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Research Article

The Myeloperoxidase Expression in Dental Abscess Lesion: Histological and Immunohistochemical Study

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Abstract

Aims: The present study aimed to examine histologically and compare the immunohistochemical expression of myeloperoxidase between dental apical abscess and granuloma.

Materials and Methods: The research involved examining teeth with periapical lesions that were treated with surgery by a skilled surgeon and then studied histologically through post-surgical biopsy samples of the affected region. A set of 60 samples. Twenty granulomas, twenty abscesses, and twenty normal controls were analyzed histologically and immunohistochemically using antibodies targeting myeloperoxidase.

Results: Histological examination showed that abscesses had a level of inflammatory cell infiltration (Grade III) higher than granulomas (Grade II) and normal control, with a p value of 0·000 indicating a notable variance in tissue damage between these two conditions; abscesses displayed more extensive tissue damage in comparison to granulomas. In addition to this finding, there was an increase in myeloperoxidase expression detected through analysis within dental abscesses when compared to granulomas; this points towards enhanced inflammatory activity, in abscesses, evidenced by elevated myeloperoxidase levels.

Conclusion: The results demonstrate a distinction in tissue damage between abscesses and granulomas, with abscesses showing more pronounced destruction compared to granulomas. The elevated levels of myeloperoxidase in abscesses indicate significant tissue damage. Moreover, analysis of myeloperoxidase could provide perspectives into the pathophysiology of these lesions.

Keywords: Abscess, granuloma, Immunohistochemical, Myeloperoxidase

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INTRODUCTION

Infections in the tooth area can lead to abscesses and granulomas (swellings). While abscesses cause symptoms like pain and swelling along with pus discharge, granulomas are usually symptom-free (1). Might have intermittent issues, from secondary infections now and then. Radiographic examinations help identify whether these lesions are cysts or abscesses accurately, since they do not always present in the way clinically (2).

Therefore, it is important to provide a sample of the tissue surrounding the tooth for examination under the microscope to understand the exact nature of the abnormality. Essentially speaking, periapical granulomas consist of tissue filled with lymphocytes, plasma cells, and macrophages, and may or may not have a layer of epithelium covering them. On the other hand, periapical abscesses are characterized by a collection of blood cells and may or may not be covered by epithelium as well (3, 4).

When dealing with abscesses and granulomas, the body's response system is mainly triggered by neutrophils. These cells become active. Release substances, from their granules like myeloperoxidase (MPO), which then produce hypochlorous acid (HOCl) along with reactive oxygen species (ROS)(5) .

Myeloperoxidase (MPO) is a type of protein found in the azurophilic granules of white blood cells, like neutrophils, and in the autophagosomes of monocytes. This enzyme is known for its properties and its capacity to generate effective bacteria-killing substances like hypochlorous acid (HOCl) using hydrogen peroxide, with halides and chloride (6,7,8). Furthermore, through its consumption of H_2O_2 myeloperoxidase safeguards the host against the impacts of H_2O_2. This makes myeloperoxidase a component of defense and an inflammatory agent (9,10). The study's objective was to investigate the presence of myeloperoxidase in abscesses and granulomas and compare them to gain insights into the function of inflammatory indicators in the inflammatory reaction linked to the formation of these lesions.

MATERIALS AND METHODS

Ethical approval:

The study was performed according to the ethical guidelines for health studies from the Research Ethics Committee at Al-Mosul University, within the College of Dentistry, Mosul, Iraq (Approval no. **UoM.Dent.23/51**).

Case Selection

A total number of 40 periapical lesions embedded in paraffin blocks were treated with apical surgery, comprising 20 granulomas and 20 abscesses, which were chosen at arbitrary from the sample log. In addition, 20 tooth samples with no periapical lesion were included as a normal control. All patients were treated in the oral surgery clinic in the College of Dentistry, University of Mosul (Mosul City/Iraq). The case group consisted of 40 blocks of individuals have lesions connected to their teeth apices, non-endodontically treated. Assemble pertinent information, patient details such as age and clinical features like anatomical position all lesions taken from upper and lower anterior teeth, were collected from case sheets, and radiographic proof of the round well describe radiolucencies of periapical tissues was needed for incorporation, teeth had been omitted whether there was a perio-endo lesion as well as a linear fracture or fissure encompassing the root. The experimental study then proceeded to obtain information on the immunoexpression of myeloperoxidase.

