of tooth movement (RTM) [3] without contributing to adverse effects such as orthodontically induced root resorption (OIRR) and to shorten the treatment period have always been the focus of many orthodontists [9, 10].

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Various surgical methods have been come to use to accelerate the RTM [11], all of which are based on the concept of the regional acceleratory phenomenon (RAP) [12], which resembles the events that could occur during fracture healing showing the recruitment of osteoblasts and osteoclasts, thus accelerating the anabolic and catabolic bone activities, respectively, and the result is faster bone remodeling [13] and low alveolar bone density [12]. Selective alveolar decortication can induce the RAP during orthodontic tooth movement (OTM) [14]. Examples of these techniques are, the invasive corticotomy [15], the less invasive approaches, the piezopuncture [16], piezocision, and corticision [17], and the flapless micro-osteoperforation (MOP) described as the least invasive, least traumatic approach among all surgical interventions [18-21]. Non-surgical adjunctive therapies have also been reported, like using a resonance vibration [22], low-level laser therapy [3], low-intensity pulsed ultrasound [23], and drugs like injection of prostaglandins [24].

The micro-osteoperforations (MOPs) fundamental characteristics of being a minimally invasive surgical technique with an accepted patient compliance were proved their success for accelerating the RTM and decreasing the treatment time (TT) via elevating the expression of inflammatory biomarkers in certain animal models and also in clinical research [11]. However, varying results and inconsistent evidences were been reported concerning the occurrence of OIRR as an iatrogenic adverse effect of MOP [18]. Chan et al. [25] noticed that the upper first premolars that were subjected to a buccally tipped force, were exhibited larger volumes of OIRR craters after MOPs, also in the Joseph et al. [26] study, an overall OIRR was much more in the treated MOP group when compared to control group, whereas in the Dos Santos et al. [27] systematic review study, they concluded that MOPs could not show any influences on OIRR.

The reason behind the induction of OIRR is the heavier activation of osteoclastogenesis via the pro-osteoclastically excessive secretion of the inflammatory cytokines [28]. These cytokines which induce the RAP to produce higher RTM can cause also higher cementoclasts activation and thus mild and severe OIRR [29]. Increased levels of such cytokines were associated with higher OIRR [30-32].

Many studies showed that interleukines (ILs) and part of cytokines could be considered as potential OIRR biomarkers, except interleukine-1 beta (IL-1β). The main 2 functioning cytokines that can be secreted locally secondary to OTM are; the pro-inflammatory pro-resorptive cytokines (increase the inflammatory response), like interleukine-1 alpha (IL-1α) [31-33] and the anti-inflammatory anti-resorptive cytokines (decrease the inflammatory response), like interleukine-10 (IL-10) [30, 33].

As a result of the mentioned literature summery, this study aimed to show the influence of different MOP approaches on OIRR through the immuno-histochemical (IHC) evaluation of certain OIRR biomarkers, the pro-resorptive IL-1α and the anti-resorptive IL-10, in response to the application of orthodontic force and following the surgical intervention with MOPs of OT.

Material and Methods

Protocol and Study Design

The current experimental study used a split mouth design of study, considering the rabbit mandibular left first premolar (LMP1) as the positive (+ve) control side and the rabbit mandibular right first premolar (RMP1) as the experimental MOP side. The Research Ethics Committee / College of Dentistry / Mosul University (UoM.Dent. 23/2) approved this study.

Sample

The rabbit lower jaw halves were collected from 45 albino, male, adult, and healthy animals, weighing from (1.5kg) to (2.2 kg), and averagely aging (24 weeks). Favorable environmental conditions that can simulate the naturals were prepared to these animals for (1 week) before assuming with the experiment. These include preparing an individualized metallic cage for every animal, preserving a constant temperature (22°C), and the availability of adequate water and commercial diet for every animal [34].

Animal Grouping

Upon the 3 MOP approaches, rabbit animals were equally and randomly grouped into 3, 15 animals in each. These in turn were further grouped equally into 3, 5 animals in each upon the time they euthanized after completing the experiment at the end of 1, 2, and 3 weeks.

Experimental and Orthodontic Procedures

Three MOP approaches were performed of different directions and injury depths near and in front of the experimental RMP1. This region was chosen because of its resemblance the human edentulous atrophied ridge [34, 35]. The first approach (OTM+MOP/2H&1V), was the new MOP approach performed via two shallow horizontal (2H) MOPs, 1 on buccal and 1 on lingual, MOP size; 1.5mm depth &1.4mm diameter, both were located below the alveolar crest by 5mm, and mesially to

RMP1 by 1mm, plus one shallow vertical (1V) MOP, MOP size; 1.5mm depth &1.4mm diameter, located at alveolar crest, and mesially to RMP1 by 1mm, plus OTM. The second approach (OTM+MOP/1V) was performed via one deep vertical (1V) MOP only, MOP size; 4.5mm depth &1.4mm diameter, located at alveolar crest, and mesially to RMP1 by 1mm, plus OTM. The third approach (OTM+MOP/3H), was performed via three shallow horizontal (3H) MOPs, all of them were on buccal side, and they were distributed vertically along the RMP1 root, MOP size; 1.5mm depth &1.4mm diameter, the first MOP was located below the alveolar crest by 1mm, and then between every 2MOPs was a 2mm distance, all MOPs were mesially to RMP1 by 1mm, plus OTM. While the positive (+ve) control (C) side (LMP1) was received only OTM (OTM/+veC). The three MOP approaches were shown in (Fig.1).

Under an intramuscular general anesthesia of xylazine (5mg/kg) + ketamine (35mg/kg), and via a single operator [34], all MOPs were performed accurately with the aid of a temporary anchorage device (TAD) self-drilling mini-screw (OSTEONIC, South Korea), dimensions of 6mm×1.4mm were as the length and the diameter, respectively. The intended MOP depth was determined by adding a stopper to this TAD [36] for better standardization in removing the same amount of bone [34]. Also, the thickness of gingival soft tissue was evaluated before assuming with the TAD drill [4] (Fig.2, A, B, and C). After completing MOPs, the same TAD was then be inserted between the 2 lower anterior teeth of rabbit via a manual screw driver (GSSEM / South Korea) (Fig.2, D). Two closed-coil springs made from nickel-titanium (NiTi) (lumen size; 0.22mm, total length; 1 m, diameter; 0.9mm, Zugfeder (758-205), Dentaurum, Germany), one on each side of mandible were used to initiate the protraction force of the 2 mandibular first premolars (MP1s) (RMP1 and LMP1), each delivering a (50g) orthodontic force. Then these 2 springs were attached anteriorly to the inserted one TAD between the 2 lower anterior teeth [12]. For a standardized work, the (50g) force was evaluated by a certain dynamometer (Hahnkolf, Stuttgart, Germany) (Fig.2, E). The final view of the placed bilateral orthodontic appliance was shown in (Fig.2, F). The rabbits then were euthanized via (ketamine of 2 doses + overdosed potassium-chloride) after finishing the experimental 3 periods of 1, 2, and 3 weeks [34]. A certain veterinary specialist (B.H.) accomplished this euthanizing procedure and in accordance to Ober et al. [37].

Immuno-histochemical Staining

Before staining with the IHC stain, the studied specimens, which were taken so that they were extended from the mandibular second premolar (MP2) to about a 10mm mesially to mandibular first premolar (MP1), were passed through the steps of tissue processing in accordance to [12, 38, 39]; thus, the specimens of the animal were firstly washed with distilled water and then fixed in 10% formaldehyde (Saudi Arabia Chemical Company- Saudi Arabia), then decalcified in 10% ethylene-diamine-tetra-acetic acid (EDTA-2Na, pH 7.4) (Scharlau, UK) at a room temperature (18–25°C), then dehydrated with graded ethanol (Scharlau, UK) of increasing concentrations, then cleared with xylene (Merk, USA), and finally embedded in paraffin (Sakura, UK). Then, the specimens were sectioned into 5-µm-thick sections (mesio-distal slices), so that they were perpendicular to the MP1' occlusal plane. Then these sections of the mesial side of the cervical, middle, and apical thirds of MP1 root were been ready for passing through the steps of the IHC stain [12] of IL-1α and IL-10 to estimate their expression as OIRR biomarkers. The IHC staining was accomplished in accordance with [12, 38, 39]. Therefore, prepared sections of the animal were deparaffinized with a series of xylene, and then hydrophilized (rehydrated) with an ethanol alcohol [12]. Polyclonal primary antibodies (Pri-Abs) were used with their specific dilution ratios: anti-IL-1α (interleukin-1 alpha, 1mg/ml concentration, IHC-Harp-Ab-DAB, Elabscience, China) and anti-IL-10 (interleukin-10, 1mg/ml concentration, IHC-Harp-Ab-DAB, Elabscience, China) to quantitatively measure the expression intensity of IL-1α and IL-10. With these **(**Pri-Abs), the sections were programmed to be incubated at (4ºC) over night, reheated at (37°C), rinsed with a sterilized phosphate-buffered saline (PBS), dried via an absorbent paper [31], added with the anti-rabbit IgG (IHC-Harp-Ab-DAB, Elabscience, China) as the secondary antibody (Sec-Ab), and incubated with an enzyme label (peroxidase) to form the (horse-radish peroxidase-conjugated Sec-Abs) at room temperature [12], which would give rise to the formation of the specific colored precipitate of brownish yellow or mostly dark brown color (primary stain) via the reaction of the antigen-antibody complex. Later, a specific light microscope (LM) (OPTIKA, Italy) was utilized to quantitatively analyze the expression intensity of IL-1α and IL-10. The LM images which were captured via a specific digital camera (Omax 18.0 MP USB 3.0, A35180U3, China), and magnified to (40x) of the compression area (mesial side of the MP1) of alveolar bone were utilized to measure the labeling of IL-1α and IL-10. The measurements were taken by 3 independent investigators, and then the labeling values were

calculated by the same investigators for accomplishing further statistical analyses of the data.

