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Research Article

Immunohistochemical study of proinflammatory markers in periapical lesions

Heba Faiz Hamodat *

Department of Basic Dental Sciences/College of Dentistry/ University of Mosul/ Iraq
* Corresponding author: hebahamodat@uomosul.edu.iq

Abstract: The current study aimed to diagnose and understand the role of various periapical granulomas and radicular cysts and their relationship to age and gender, as well as CD68 and TNF proteins. **Materials and Methods**: 26 patients were diagnosed with RCs, 14 cases with PGs, and 10 cases served as a control group, for a total of 40 cases. A sample size of 40 could provide preparatory information, and increasing the sample size greatly enhances the reliability and toughness of the study's findings. The necessary information on the patients' age, gender, and anatomical site of the infection was obtained. **Results**: 35% of cases had radicular cysts, and 65% had granulomas. This reflects the increased prevalence of granulomas among patients. The percentage of females in the cysts group was greater, at 71.4%, compared to 50% in the granulomas group. While the proportions of males and females in the granulomas group were close. The periapical lesions were more common between the ages of 13 and 20 years, with 42.3% of PGs and 35.7% of RCs occurring between these ages. CD68 protein expression was substantially higher in the PG group, with a mean score of 3.27, than in the RC group, with a mean score of 2.36. TNF was substantially higher in the PG group. **Conclusion**: The patients in the study have a higher prevalence of granulomas, and gender and age differences play a role in the kind of infection. TNF-α plays distinct roles in the pathophysiology of PGs and RCs. In PGs, it sustains a strong inflammatory response leading to tissue damage, while RCs show less inflammatory response dominated by epithelial proliferation and cyst growth. These differences influence remedy. High CD68 expression in PGs indicates a robust immunological response mediated by macrophages, which may aid in tissue repair and remodeling. Lower expression of CD68 and TNF alpha in RCs may explain the cysts' delayed growth and encapsulation.

Keywords: CD68, Immunohistochemistry, Periapical granuloma, Radicular cyst.

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INTRODUCTION

Periapical lesions are a collection of inflammatory diseases that affect the apical areas of teeth. Periapical granulomas and radicular cysts are the most prevalent lesions in this environment [1].

These lesions are caused mostly by chronic oral infections, which trigger complicated immunological and inflammatory responses that play major roles in their creation and progression [2.]

Human periapical lesions refer to a range of health issues that occur at the tip of teeth. These lesions can be caused by root infections, traumas, and various disorders affecting the roots and surrounding areas. Examples of lesions include radiculitis, radicular cysts, and gingival root nodules[3].

Dentists and oral maxillofacial surgeons must assess and treat periapical lesions[4]. Inflammatory mediators play a role in the development and progression of these lesions in roots. The systems response to inflammation in the tooth root region involves cytokines, prostaglandins, interleukins, and other inflammatory substances that're essential for this process [5].

When the tooth root becomes inflamed owing to reasons such as decay or injury, the immune system is stimulated to tackle the inflammation and potential biological threats [5].

Periodontal radiculitis can lead to the production of periradicular lesions such as radicular cysts, periodontal cysts, and gingival tumors if it is not treated effectively or if it persists for an extended period. Deterioration of the bone around the root and adjacent teeth can result in other health issues, such as bone loss and decreasing bone level surrounding the tooth, which may necessitate surgical intervention to treat [6].

To explain, periapical granuloma is typically not cancerous or non-malignant but rather a chronic inflammatory reaction in the tooth root caused by inflammation in the tooth [7].

CD68 cells include macrophages, which help to remove dead cells and toxic substances from the environment. TNF-alpha also contributes to the clearance process by boosting the inflammatory response [8].

Periapical granulomas and radicular cysts are periapical inflammatory lesions characterized by a dynamic interplay between immune cells, cytokines, and growth factors such as CD68 and TNF [9]. In this research, we will delve deeper into the roles being explored. When studying lesions through immunohistochemistry to analyze protein markers based on gender and age, the null hypothesis often assumes that there is no significant presence of these markers in the examined tissues. This lack of

presence could be attributed to chance. It may suggest that protein inflammatory markers do not play a significant role in this clinical scenario.