Histopathological analysis

Thick tissue sections measuring 5 micrometers, which were derived from paraffin-deeply embedded blocks of dental granuloma, abscess, and normal samples, were prepared on glass slides for further analysis. To examine the histopathology of these conditions, the sections were subjected to H&E staining and subsequently visualized under a light microscope. The main objective was to prove the existence of unique criteria that distinguish among groups. The strength of inflammation was recorded according to the methodology applied. Every sample was recorded attuned to the inflammatory reaction, in three sequential microscopical domains, starting with

the inner side of the sample and commencing wider into the connective tissues, each sample was scored at 400X optical zoom:

Grade I inflammation cell infiltration confined to (1) microscopical ground, Grade II inflammation cell infiltration expands to (2) microscopical ground, Grade III inflammation cell infiltration throughout all (3) microscopically ground (11)

Immunohistochemistry of myeloperoxidase

This research concentrated on analyzing the immunohistochemistry dyeing structure of myeloperoxidase expression in microscope. The specimens were embedded in paraffin, and thin sections were collected. Applied to polished glass slides using an adhesive. An antibody called anti-MPO was employed for marker detection. Once the primary antibody was applied, a secondary antibody was utilized to further improve visibility. A streptavidin biotin complex was used to boost the signal. The target antigen site was visually identified through a reaction with 3,3' diaminobenzidine DAB. We used Mayers hematoxylin as a counterstain and Intellan as a mounting medium for long-term storage for visualization. In quantitative statistical analysis, the true positive absolutely brilliant were classified as weak (+), moderate (++), intense (+++), or very intense (++++) based on the intensity of the dyeing (13).

Statistical analysis

The data was entered using a statistical application (SPSS 23), which was designed particularly for social scientists. The standard deviation (SD) or mean is part of the overall descriptive statistical examination. A one-way analysis of variance (ANOVA) was conducted for more than two means, and a Tukey HSD post hoc test was applied to compare two means. The significance level in all statistical evaluations was determined at $p < 0.05$.

RESULTS

Demographic data

Out of the 60 cases studied, 33.3% (20 cases) were identified as abscess, granuloma 33.3% (20 cases) as granuloma, and normal control 33.3% (20 cases). All specimens were taken from male patients only, with an age range between 20 and 45 years.

Histopathological study:

The histological image of a dental abscess and granuloma is shown in Figure 1. In the abscess formation, represented by severe infiltration of inflammatory cells in the center, represented by predominant mononuclear inflammatory cells surrounding by necrotic tissue (figure 1). In granuloma lesions showing chronic inflammation, the granuloma is represented by necrotic tissue, severe infiltration of chronic inflammatory cells surrounded by fibrous tissue (Figure 1).

The average histological score of apical abscesses (2.90±0.316) indicates significant tissue damage in the periapical area in the case of an abscess, reflecting acute inflammation and substantial tissue destruction when compared with apical granulomas and normal control. Whereas, the histological score of apical granulomas (1.70±0.483) is lower compared to the abscess and normal control, indicating less severe tissue damage in apical granuloma as revealed in Table (1). This reflects the chronic nature of apical granuloma, which is usually associated with a less acute inflammatory reaction.

With a p-value of 0.000, this indicates that the differences in histological scores between the periapical abscess and apical granuloma are highly statistically significant. Therefore, it can be concluded that there is a notable difference in tissue damage between the two conditions, with more pronounced damage in the abscess.