Statistical Analysis

Software of the Statistical Package for the Social Sciences (SPSS 22.0, Chicago, IL) was utilized to perform all the analyses of the statistics. The data of the IHC of the scores of ILs expression in the current study were non-parametric, thus, they were statistically analyzed as the median and as the interquartile range (IQR) via using the test of Kruskal-Wallis H for multiple comparing among different experimental groups and then using the Friedman test for multiple comparing among different periods. At $P \leq 0.05$, the level of the statistical significance was applied.

Furthermore, both class calibrations (inter- and intra-) were set in the present study. For calibrations of the inter-class; the same rabbit samples were investigated by 2 different researches (M.G., A.M.), the Dahlberg equation was utilized to caliber the random errors, and the paired t test was used to caliber the systemic errors. For calibrations of the intra-class; individual investigators examined about 10 rabbit samples, two times and at least of two weeks interval. The outcomes of the inter-class were; (0.081), (0.71) for the random errors and systemic errors, respectively, while of the intra-class they were; (0.065), (0.73) for the random errors and systemic errors, respectively. This means that both class calibrations were non-significant statistically.

Results

Along the whole experimental study, the closed coil NiTi springs of all animals were existed in their places and without any damage. In all groups, the MP1 moving mesial pressed side, as it was correctly moved, at both mandibular sides was examined for the labeling of both OIRR biomarkers; IL-1α and IL-10.

Immuno-histochemical Analysis

In the current study, the expression of both ILs (IL-1α and IL-10) was founded on the compression area of the moving MP1 in the PDL and alveolar bone of all groups (Figures 3-10). The expressive levels of IL-1α and IL-10 in all MOP groups were showed more expression and significant increase when compared with the control groups ($P < 0.001$ to $= 0.041$). The new approach (OTM+MOP/2H&1V) showed moderate ILs expression between the higher group, the (OTM+MOP/1V) and the lower group, the (OTM+MOP/3H). Also, significant differences between weeks 1 and 2, and 1 and 3 ($P = 0.002$ to 0.036 for IL-1α and $P = 0.002$ to 0.040 for IL-10) have been reported. However, non-significant differences between weeks 2 and 3 have been depicted regarding these ILs through the IHC evaluation. Moreover, both ILs showed their highest expression at the middle and cervical thirds of MP1 root and they were significantly higher ($P = 0.002$ to 0.036 for IL-1α and $P = 0.002$ to 0.040 for IL-10) than the apical third in all groups (Tables 1 & 2).

Discussion

In the present study, the MOPs numbers, locations, depths, and diameters were designed and also modified in accordance to numerous past studies in rats [21, 40, 41] and in rabbits [12, 34, 35]. Diameters of MOPs of 0.25mm, 0.5mm, and 1.2mm were made in rats [21, 40, 41], while in albino rabbits, diameters of MOPs of 1mm and 1.4mm [12, 34, 35], and depths of 1.5mm and 4.6mm were made in these animals [34]. According to Uribe et al. [42]; a 1mm depth of corticotomy injury might not be enough to induce the response of the RAP. Thus, the current study performed deeper MOPs that nearly matched the studied dimensions of [34]; 1.5mm and 4.5mm as MOP depths and 1.4mm as MOP diameter in rabbits. The rabbit experimental region of the study (MP1) agreed with the above mentioned studies [12, 34, 35]. Also the orthodontic appliance design [12] and the amount of the traction force (50g) matched many studies [12, 21, 43-45]. Moreover, anchoring to TAD [12] can give successful direct anchorage [46] and efficient protraction of the MP1, and similarly can eliminate the need to use the somewhat small and less retentive lower anterior teeth [12].

The surgical techniques influence on accelerating the RTM and the subject of OIRR previously and till now is unclear. It was depicted that one of the aims of the (Corticotomy-Assisted Orthodontics) is the prevention of OIRR [47]. However, the influence of these techniques on OIRR showed numerous contradictory findings [25], particularly for discovering the cause of OIRR, i.e.; whether OIRR is the result of the remodeling process on the root surface or the result of the incorrect use of the surgical intervention that can directly traumatize the root [17]. Many studies showed that the ILs can play an important role in the process of OIRR [33] together with the receptor activator of nuclear factor kappa B ligand / receptor activator of nuclear factor kappa B / osteoprotegerin system (RANKL/RANK/OPG), although the exact odonto-/osteoclasts functional regulation remained to the present without any definite or clear interpretation [48]. Highly confirmed evidence corroborates that MOP can initiate the biological changes via the

increase in chemokines and cytokines biomarkers release that could induce the aseptic inflammatory reaction and differentiation of osteoclasts, which in turn accelerates RTM [10, 20, 34]. Moreover, the normally expressed inflammatory markers through OTM could be efficiently amplified by the RAP induced via MOP [49]. Alikhani [24] suggested that the MOP depth into bone should be of (3-7mm) to be sufficient for obtaining the intended catabolic effect and high inflammatory markers to accelerate RTM in humans. Chen et al. [50], upon their study on rats reported that the amount of the OIRR could have a positive (+ve) correlation with the surgical injury depth or extent performed for the aim of accelerating OTM. This may agree with the current study, as all MOP groups had higher expressions of IL-1α and IL-10 than the (OTM/+veC) group.

Furthermore, this might go to show that the MOP group that its holes were performed with a more deep injury, could also be characterized by the presence of the highest ILs expression, fastest OTM, and the greatest OIRR. According to this, the new approach, the (OTM+MOP/2H&1V) group which was performed in such way that the 2 horizontal MOPs could create a deep continuous hole of nearly (3mm) of MOP depth, which agreed with Alikhani [24], exhibited also an intermediate ILs expression between the higher, the (OTM+MOP/1V) group and the lower, the (OTM+MOP/3H) group. In another word, a moderate OIRR and an acceptable moderate OTM acceleration could be produced through this intervention. Thus, the highest ILs expression and OIRR and also faster OTM were noticed in the (OTM+MOP/1V) group, owing to the greater MOP depth (4.5mm). At the same time, the lowest ILs expression and OIRR and also the least acceleration of OTM were showed in the (OTM+MOP/3H) group, owing to the presence of shallow MOP depths of (1.5mm) when comparing among the MOP groups, which may come in agreement with the studies of Bakr et al. [4] and Wagh et al. [6]; as they concentrated on the effect of the surgical insult and suggested that a minimal insult might not be able to induce the inflammatory response for triggering the RAP or the expression of different cytokines. Almog et al. [51] suggested that the IL-1α is the most important cytokine that is usually derived from the (monocyte-macrophage lineage). Gregorczyk-Maga et al. [31, 32] claimed also that this cytokine might be considered as a novel non-invasive potential biomarker for the early detection of OIRR. Xiao et al. [52] confirmed this, suggesting that the OIRR may be induced during OTM because IL-1α does not only regulate the maturation and differentiation of osteoclasts, but also the OIRR cells, the odontoclasts. Because IL-1 major role involved in the osteoclasts activation and bone loss during OTM, it can be mainly expressed at the compression side of the moved tooth root [53]. IL-1α can be found in every OIRR site in a rat model [54].

The IL-10 which is mainly secreted by innate immune cells, like T-helper 2 (Th2) cells [55], dendritic cells (DCs), and macrophages, can function as anti-inflammatory by inhibiting RANK nuclear translocation, and by consequence, the pro-inflammatory genes expression. Nevertheless, IL-10 can equally induce the shutdown or the propagation of the responses of inflammation [56]. The findings of the current study came in agreement with many previous investigations; most of them demonstrated the resultant of a considerable and an overall volumetric OIRR craters in the MOP experimental side [6, 9, 19, 20, 24, 26, 50]. Chan et al. [24] upon their study concluded that MOP can result in a greater amount of OIRR on (day 28) during OTM when applying buccal tipping force to the upper first premolars in humans. Chan [24] performed MOPs with a depth of (5mm) and related this resorption to the RAP caused by the improved turnover of the alveolar bone that was correlated by an elevation of osteoclastic activity, which may exacerbate OIRR [57]. Furthermore, it is likely that the exaggerated regional osteoclastic activation, as shown after MOP [24, 26], may result in an elevation of the odontoclastic activity also [58]. Wagh et al. [6] demonstrated that the increase in the percentage of OIRR of canines with MOP was much higher than those treated with mechanical vibration, they related this to the increase in the inflammatory biomarkers by the RAP which were responsible also for the increase in the RTM, although, it was a non-significant difference in OIRR. They used orthopantomography (OPG) for measuring which might be not so accurate. Chandorikar and Bhad [19] reported the presence of an overall OIRR after MOPs more than the control (although it should be repeated). Nevertheless, further researches may be needed to highlight this subject and to show the OIRR repaired craters after MOPs.