The main goal of this study is to identify granulomas and radicular cysts, assess their occurrence across genders and age brackets, and examine how CD68 and TNF proteins contribute to diagnosing and managing these lesions. Additionally, it offers an examination of the features and immunohistochemical expression related to periapical lesions in the oral cavity. Such insights can be valuable for understanding, diagnosing, and treating these conditions.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Oral Surgery at the College of Dentistry, University of Mosul (Mosul City/Iraq) from January 2022 to January 2023. These patients were referred for apical surgery at the College of Dentistry Teaching School.

Patient criteria

Selected patients must meet the following criteria and sign an informed consent form specifying ages, presence or absence of a systemic disease, does he take antibiotics, whether the patient is breastfeeding, pregnant, or undergone endodontic treatment, are there fractures in the tooth roots, does the patient take Immunosuppressants.

The apical lesion Criteria

- Apical lesion on permanent teeth with deep caries in the upper jaw or lower jaw.
- Confirm the presence of the apical lesion using periapical radiography, based on which it is selected as a granuloma or a non-granuloma.
- Dentinal granuloma is radiolucent with a clear margin at the apex of the tooth.
- The dentinal granuloma was enlarged by apical gingivitis, and the apical teeth appeared radiolucent.
- Indicating existing deep cavities for remedy after taking consent from the patients.

Surgery and sample collection

After the flap was elevated and the osteotomy was done, the location of the root end and the periapical tissue lesion was determined, the incision was made for the purpose of resection, and the pathological tissue and the restricted end of the root were treated. Finally, the pathological tissues associated with the resected root tip were collected.

Experiment design

A total of 40 periapical lesions, comprising 26 PGs and 14 RCs, were chosen randomly from the biopsy records. In addition, 10 tooth samples with no peripheral lesions were included as controls. Formalin-fixed, paraffin-embedded tissue slices were immunohistochemically stained for CD68 and TNF alpha. Patient information from records, such as age, gender, and location (anatomical site), was gathered. Compared with the results of immunohistochemistry tests.

Histological Analysis

To further explore the matter, thin tissue slices measuring 5 mm were created on glass slides from blocks of paraffin-embedded PGs and RCs. The histology of these cases was studied by examining stained sections using hematoxylin and eosin under a microscope. The primary aim was to validate criteria that distinguish PGs from RCs.

Immunohistochemical Staining for CD68 and TNF α:

The immunohistochemical staining pattern distribution of CD68 and TNF-α antigens was assessed by researchers using microscopy.

Thin sections of specimens embedded in paraffin were placed on glass slides with an adhesive. Antibodies named anti-CD68 and anti TNF α were used to detect markers. Following the application of the antibody, a secondary antibody was used to enhance visualization. Streptavidin biotin complex was utilized to amplify the signal. A visual signal indicating the target antigen site was generated through a reaction with 3,3' diaminobenzidine DAB. Mayer's hematoxylin served as a counterstain for visualization, while Entelan was applied as a long-term storage mounting media.

By using this approach, we were able to identify and study cells that show CD68 positivity in tissue samples, giving us insights into functions and disease conditions. We assigned a score to each sample based on the level of staining in cells and the intensity of the stain as in Table 1.

Table (1): Immunohistochemical scoring

0	No cells staining
1	Mild staining in 10% of cells
2	Moderate staining in 10-25% of cells
3	Severe staining in 25-50% of cells
4	very intense > 50%

Statistical analysis

The SPSS statistical system, version 16, was used from the US, Chicago, and the analysis used was ANOVA. The nonparametric Kruskal-Wallis H test was used to examine the mean difference between more than two groups, followed by the nonparametric Mann-Whitney test to analyze the mean difference between any two groups. Probability less than $P \leq 0.05$.

RESULTS

Demographic data

The findings revealed that granulomas accounted for 65% of the total infections in the study, confirming that granulomas are more common than radical cysts because PGs formation led to immediate body's defense mechanism against infection, while cyst requires additional pathological changes and may evade detection due to their symptomatic nature.