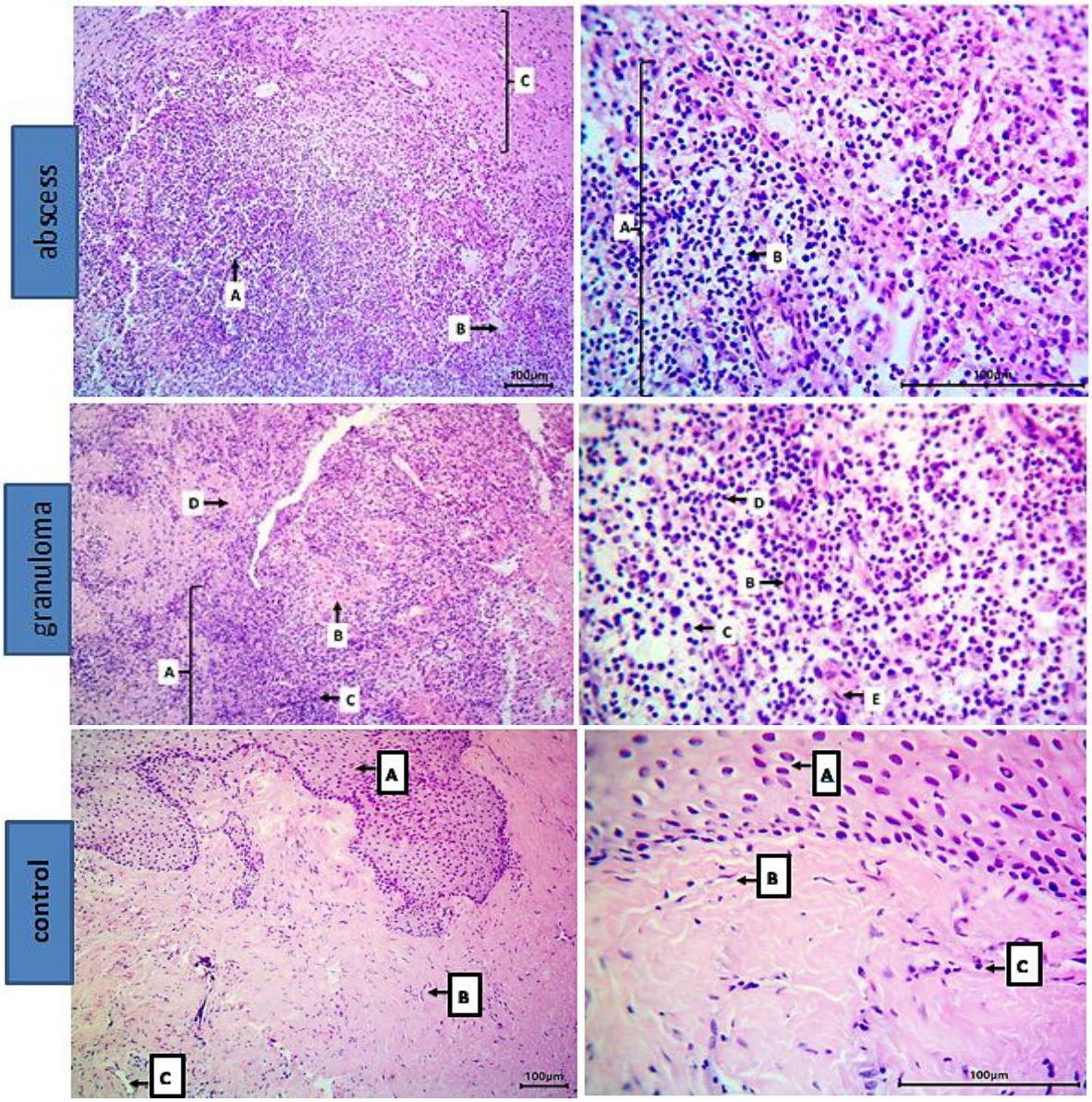

Figure (1): Histological section of periapical tissue: Histological section of abscess representing by severe infiltration of inflammatory cells in the center, representing by predominant mono-nuclear inflammatory cells (A), surrounded by necrotic tissue (B) and (C). Histological section of granuloma showing: chronic inflammatory granuloma (A) represented by necrosis (B), severe infiltration of chronic inflammatory cells (C) surrounded by fibrous tissue (D). Histological section of the control group showing normal histological architecture represented by epithelial layer (A), and connective fibrous tissue (B) with blood vessels (C). H&E stain, 100X, 400X.

Table (1): Histopathological score in study groups

Marker	Study groups (mean ± standard deviation)			
	Control	**Periapical abscess**	**Apical granuloma**	**Sig.(p-value)**
Histopathological score	0.00±0.0.00 [a]	2.90±0.316 [b]	1.70±0.0.483[c]	0.000

Comparison is represented by letters; different letters reveals significant difference, value set as Mean±SD. P-value≤0.05

Immunohistochemistry analysis for myeloperoxidase

Immunohistochemical analysis plays a crucial role in understanding the expression patterns of specific proteins in various pathological conditions. The average MPO expression (3.90±0.316) of periapical Abscess indicates a high level of this enzyme in the affected area, reflecting significant activity of neutrophils, which produce MPO, indicating the presence of acute and strong inflammation in this region. MPO expression of apical granuloma (2.80±0.422) is relatively lower, suggesting that inflammation in this case is not as intense as in the periapical abscess, but there is still considerable inflammatory activity (Figure 2 and Table 2).