Alikhani et al. [59] noticed during OTM with and without MOP that the released IL-1α (major pro-inflammatory cytokine) was the unique biomarker that can remain significantly in a higher level after 4 weeks (28 days) of retraction when compared to before retraction in human, higher in the MOP group (by 5 times), favoring the osteoclastic activity and recruitment, while all other cytokines were decreased with the progress of time. This conclusion may come in agreement with the current study, as this cytokine was higher in the MOP groups than the (OTM/+veC)

group. Moreover, Ahuja et al. [29] depicted a significant raise (at day 28 in human) in the pro-resorptive cytokines and OIRR secondary to OTM at the distal and mesial sides of the middle third of the moved teeth. Nevertheless, the current study showed the IL-1α expression after the (4 weeks) which matches the (9 days) in rabbits, and depicted a reduction in this cytokine in all groups overtime, and an elevation of the IL-10 in the MOP groups and a reduction of IL-10 in the (OTM/+veC) groups over time (although weeks 2 and 3 showed the same expression of IL-10 at the control) which was not studied by Alikhani [59] and Ahuja [29]. Sunny et al. [58] conducted a study in human and found a significant osteoclastic activity in the site of the MOP. Besides, the ILs produced during OTM, at inflammation and cell death phases may lead to the secretion of certain enzymes which later can signify the results of the osteoblasts and osteoclasts number, and thus increase the remodeling of bone and root, that can be amplified with the MOP [58].

Moreover, certain animal studies [34, 40] suggested a significantly high increase in the osteoclasts and inflammatory biomarkers level at the MOP groups, in variable sessions of follow-up. Cheung et al. [40] and Sugimori et al. [21] found a no significant difference of OIRR in the intergroup (MOP and control) in rats. Also Kim et al. [34] demonstrated a non-significant OIRR in either the single vertical MOP (SV-MOP) or the multiple horizontal MOP (MH-MOP) group in rabbits. Gemert et al. [60] noticed that MOP did not induce OIRR in beagle dogs. The current study may come in a strong disagreement with Erdenebat et al. [10]; they concluded that MOP did not significantly affect OIRR of the upper first molar, whereas; it may induce a constituent cementogenesis process after 2 weeks in mice.

Also, the current study may be disagreed with some human studies [61-63]; they found a non-significant difference between the MOP and the control group. The disagreement and the disparity of the findings in addition to the follow-up period variation, might be due to the biological variability present between humans and animals (rabbit bone metabolism is 3 times faster than that of human) [20], different techniques administered for OIRR record, different protocols of the studies [34], different genetic susceptibility towards OIRR (patients may have low or high risk of OIRR) [64], magnitude and direction of forces [35], and time elapsed between tooth extraction and MOP performance, according to Alikhani et al. [59] and Wagh et al. [6]; MOPs should be delivered (6 months) after tooth extraction to eradicate the inflammatory biomarkers of the extraction site from those triggered by MOPs which may obscure the MOP effect, giving false results. Further researches concerning the influence of the RAP via MOPs on OIRR during OTM and the use of three-dimensional (3D) volumetric OIRR measure with longer follow-up periods are recommended.

In the current study, the IL-1α showed its highest expression at week 1 in all experimental groups, which may come in agreement with Bober et al. [65]; they suggested the presence of an ongoing inflammatory response when it is stimulated by the orthodontic force application that can overcome (21 days) in humans, and it is characterized by an elevated levels of (α-defensins) inflammatory biomarkers. The (21 days) in humans can nearly match the (1 week) in rabbits, and so can be agreed with the current study. In addition, Raghav et al. [49] observed in the first month after MOP, a marked raise in the activity of the biomarkers in humans which may agree with the current study. Alikhani et al. [59] and Kapoor et al. [33] confirmed these findings; they noticed that the concentration of the inflammatory biomarkers can be maximized through the first (4 weeks) after surgical injury or orthodontic force application in humans. Alfawal et al. [66] agreed and showed also the termination of the RAP effect which was at the end of the second month in human, which may come in agreement with the current study. Also, at the MOP groups, frequent MOP performance was not achieved in the current study, and this also may explain the reduction in the expression of IL-1α.

Overtime, in the current study, the expression of IL-1α was slightly decreased in response to the increased expression of IL-10 at the MOP groups, which may come in agreement with Aghelan et al. [67] who suggested that the levels of anti-inflammatory IL-10 and pro-inflammatory IL-1α could be inversely related with the presence of chronic stimulation and chronic conditions. Both can be detected in chronic inflammations [68]. According to Chen et al. [69]; IL-10 can function efficiently in bone metabolism. In the current study, the RAP induced by the MOP may produce this insistent stimulation, elongation of the inflammation and the remodeling process of bone and root, and also a frequent resorptive-repair process by the frequent exposure to bone and root turnover, which may exacerbate OIRR. Yashin et al. [30] showed the effect of the anti-inflammatory anti-resorptive cytokines, like IL-10 by inducing a stable bone remodeling state upon their role in the defense mechanism of the body, and noticed a significantly increased levels of the IL-10 during OTM with (moderate to severe) OIRR group when compared to the control group, which agreed with the current

study. Also, Saraiva et al. [56] suggested that the IL-10 can function in both the propagation and the shutdown of the inflammatory response. Nugraha et al. [44] reported that the IL-10 can function in resolving the inflammation, but can causes also a cellular differentiation and Rojas et al. [70] reported the late secretion of the IL-10 when compared to the early release of IL-1α. According to this, the current study may go to show that the increased expression of IL-10 can be considered as a biomarker for the detection of late OIRR. Besides, the increased expression of IL-1α may be believed to be as an early detector of OIRR, which agreed with Gregorczyk-Maga et al. [31, 32]. De Molon [71] et al. examined the early immune-inflammatory process of cytokines during alveolar bone loss at (0-14 days), and the late immune-inflammatory process at (14-21 days) in a rat model. Besides, Yang et al. [72] by the scanning electron microscope (SEM) of the compression side; noticed the presence of RR craters in the roots of rat molars after (2 weeks) of traction. Furthermore, Sugimori et al. [21] stated the presence of OIRR and bone resorption at the compression side during OTM alone and with MOPs between (day 1) and (day 14) in rats. Besides, Craveiro et al. [73] depicted a continuous availability of the inflammatory markers and intensified bone / root remodeling on the compression side after (1, 3 and 5 weeks) of OTM in mice. According to Ratanasereeprasert et al. [74]; the IL-10 showed a different time-dependent expression (increase or decrease) collected from the compression side between (day 1) and (day 28) of OTM in humans, indicating different tendencies towards RR among the tested groups.

Moreover, the presence of the non-significant results of the RR biomarkers between week 2 and 3 at the MOP groups might be due to the slight beginning of the termination of the MOP effect, which may come in agreement with the conclusions of Raghav et al. [49]; Raghav stated that after (8 weeks) of MOPs in humans, there was no longer influence of the RAP induced by MOPs. The (8 weeks) in humans may come close to the (2.5 weeks) in rabbits, which could agree with the current study.

Overtime, at the (OTM/+veC) groups in the current study, both ILs (IL-1α and IL-10) were decreased. The IL-10 also showed the same expression at weeks 2 and 3. According to Schröder et al. [75]; the inflammatory biomarker levels would differ mainly through the initial early days of OTM, and then stabilize and nearly return to normal levels after the first week of OTM. The surrounding local tissues in the stages of late force application may show an adaptation to these forces, and so the cytokines (systemic & local) might not be changed also [76, 77], and this may explain the reduction of the ILs in the (OTM/+veC) group at week 2 and 3 in the current study. Moreover, the light force used in the current study causes a skip in the second phase of OTM, thus the reaction of the inflammation might be reduced also in response to [78]. Besides, Alikhani et al. [79] reported the increase of cytokines at (day 14 in human is nearly (≈) day 5 in rabbit) after OTM, and later there was a decline in these cytokines, which may agree with the current study. Reiss et al. [80] agreed, they noticed that the inflammatory biomarkers and bone turnover levels could show no alteration in saliva during OTM at every month of follow-up in human. Moreover, Karaduman et al. [81] showed a reduction of (IL-10) after (28 days) of OTM in humans. Furthermore, upon the study of Trelenberg-Stoll et al. [82] on mice, they observed the presence of RR volume during OTM by the micro-computerized tomography (micro-CT) and pointed out a significant elevation of RR after (7 days) in rats and (11 days) in mice. This may also explain the lowering of the expression of RR biomarkers of the (OTM/+veC) group in the current study at week 2 and 3 with the absence of the RAP induced by the MOP. In another word, there was an early reduction in the root resorptive process shown at weeks 2 and 3 of the (OTM/+veC) group.