Females were impacted by RCs at a rate of 71.4%, whereas males were affected at a rate of 28.6 %. Some studies suggest a higher prevalence in females may be due to hormonal factors, healthcare seeking which leads to earlier detection, and genetic factors. While the proportions of males and females in the granulomas group were close. Human periapical lesions were found to be more common in patients aged 13 to 20 years, accounting for 42.3% of granuloma cases and 35.7% of radicular cyst cases. This implies that young men and women are more vulnerable to these lesions.

Periapical lesions granulomas had a 67% frequency in the maxillary areas compared to the rest of the mandibular regions, while radicular cysts had a 50% incidence, as shown in Table 2. This may be due to anatomical variation between the maxilla and mandibular. The maxilla has a more porous bone structure and a richer blood supply compared to the denser cortical bone of the mandible, in addition to the proximity of the maxillary teeth to the sinus.

Table (2): Data proportions for the study

Characteristics		Periapical lesion n%		Cyst n %		Granuloma n%		Control n%	
Gender	Male	17	42.5%	4	28.6%	13	50%	5	50%
	Female	23	57.5%	10	71.4%	13	50%	5	50%
Age	13-20	16	40%	5	35.7%	11	42.3%	5	50%
	21-30	13	32.5%	4	28.6%	9	34.6%	3	30%
	31-45	11	27%	5	35.7%	6	23.1%	2	20%
Site	Maxillary anterior	26	67%	7	50%	19	73.1%	4	40%
	Maxillary posterior	6	15%	5	35%	1	3.8%	2	20%
	Mandibular anterior	7	17%	2	14.3%	5	19.2%	3	30%
	Mandibular posterior	1	2.5%	0	0%	1	3.8%	1	10%

Histopathological study

Signs of persistent inflammation appear in the area of granulomas with the presence of granulation tissue. This tissue is made up of a fibrous wall and a range of cells, such as macrophages, lymphocytes, plasma cells, mast cells, giant cells, and fibroblasts. Radicular cyst lesions, on the other hand, suggest chronic inflammation without granulation tissue. Cells like macrophages, lymphocytes, plasma cells, mast cells, and fibroblasts are present in the cyst. These cellular components play a role in confirming the diagnosis of granuloma and radicular cysts, and this difference would affect on the progression and treatment as in granuloma the granulation tissue leads to fibrosis overtime and the remedy focuses on medical management and surgery if need, while in RCs will cause bone resorption but less aggressive and treated by endodontic remedy or removed by surgery. They also help in understanding the characteristics of these conditions when viewed under a microscope as in figure 1.