With a p-value of 0.000, the differences in MPO expression between the periapical abscess and apical granuloma are statistically significant, meaning that the inflammatory activity (reflected in MPO expression) is much higher in the abscess compared to the granuloma.

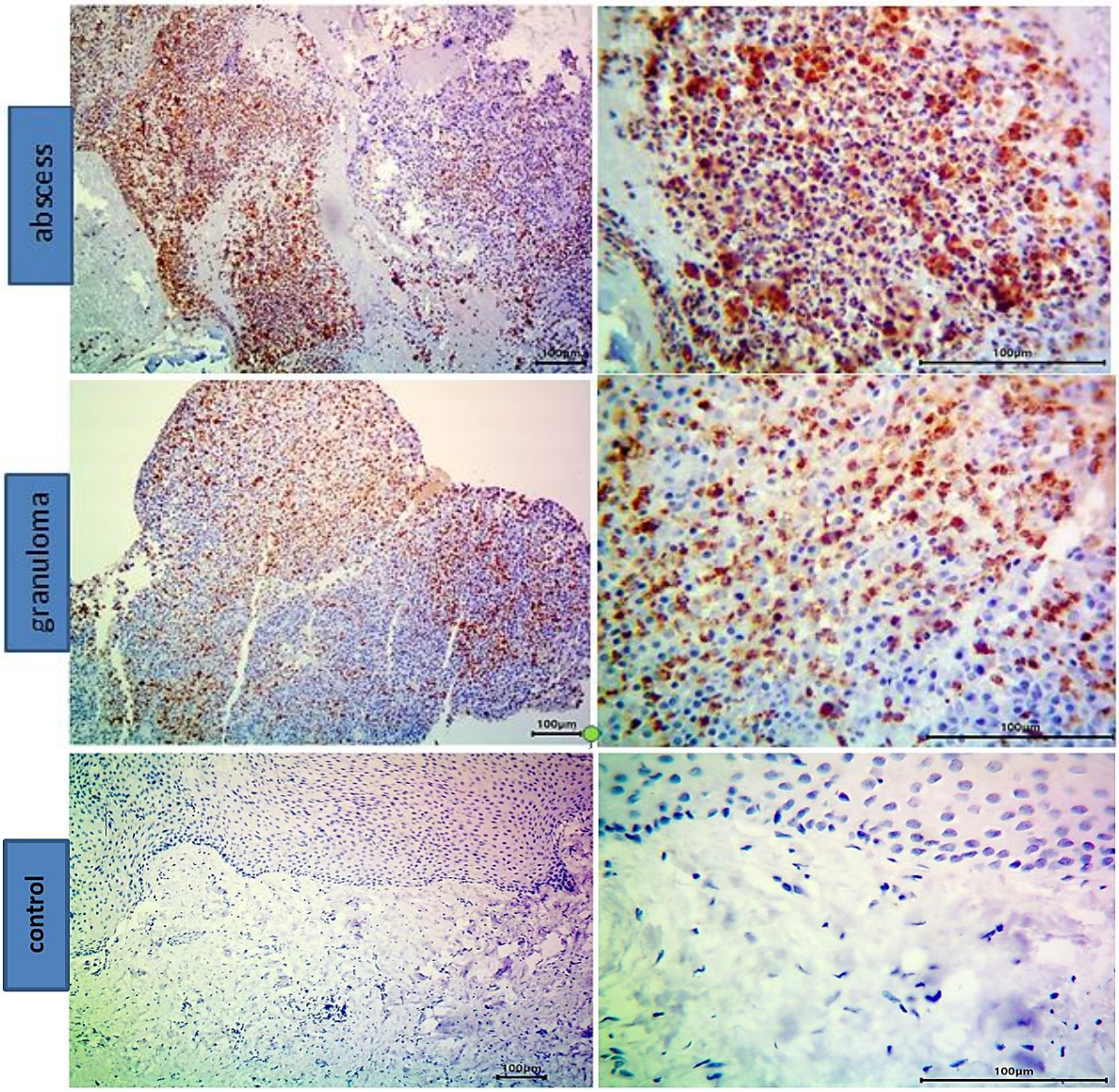

Figure (2): Immunohistochemical expression of MPO in the periapical tissue. Immunohistochemical expression of MPO in the abscess showing very intense expression (score ++++4); Immunohistochemical expression of MPO in the periapical granuloma showing intense expression (score +++3); Immunohistochemical expression of MPO in the control group showing negative expression (score -0); hematoxylin; 100X, 400X.