In the current study, both ILs were highly expressed at the middle and cervical thirds of the MP1 root, and they were significantly higher than the apical third, this might go to show that these regions may be more prone to exhibit RR (i.e., lateral RR was more than the apical RR), which may come in agreement with multiple studies [83-86]. All of these studies demonstrated that the most stressed region of periodontal ligament (PDL) at the compression side, which were the cervical third [83, 85, 86] or the middle and cervical thirds of tooth root [84], could show also higher RR and resorption craters in rats after (1 week) of OTM [83] or (3 weeks) [84], and also in humans induced by the continuous pressure with spring [85, 86]. Besides, according to Verna et al. [87]; the shifting of the center of rotation (C_{Ro}) of the moved teeth towards the apical region via MOPs could make the middle and cervical thirds of the root to be pressed against the alveolar bone more than the apex, thus causing more RR at these regions, which agreed with the current study. Moreover, when protracting the MP1, it may undergo a slight rotational movement, as the tooth movement is a 3D motion in the space, and in the early stages of OTM, a considerable tooth rotation cannot be avoided [88], and a combination of (rotation and translation) may be definitely and simultaneously occurred during OTM [72, 89]. Thus, in accordance to [90]; the resorption lacunae could be prominently observed at

the root middle third during rotational movement. Furthermore, according to Maués et al. [91]; teeth with closed apices (complete root formation) might be subjected to external apical root resorption (EARR) more than teeth with open apices during OTM. This was strongly showed by the absence of the IL-10 expression (zero) at the apical third region of the 2 weeks and 3 weeks of the (OTM/+veC) groups. Also, Phillips [92] and Dindaroğlu and Doğan [93] found the absence of a direct correlation between the angular or sagittal tooth root apex movements and the RR. According to many studies [94-97]; the occurrence of EARR during OTM was mainly presented at the anterior teeth roots.

Nevertheless, multiple studies demonstrated the presence of different levels of cytokines and other biomarkers in patients undergoing OT. The variability of the results may be due to the presence of the heterogeneity in the methods used for recording OIRR [34]. Moreover, the presence of inflammation may change the biomarker value when measured for e.g. in the bio-fluids [29, 30]. In addition, a clear cascade of both inflammatory biomarkers (anti- and pro-) occurred during the healing of the fractures may not be occurred during OTM [77]. However, there are till now some difficulties to look for the precise OIRR biomarker, as the osteoclasts are also stimulated. Thus, further studies are needed to highlight the interesting hypothesis of OIRR and OTM with MOP, and the molecular mechanism of IL-1α and IL-10 on OIRR, with the introduction also of larger samples and the use of more standardized methods for recording OIRR, if at future investigated, it would be efficient to surpass the drawbacks of the studies.

This break-through investigation was introduced to show for the first time evidence regarding a new MOP modality for causing faster OTM with an acceptable OIRR, these results were reached successfully via the introduction of numerous morphological parameters and multiple differences among groups, which showed clear significant results, even within a small sample. However, future studies with a relatively larger sample and a new proposed MOP modality may be essential for deeper knowledge about its effect on OTM and the side effect on OIRR and so to be more applicable in the clinical studies.

Limitations

The current study showed an extended methodology which might produce a comprehensive insight regarding the MOP effect on OIRR. However, the following research limitations were observed:

1. Detecting OIRR was only performed via an IHC investigation, and some of the ILs reactions might be masked within tissues or they may be entirely not reacted.
2. Rabbit teeth are described as an elodont type of teeth with a patent apex and a continuous eruption phenomenon, thus the root apical third region outcomes of the OIRR biomarkers might not be so accurate due to the continuous remodeling at this region.

Conclusion

Within the limitations of the present study, the following conclusions were noticed:

-All the studied micro-osteoperforation (MOP) approaches could significantly increase the OIRR at the compression side of rabbit root detected by the higher expressions of IL-1α and IL-10 in the MOP group.

-The proposed new approach, the (OTM+MOP/2H&1V) induced moderate OIRR, between the highly induced group (OTM+MOP/1V) and the less induced group (OTM+MOP/3H), thus this new approach might be chosen for stimulating faster OTM, because it causes less OIRR than the (OTM+MOP/1V) group, and slightly higher than the (OTM+MOP/3H) group when examined in rabbits.

-Increased expressions of IL-1α may be considered as an early detector of OIRR, while increased expressions of IL-10 can be considered as a biomarker for the detection of late OIRR.

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Declaration of Conflict of Interest

The investigators declare the absence of any conflict of interest.

TABLE 1. The scores of the *IL-1α* in all groups after 1, 2 and 3 weeks of orthodontic tooth movement (OTM) or orthodontic tooth movement plus micro-osteoperforation (OTM+MOP)

Periods	Groups Regions	OTM+MOP/1V	OTM+MOP/2H &1V	OTM+MOP/3H	OTM/+veC	*P*-Value
1 week	Apical	2.5 (2) A b	2 (1) B b	1.5 (1) C b	1 (0) D b	<0.001
	Middle	3 (1) A a	2.5 (0.5) B a	2 (1) C a	1.5 (0) D a	0.002
	cervical	3 (1) A a	2.5 (2) B a	2 (0) C a	1.5 (0.5) D a	0.019
2 weeks	Apical	2 (0) A c	1.5 (0) B c	1 (1) C c	0.5 (0) D c	0.024
	Middle	2.5 (0.5) A b	2 (1) B b	1.5 (0) C b	1 (0) D b	0.036
	cervical	2.5 (1) A b	2 (0) B b	1.5 (0) C b	1 (1) D b	0.036
3 weeks	Apical	1.75 (1) A c	1.25 (0) B c	0.75 (1) C c	0.25 (0) D c	0.041
	Middle	2.25 (1) A b	1.75 (1) B b	1.25 (0) C b	0.75 (0) D b	0.029
	cervical	2.25 (1) A b	1.75 (0) B b	1.25 (0) C b	0.75 (0) D b	0.029
***P*-Value**		0.002	0.026	0.021	0.036	

Data expressed as Median and Interquartile Range (IQR) (Number (N) = 5 animals).
Different capital letters mean there is significant difference among groups at $p \leq 0.05$.
Different small letters mean there is significant difference among periods at $p \leq 0.05$.
H: horizontal MOP, V: vertical MOP, +veC: positive control.

TABLE 2. The scores of the *IL-10* in all groups after 1, 2 and 3 weeks of orthodontic tooth movement (OTM) or orthodontic tooth movement plus micro-osteoperforation (OTM+MOP)

Periods	Groups Regions	OTM+MOP/1V	OTM+MOP/2H &1V	OTM+MOP/3H	OTM/+veC	*P*-Value
1 week	Apical	1.75 (1) A c	1.25 (1) B c	0.75 (0) C c	0.25 (0) D b	0.036
	Middle	2.25 (1) A b	1.75 (1) B b	1.25 (0) C b	0.75 (0) D a	0.004
	cervical	2.25 (2) A b	1.75 (1) B b	1.25 (1) C b	0.75 (1) D a	0.004
2 weeks	Apical	2.25 (2) A b	1.75 (1) B b	1.25 (1) C b	0 (0) D c	0.021
	Middle	2.75 (1) A a	2.25 (2) B a	1.75 (1) C a	0.25 (1) D b	<0.001
	cervical	2.75 (1) A a	2.25 (2) B a	1.75 (1) C a	0.25 (0) D b	<0.001
3 weeks	Apical	2.5 (1) A b	2 (2) B b	1.5 (0.5) C b	0 (0) D c	0.011
	Middle	3 (1) A a	2.5 (0.5) B a	2 (1) C a	0.25 (0) D b	0.001
	cervical	3 (3) A a	2.5 (1) B a	2 (2) C a	0.25 (0) D b	0.001
***P*-Value**		0.039	0.038	0.002	0.040	

Data expressed as Median and Interquartile Range (IQR) (Number (N) = 5 animals).
Different capital letters mean there is significant difference among groups at $p \leq 0.05$.
Different small letters mean there is significant difference among periods at $p \leq 0.05$.
H: horizontal MOP, V: vertical MOP, +veC: positive control.