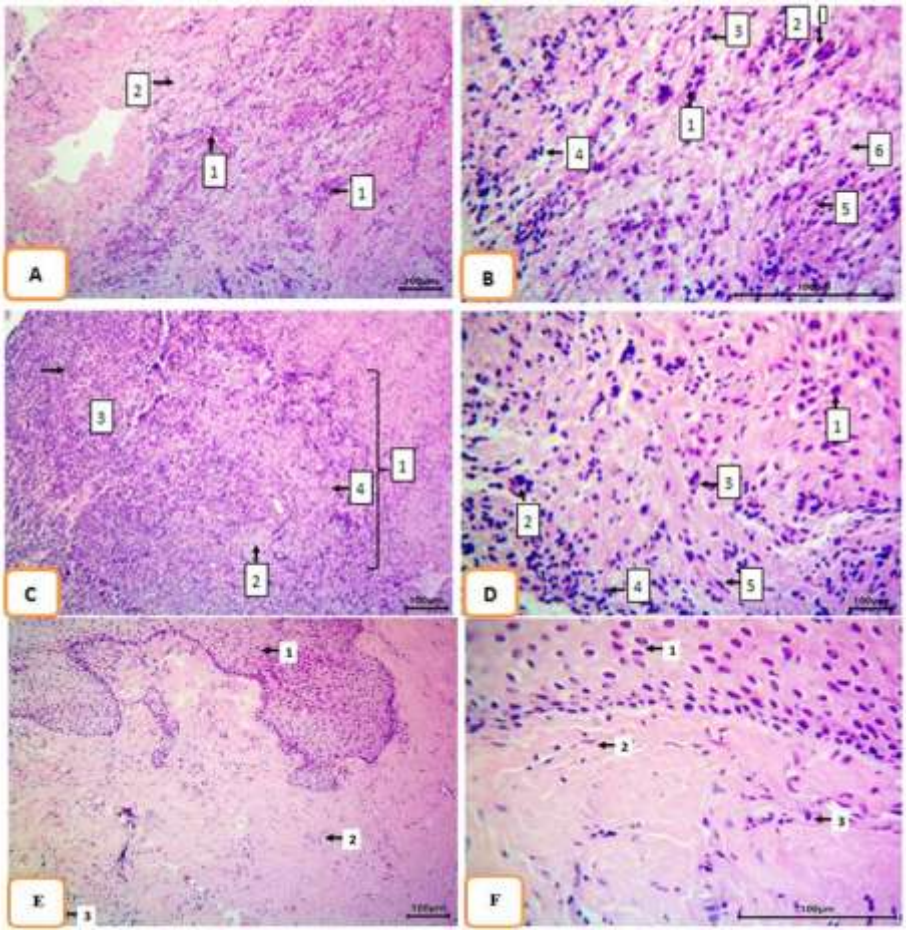

Figure (1): Histological section of periapical lesions, 100X, 400X A Radicular cyst showing chronic inflammatory cells 1, fibrous tissue 2. H&E stain**.** B cyst showing macrophages 1, giant cells 2, plasma cells 3, lymphocytes 4 fibroblasts 5, fibrous tissue 6. H&E stain.C Periapical granuloma lesions showing: granuloma 1 necrosis 2, chronic inflammatory cells 3 fibrous tissue 4. H&E stain. D granuloma showing macrophages 1, datia langhans giant cells 2, plasma cells 3, lymphocytes 4, and fibroblasts 5. H&E stain,.E control group showing normal histological architecture represented by epithelial layer 1, and connective fibrous tissue 2 with blood vessels 3. H&E stain. F control group showing stratified squamous epithelial cells 1 connective fibrous tissue with fibroblasts 2 and few lymphocytes 3. H&E stain.

The analysis using immunohistochemistry revealed the presence of CD68 and TNF-α in both cyst formations, which revealed a significant filtration of macrophage cells. However, CD68 and TNF expression levels were higher in the granuloma group 3.27±0.83; 3.65± 0.49 than in the radicular cyst 2.36 ±0.0.49; 2.21±0.43 and control 0.0±0.0; 0.0±0.0. There were substantial differences between the groups' P value of 0.05, as shown in Figure 2 and Table 3.