Table (2): Immunohistochemistry for myeloperoxidase in study groups

Marker	Study groups (mean ± standard deviation)			
	Control	Periapical abscess	Apical granuloma	Sig.(p-value)
Myeloperoxidase	0.00±0.0.00 [a]	3.90 ± 0.316[b]	2.80±0.422[c]	0.000

Comparison is represented by letters; different letters reveal a significant difference, value set as Mean±SD. P-value≤0.05

DISCUSSION

The inflammatory process affecting periapical tissues results from a complex interaction that depends directly on the cellular relationship between the immune system and the microorganisms present in the root canal system. Structural and physiological changes occur in periapical tissues as a response to this process, driven by ongoing immuno-inflammatory events (14). Reactive oxygen species (ROS) play a key role in pathogen control in diseases involving phagocyte infiltration and bone turnover, serving as host defense mechanisms against invading microbes (15).

One of these reactive oxygen species is myeloperoxidase (MPO), a potent tissue-damaging enzyme primarily expressed by neutrophils and monocytes. Elevated circulating levels of MPO are associated with inflammatory processes and the generation of ROS (16,17). In our study, we observed the highest MPO levels in abscesses compared to granulomas and normal controls, which is consistent with findings by Veiko *et al*. (18). Research on the expression of myeloperoxidase in periapical granulomas remains limited (19).

The findings suggest distinctions between abscess and apical granuloma regarding tissue injury and inflammation levels. Periapical abscess exhibits histological damage and MPO expression indicative of acute inflammation linked to the buildup of pus and increased immune response from neutrophils (20). On the other hand, in the case of apical granuloma, both tissue damage and inflammation levels are comparatively lower, correlating with the aspect of this condition that typically involves a milder yet ongoing immune reaction (21).

The variation in these occurrences can be explained by the characteristics of each situation; abscesses usually result from bacterial infections, causing intense inflammatory reactions, while granulomas are a result of long-term immune responses developing gradually from milder but consistent stimulation (22) .

An apical abscess is identified by inflammation caused by an infection. When there is an infection in the area around the root of a tooth, cells from the system, like macrophages and dendritic cells, identify the harmful invaders using certain receptors

known as pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) (23). These receptors start a chain of chemical reactions involving molecules like NF kappa B and MAP kinases that lead to the production of substances such as IL beta 1, TNF alpha, and IL-6 (24). These signaling molecules attract white blood cells called neutrophils to the infection site, where they help combat inflammation by releasing enzymes and proteins that fight off bacteria and repair damaged tissues effectively (25).

Myeloperoxidase (MPO), being one enzyme released by neutrophils, is crucial in the process as it facilitates the production of acid (HOCl) (26). This acid is generated from hydrogen peroxide (H_2O_2), an oxidizing agent that aids in killing bacteria and breaking down tissue (27). The intense MPO activity plays a role in causing tissue damage seen in periapical abscesses (28). The oxidative harm triggered by HOCl and other reactive molecules does not eliminate bacteria. Also harms the nearby tissues significantly, resulting in ulceration and the formation of pus typical of abscesses.

In instances of granuloma, the inflammatory reaction is milder and better controlled when compared to abscesses (29). During this stage of prolonged response caused by irritants, like leftover infections or foreign substances in the body's system, triggers an activation process involving macrophages that play a role in engulfing and secreting anti-inflammatory substances, such as IL10 and TGF Beta, to aid in creating granulation tissue rather than causing immediate tissue damage as seen in acute conditions (30). In this prolonged phase of the response cycle, fibroblasts and macrophages are stimulated to generate components of the matrix (ECMs), including collagen (31).

This method supports the healing of tissues and the development of granulation tissue near the inflamed area to minimize the impact of the system's response (32). MPO is still present. In amounts compared to acute abscesses, chronic inflammation reduces neutrophil function and promotes the activity of T cells and plasma cells involved in tissue repair (33). During acute abscess episodes, a surge in inflammatory

cytokines, such as TNF alpha and IL beta affecting extensive recruitment of neutrophils and activation of MPO (34).