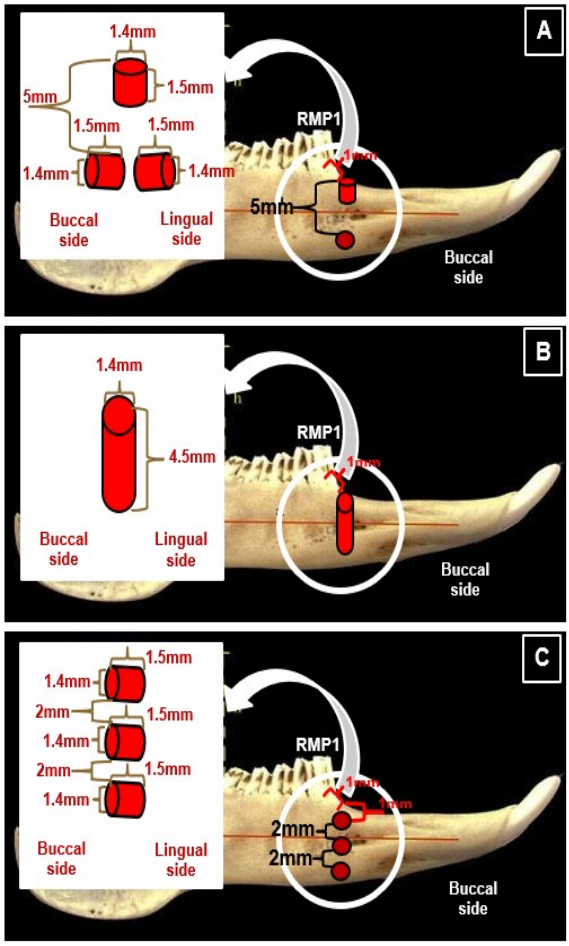

Fig. 1. The right side of rabbit mandible illustrating: A. A new MOP approach of horizontal and vertical MOPs (OTM+MOP/2H&1V) B. Vertical MOP approach (OTM+MOP/1V) C. Horizontal MOP approach (OTM+MOP/3H).

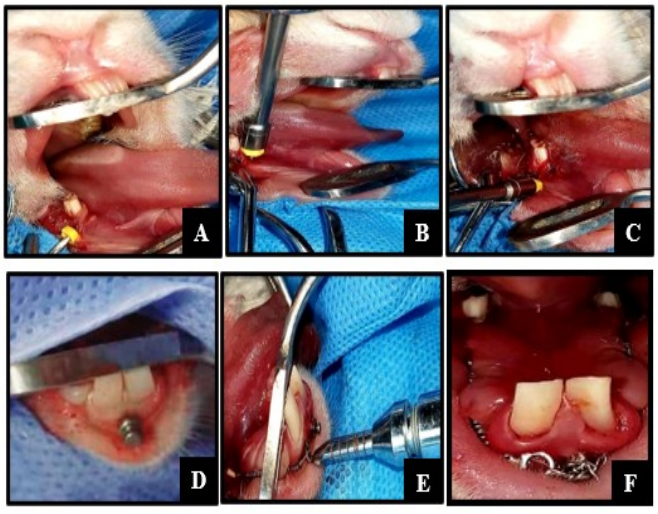

Fig. 2. A. Evaluating the soft tissue thickness or gum depth with a stopper added-periodontal probe B. Creating vertical MOP with a stopper added mini-screw (TAD) by placing this TAD parallel to the RMP1 long axis C. Creating horizontal MOP with a stopper added mini-screw (TAD) by placing this TAD at 90° to the soft tissue of the alveolar bone D. Final positioning of TAD in between the 2 lower anterior teeth E. Using a dynamometer for evaluating the amount of the orthodontic traction force of (50g) F. Final view of the bilateral orthodontic appliance.

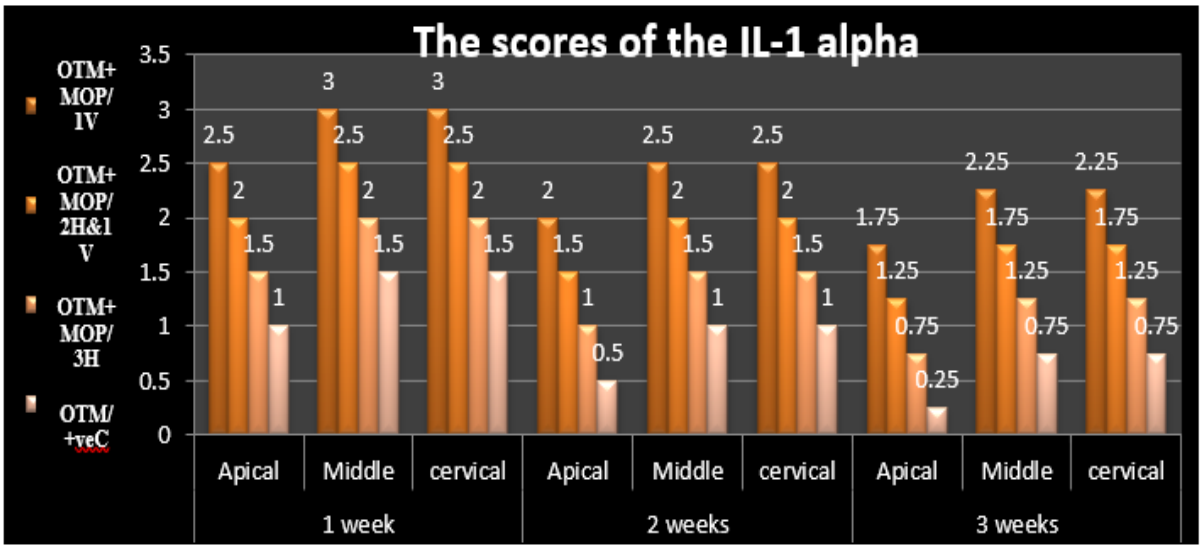

Fig. 3. Histogram of the scores of the *IL-1α* in all groups after 1, 2 and 3 weeks of orthodontic tooth movement (OTM) or orthodontic tooth movement plus micro-osteoperforation (OTM+MOP).
H: horizontal MOP, V: vertical MOP, +veC: positive control.

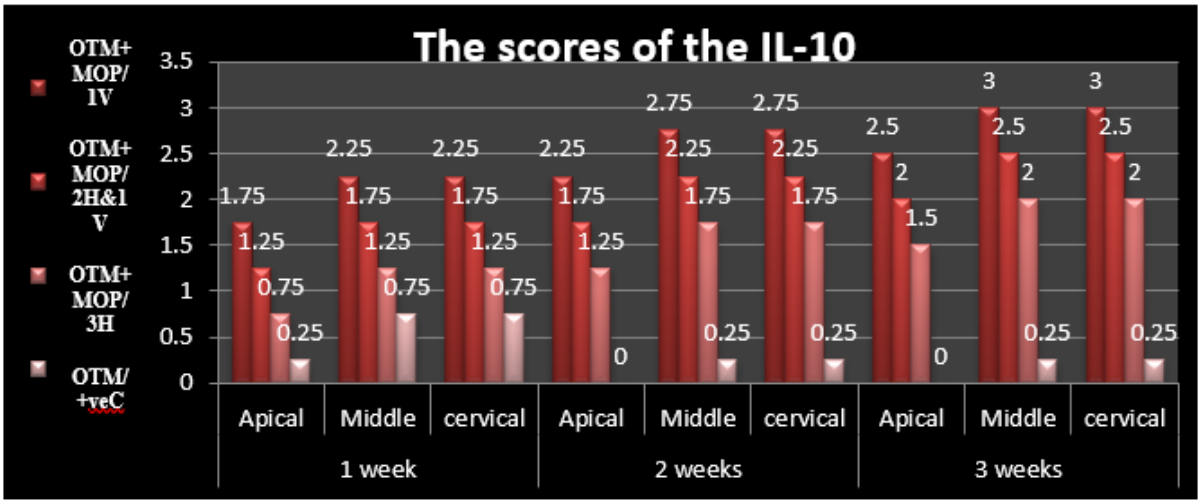

Fig. 4. Histogram of the scores of the *IL-10* in all groups after 1, 2 and 3 weeks of orthodontic tooth movement (OTM) or orthodontic tooth movement plus micro-osteoperforation (OTM+MOP).
H: horizontal MOP, V: vertical MOP, +veC: positive control.