On the other hand, apical granulomas strike a harmony between inflammatory and anti-inflammatory proteins like IL. 10, diminishing the intensity of inflammation (35). Moreover, TGF Beta contributes to curbing the actions of neutrophils and encouraging the creation of tissue within granulomas result in decreased levels of myeloperoxidase (MPO) and reduced tissue harm (36).

The variations in ratings and MPO levels can be clarified through these mechanisms (37). Instances of abscess demonstrate an inflammatory reaction with significant MPO activity and extensive tissue damage; contrastingly, apical granuloma signifies a well-coordinated persistent inflammatory response that strikes a equilibrium between tissue damage and regeneration, resulting in less tissue harm and diminished MPO levels (38, 39).

CONCLUSIONS

Within the limitations of the current study, it is possible to conclude that:

The myeloperoxidase is an important pathological marker in periapical diseases, including periapical abscess and granuloma. The dental abscess tissue in the periapical region shows there is an increase in myeloperoxidase expression compared with the dental granuloma. Understanding the role of myeloperoxidase might provide additional insights into the process of periapical lesion development.

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Authors' Contribution

Attarbashee RK completed the conceptualization, data curation, formal analysis, finding acquisition, investigation, resources, software, validation, visualization, writing- original draft, writing- review, and editing. AL-kazzaz SG completed the Conceptualization, Investigation, Methodology, Project administration, Supervision, Visualization, Writing, Writing- review & editing. Mammdoh JK finished the Conceptualization, investigation, methodology, project administration, supervision, visualization, and writing- review & editing.

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Ethical statement: The study was performed according to the ethical guidelines for health studies from the Research Ethics Committee at Al-Mosul University, within the College of Dentistry, Mosul, Iraq (Approval no. **UoM.Dent.23/51**).

Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript

Availability of data and materials: All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declaration of Generative AI and AI-assisted technologies

During the preparation of this work, the authors used a grammar tool to improve the readability of the manuscript. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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التعبير عن إنزيم الميلوبيروكسيداز في آفة الخُراج السِنّي: دراسة نسيجية ومناعية نسيجية

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الملخص

الأهداف: هدفت الدراسة الى تحليل الفحص النسيجي ومقارنة الكيمياء المناعية لإنزيم الميلوبيروكسيديز (MPO) بين الخراجات والأورام الحبيبية ماحول ذروة الاسنان. **المواد وطرائق العمل**: تضمن البحث فحص الأسنان المصابة بآفات ما حول ذروة الاسنان تم علاجها جراحيا ومن ثم دراستها نسيجيا من خلال أخذ عينات (خزعة) بعد جراحة المنطقة المصابة. تألفت مجموعة الدراسة من 60 عينة. تم تحليل 20 ورماً حبيبياً و20 خراجاً ما حول ذروة الاسنان و20 عينة كمجموعة ضابطة بواسطة الكيمياء المناعية باستخدام الأجسام المضادة التي تستهدف الميلوبيروكسيديز. **النتائج**: أظهر الفحص النسيجي أن الخراجات لديها مستوى عال من الخلايا الالتهابية (الدرجة الثالثة) مقارنة بالأورام الحبيبية (الدرجة الثانية) والمجموعة الضابطة مع وجود تباين ملحوظ في ضرر الأنسجة بين هاتين الحالتين. بالإضافة إلى انه كانت هناك زيادة في تعبير الميلوبيروكسيديز المكتشف من خلال التحليل داخل خراجات الأسنان بالمقارنة مع الأورام الحبيبية وهذا يشير إلى زيادة النشاط الالتهابي في الخراجات التي يتضح من ارتفاع مستويات الميلوبيروكسيديز. **الاستنتاجات**: اظهرت النتائج اختلافًا في ضرر الأنسجة بين الخراجات والأورام الحبيبية ما حول ذروة الاسنان، حيث تظهر الخراجات ضررا ذو فرق معنوي مقارنة بالأورام الحبيبية. تشير المستويات المرتفعة من الميلوبيروكسيديز في الخراجات ضررا كبيرا في الأنسجة ما حول ذروة الاسنان. علاوة على ذلك، فإن تحليل الميلوبيروكسيديز يمكن أن يقدم تصور أكثر حول الوظيفة المرضية لهذه الآفات.

الكلمات المفتاحية: خراج، ورم حبيبي، مناعي نسيجي، بيروكسيداز النخاع