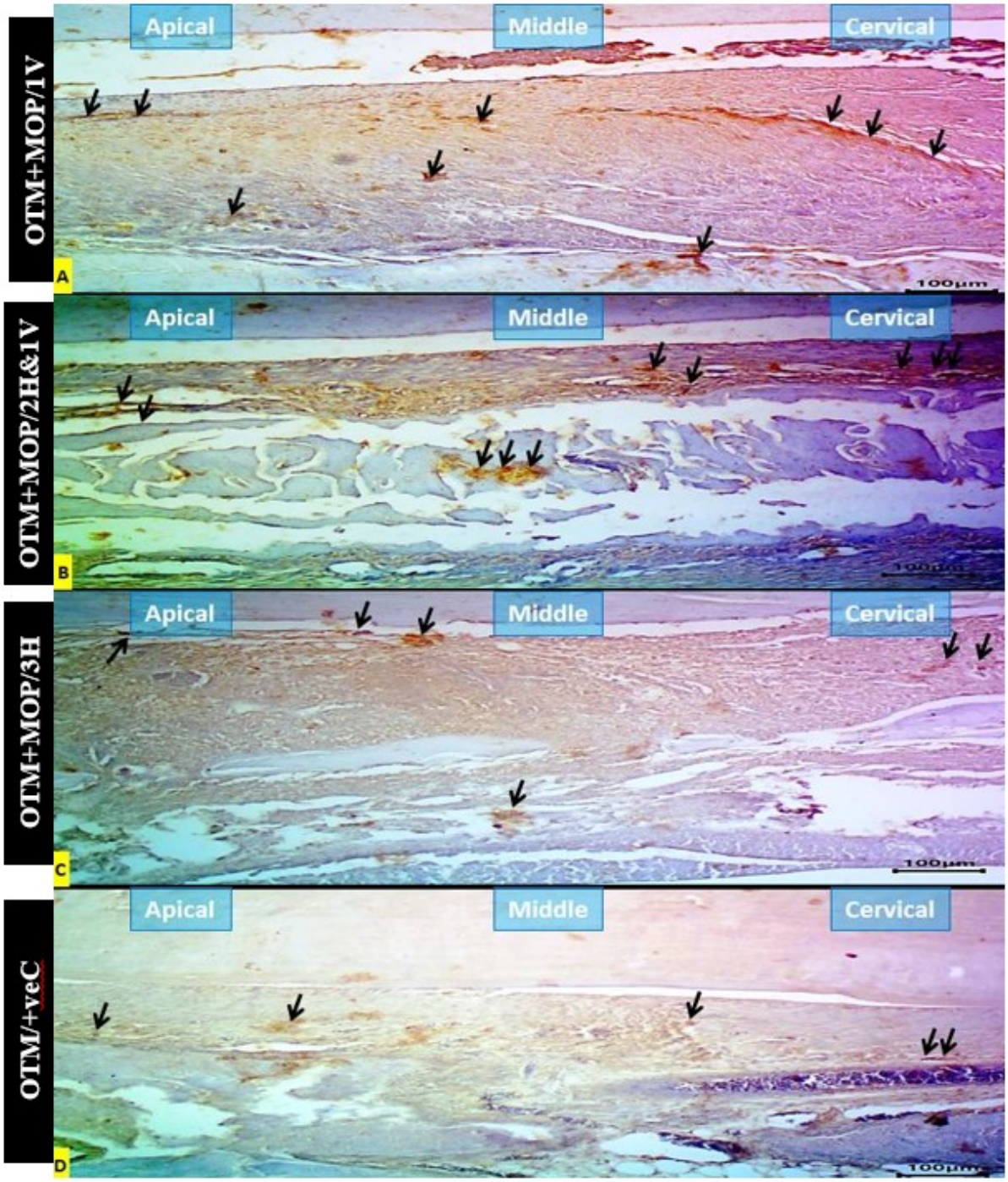

Fig. 5. Immunohistochemistry expression of the *IL-1α* in the rabbit compression mesial side of MP1 tooth root after removal of orthodontic appliance at *1 week* from the [A]: OTM+MOP/1V group showing intense positive (+ve) reaction [B]: OTM+MOP/2H&1V group showing moderate +ve reaction [C]: OTM+MOP/3H group showing weak +ve reaction [D]: OTM/+veC group showing very weak +ve reaction. In all groups, the *middle* and *cervical* third regions showed the higher expression than the apical third. (A, B, C, D: 40X), Scale bar=100µm.

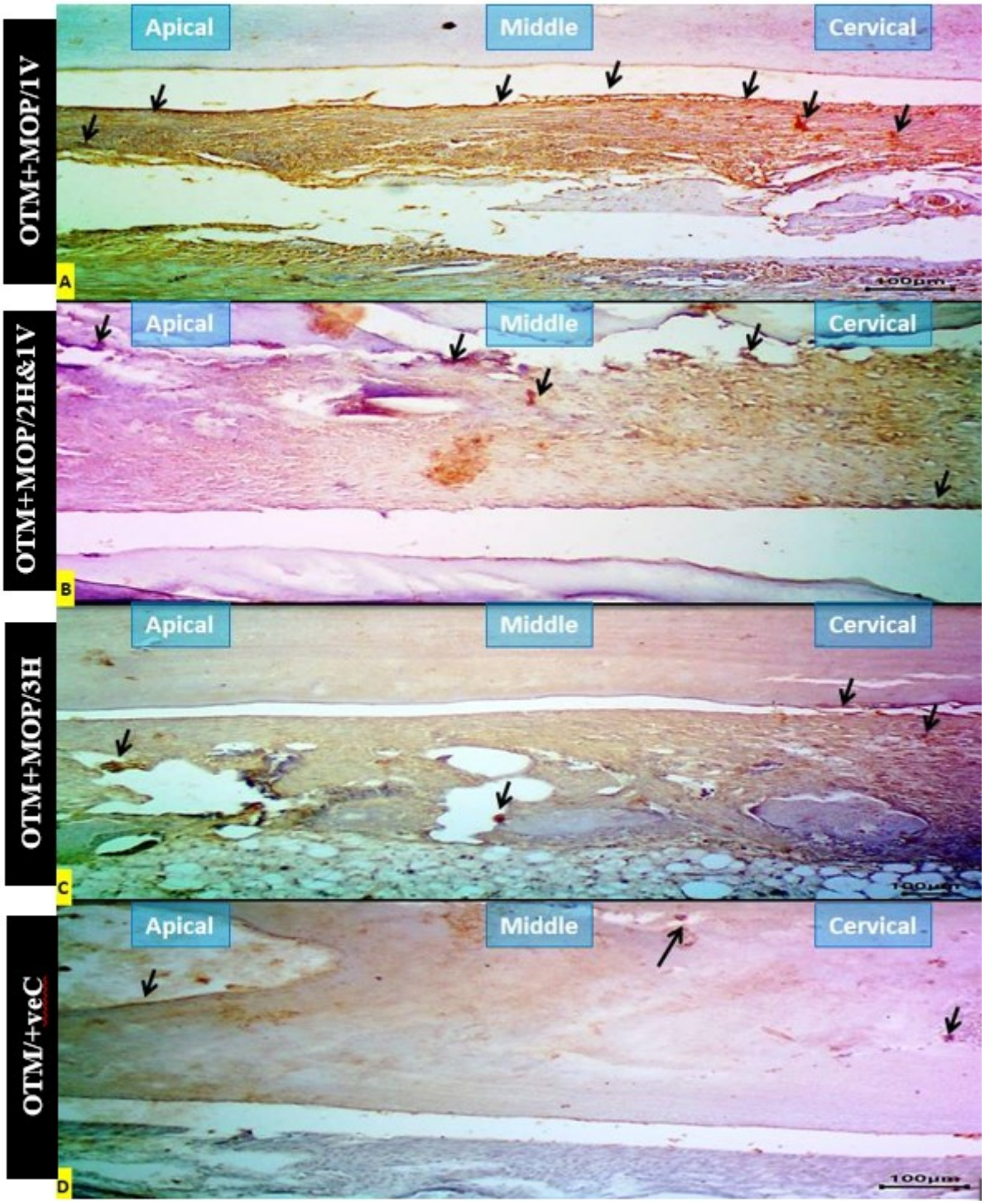

Fig. 6. Immunohistochemistry expression of the *IL-1α* in the rabbit compression mesial side of MP1 tooth root after removal of orthodontic appliance at *2 weeks* from the [A]: OTM+MOP/1V group showing intense positive (+ve) reaction [B]: OTM+MOP/2H&1V group showing moderate +ve reaction [C]: OTM+MOP/3H group showing weak +ve reaction [D]: OTM/+veC group showing very weak +ve reaction. In all groups, the *middle* and *cervical* third regions showed the higher expression than the apical third. (A, B, C, D: 40X), Scale bar=100µm.

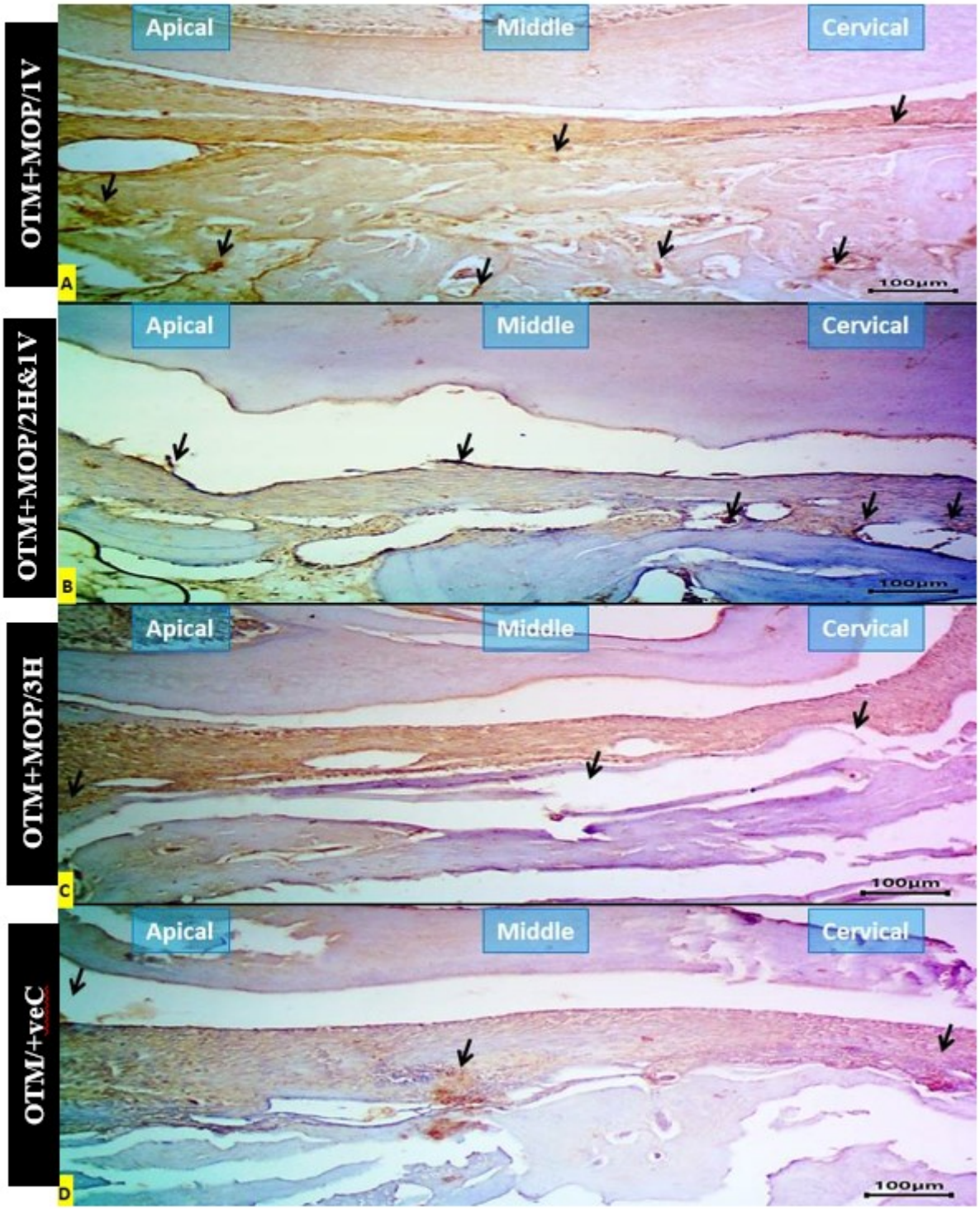

Fig. 7. Immunohistochemistry expression of the *IL-1α* in the rabbit compression mesial side of MP1 tooth root after removal of orthodontic appliance at *3 weeks* from the [A]: OTM+MOP/1V group showing intense positive (+ve) reaction [B]: OTM+MOP/2H&1V group showing moderate +ve reaction [C]: OTM+MOP/3H group showing weak +ve reaction [D]: OTM/+veC group showing very weak +ve reaction. In all groups, the *middle* and *cervical* third regions showed the higher expression than the apical third. (A, B, C, D: 40X), Scale bar=100µm.

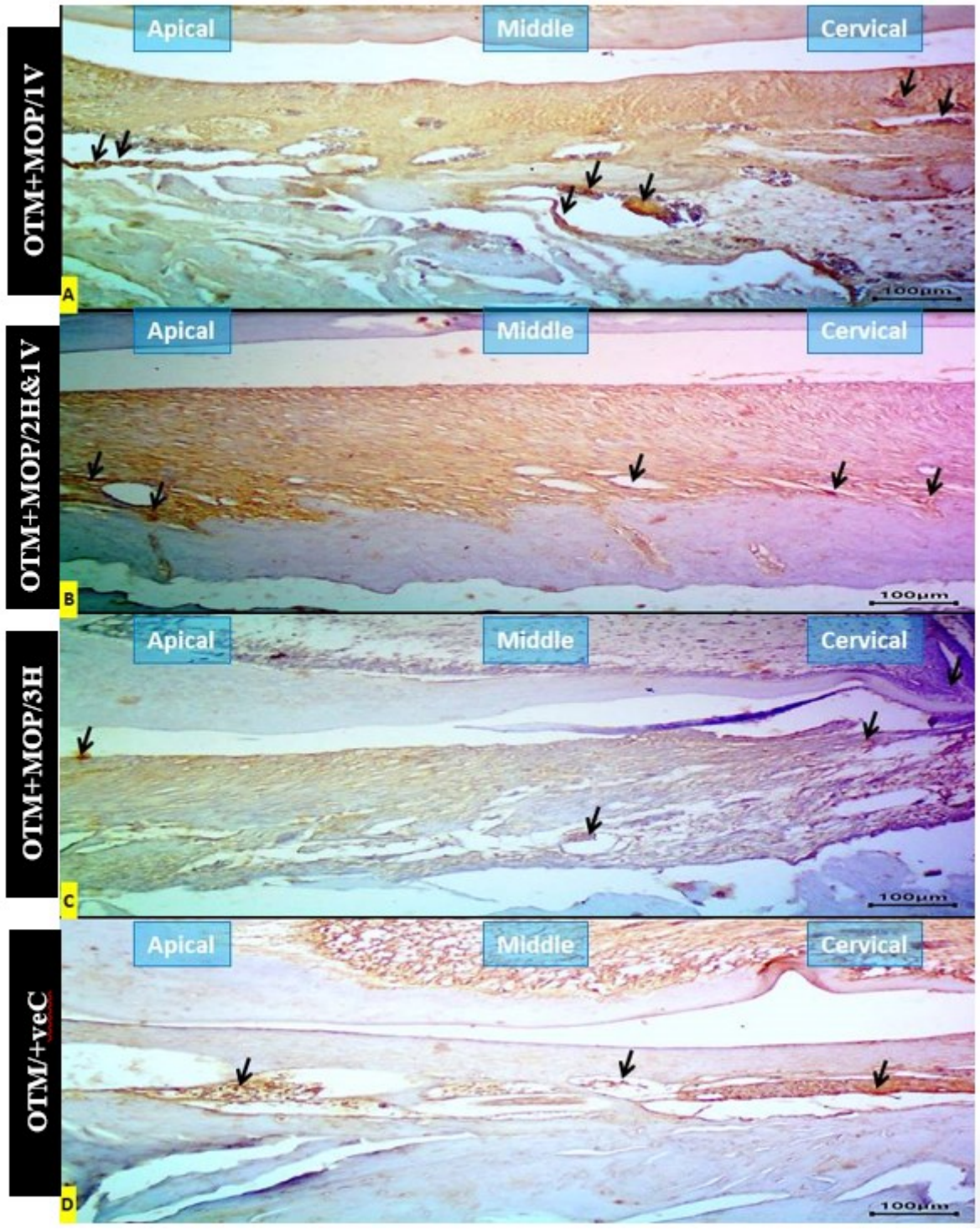

Fig. 8. Immunohistochemistry expression of the *IL-10* in the rabbit compression mesial side of MP1 tooth root after removal of orthodontic appliance at *1 week* from the [A]: OTM+MOP/1V group showing intense positive (+ve) reaction [B]: OTM+MOP/2H&1V group showing moderate +ve reaction [C]: OTM+MOP/3H group showing weak +ve reaction [D]: OTM/+veC group showing very weak +ve reaction. In all groups, the *middle* and *cervical* third regions showed the higher expression than the apical third. (A, B, C, D: 40X), Scale bar=100µm.

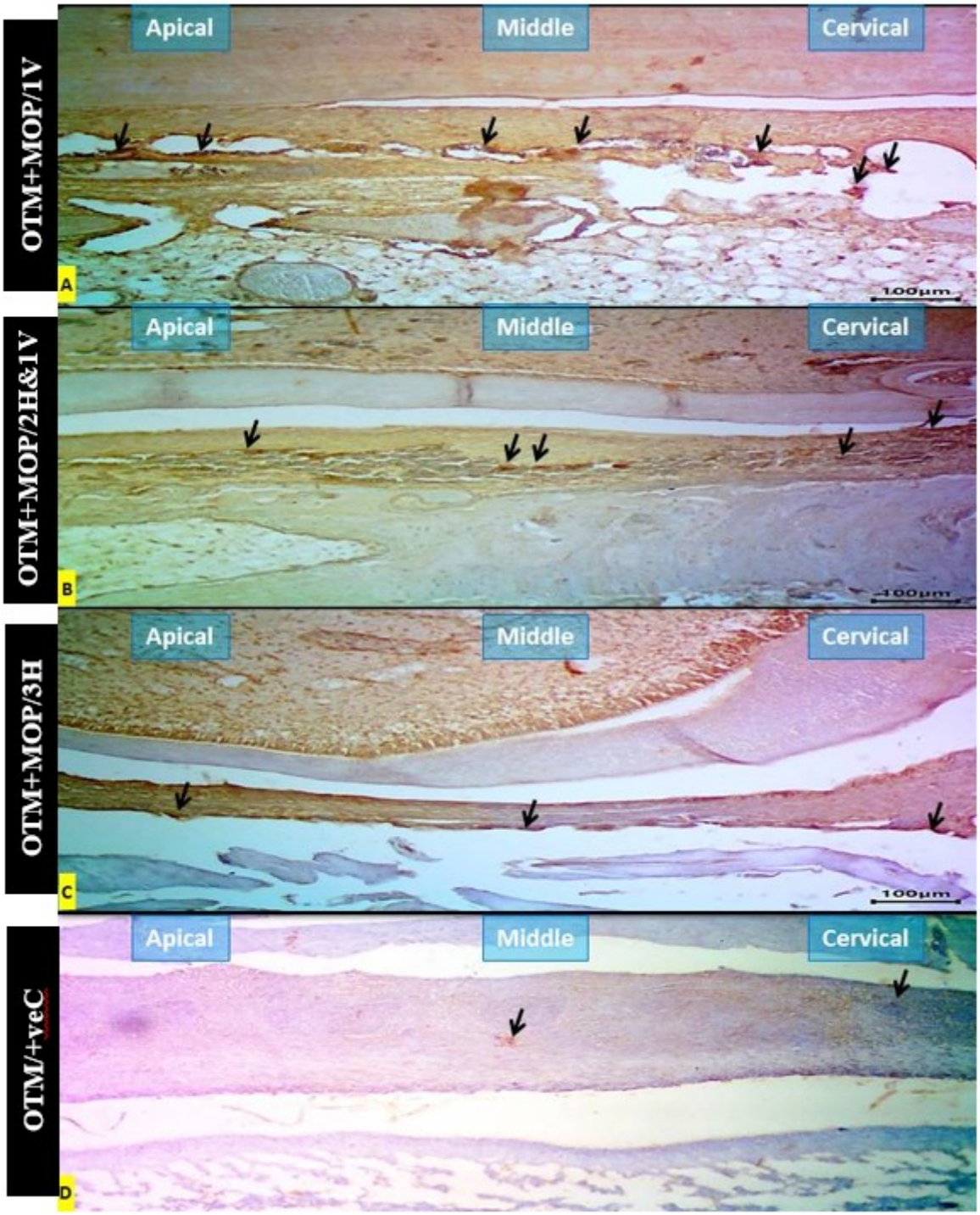

Fig. 9. Immunohistochemistry expression of the *IL-10* in the rabbit compression mesial side of MP1 tooth root after removal of orthodontic appliance at *2 weeks* from the [A]: OTM+MOP/1V group showing intense positive (+ve) reaction [B]: OTM+MOP/2H&1V group showing moderate +ve reaction [C]: OTM+MOP/3H group showing weak +ve reaction [D]: OTM/+veC group showing very weak +ve reaction. In all groups, the *middle* and *cervical* third regions showed the higher expression than the apical third. (A, B, C, D: 40X), Scale bar=100µm.

Fig. 10. Immunohistochemistry expression of the *IL-10* in the rabbit compression mesial side of MP1 tooth root after removal of orthodontic appliance at *3 weeks* from the [A]: OTM+MOP/1V group showing intense positive (+ve) reaction [B]: OTM+MOP/2H&1V group showing moderate +ve reaction [C]: OTM+MOP/3H group showing weak +ve reaction [D]: OTM/+veC group showing very weak +ve reaction. In all groups, the *middle* and *cervical* third regions showed the higher expression than the apical third. (A, B, C, D: 40X), Scale bar=100µm.

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تأثير عمل نهج مفترض من التثقبات العظمية الدقيقة على تآكل جذر السن الموجب تحريكه أثناء أجراء العلاج التقويمي للأسنان: دراسة كيميائية نسيجية مناعية في الارانب

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الخلاصة

سعت هذه الدراسة لأختبار ومقارنة تأثير نهج جديد من التثقبات العظمية الدقيقة مع نهجين آخرين من هذه التثقبات المدروسة سابقا على تآكل جذر الأسنان المرافق لعلاج تقويم الأسنان في الأرانب. العينة لهذه الدراسة تكونت من ٤٥ أرنب أبيض ذكري بالغ وبعمر (٢٤ أسبوع) تم تقسيمهم عشوائيا وبشكل متساو الى ثلاث مجاميع بحسب النهج المجرى من التثقبات العظمية الدقيقة، المجموعة الأولى والتي هي النهج الجديد أجرى لها ثقبان عظميان دقيقان سطحيان أفقيان وثقب واحد عظمي دقيق سطحي عمودي وكذلك العلاج التقويمي، المجموعة الثانية أجرى لها ثقب واحد عظمي دقيق عميق عمودي وكذلك العلاج التقويمي، المجموعة الثالثة أجرى لها ثلاثة ثقوب عظمية دقيقة سطحية أفقية وكذلك العلاج التقويمي. جميع هذه النهج أجريت على منطقة العظم من أمام الضرس الأولي في الفك الأسفل الأيمن للأرنب والتي أعتبرت هذه المجاميع على أنها (المجاميع التجريبية)، بينما أعتبرنفس الضرس ولكن في الجانب الأيسر من الفك الأسفل للأرنب على أنه (المجموعة القياسية) والتي وضع لها العلاج التقويمي فقط. تم سحب الضرس الأولي من كلا جهتي الفك تقويميا وللأمام بواسطة نابضين حلزونيين مفتوحين من النيكل والتيتانيوم، نابض حلزوني لكل ضرس وكذلك كل نابض يعطي قوة سحب بمقدار (٥٠غم)، وتم تثبيت كلا النابضين بواسطة زرعة تقويمية واحدة وضعت بين السنين القاطعين السفليين. بعد ثلاث فترات أختبارية مختلفة وهي بعد (١، ٢ و ٣ أسابيع) تم التضحية بالأرانب لأجل أختبار تآكل جذر الأسنان للضروس الأولية بواسطة فحص علامات حيوية خاصة لأظهار مقدار تآكل الجذرتسمى بالأنترلوكينز (أنترلوكين١ ألفا و أنترلوكين ١٠) من خلال الكيمياء المناعية النسيجية للمقاطع المأخوذة من المناطق الحول السن من جهة الأمام المضغوطة تقويميا (الثلث العنقي، الثلث الوسطي و الثلث القمي) من جذرالضرس الأولي لجهتي الفك السفلي. أظهرت النتائج وجود زيادة معنوية في مستويات الأنترلوكينز (أنترلوكين١ ألفا و أنترلوكين ١٠) في منطقة ما حول الضرس الأولي الأمامية المضغوطة تقويميا لمجاميع الثقوب العظمية الدقيقة (<٠٠١. الى = ٠٤١.). أضف الى ذلك، الثلث الوسطي والثلث العنقي من جذرالضرس الأولي لجهتي الفك السفلي أظهرا زيادة معنوية (<٠٥.) عن الثلث القمي في كل المجاميع. كذلك أظهرت مجموعة النهج الجديد (ثقبان عظميان دقيقان سطحيان أفقيان وثقب واحد عظمي دقيق سطحي عمودي وكذلك العلاج التقويمي) وجود مستويات متوسطة من الأنترلوكينز ما بين العالية، للمجموعة (ثقب واحد عظمي دقيق عميق عمودي وكذلك العلاج التقويمي) والواطئة، للمجموعة (ثلاثة ثقوب عظمية دقيقة سطحية أفقية وكذلك العلاج التقويمي). أستنتجت هذه الدراسة أنه من المرجح أن جميع مجاميع الثقوب العظمية الدقيقة ممكن أن تحفز تآكل جذر السن خلال العلاج التقويمي في منطقة ما حول السن المضغوطة تقويميا، ولكن بمديات مختلفة. كذلك أظهرت هذه الدراسة أن مجموعة النهج الجديد (ثقبان عظميان دقيقان سطحيان أفقيان وثقب واحد عظمي دقيق سطحي عمودي وكذلك العلاج التقويمي) على الأرجح أن تكون هي المرغوبة عمليا في تسريع حركة الأسنان تقويميا لأنها أظهرت معدلات متوسطة من قابلية تحفيز تآكل جذر السن والتي كانت ما بعد المجموعة الأقل تحفيزا لتآكل الجذر وهي المجموعة ذات (الثلاث ثقوب عظمية دقيقة سطحية أفقية وكذلك العلاج التقويمي) عندما أختبرت في الأرانب.

الكلمات المفتاحية: علامات حيوية، الكيمياء النسيجية المناعية، التثقب العظمي الدقيق، حركة السن التقويمية، تآكل جذر السن.