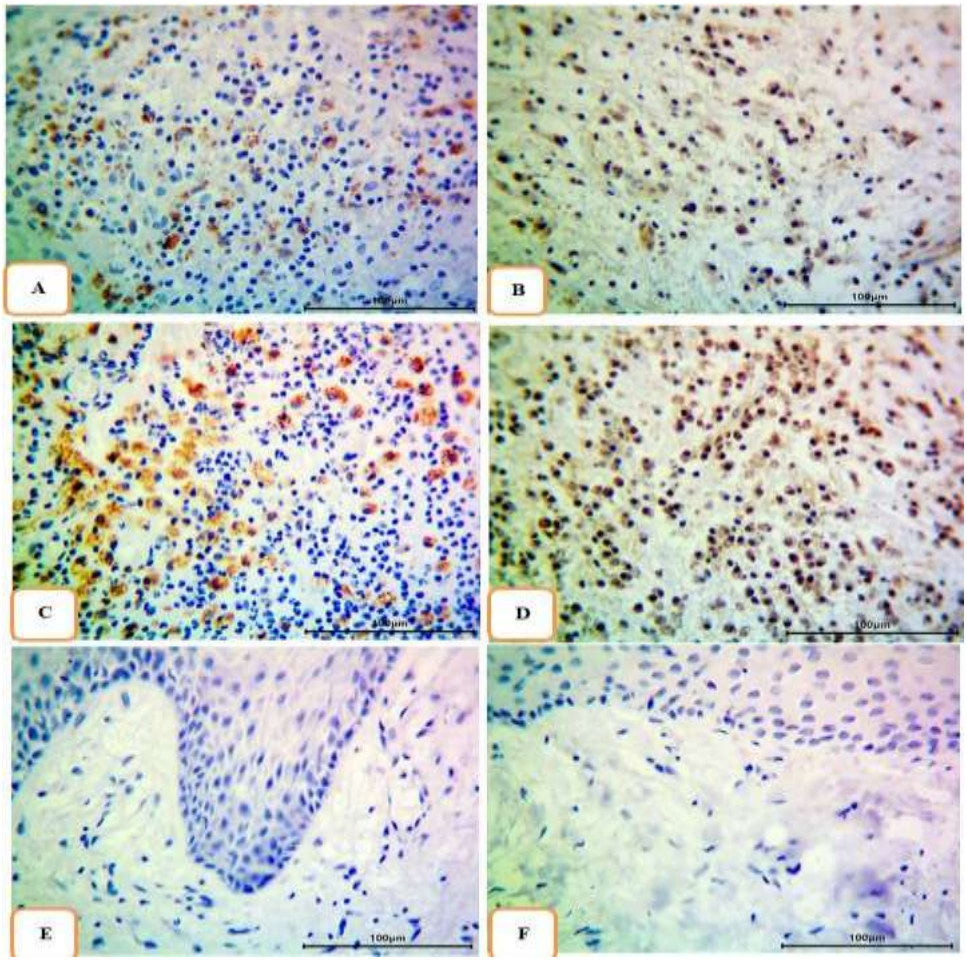

Figure 2: (A) Immunohistochemistry of CD68 showing moderate expression; (B) Immunohistochemistry of TNF-α showing moderate expression; (C) Immunohistochemistry of CD68 showing intense expression; (D) Immunohistochemistry of TNF-α showing very intense expression; (E) Immunohistochemistry of CD68 showing negative expression (F) Immunohistochemistry of TNF-α showing negative expression, hematoxylin; 400X.

Table (3): Immunohistochemical score for proinflammatory markers

IHC parameter	PG	RC	Control
CD68	3.27±0.83 **a**	2.36±0.0.49 **b**	0.0±0.0 **c**
TNF-α	3.65 ± 0.49 a	2.21±0.43 **b**	0.0±0.0 **c**

Values are set as Mean±SD. P-value≤0.05; dissimilar letters mean significant differences

This implies that the periapical lesions contain different degrees of CD68 and TNF- expression. In the granulation tissue of PGs, CD68-positive macrophages were

numerous, indicating active phagocytic activity, which play a role in containing or eliminating the causative agent.

TNF-expression was also high, indicating an inflammatory environment. RCs, on the other hand, had a distinct pattern, with reduced CD68 expression and less TNF present. Both markers were barely stained in the control samples.

Table 3 and Figure 2 show that tissue analysis employing immunohistochemistry IHC plays an essential role in detecting the expression of particular proteins CD68 and TNF- in the peripheral granuloma and radicular cyst groups.

CD68 expression and TNF-α expression

CD68 protein expression was found to be substantially greater in the peripheral granuloma PG group with a mean score of 3.27 compared to the radicular cyst RC group with a mean score of 2.36. In the control group, no CD68 protein expression was observed.

TNF protein expression: Similar to the previous study, TNF- protein expression was considerably higher in the peripheral granuloma PG group with a mean score of 3.65 compared to the radicular cyst RC group with a mean score of 2.21. As in the prior example, no TNF- protein expression was detected in the control group.

These differences were also statistically significant, with a *P*-value of 0.05 suggesting that there were substantial differences in TNF-expression between groups.

DISCUSSION

These findings show that periapical granulomas PG have higher levels of CD68 and TNF- protein expression than radicular cysts RC and the control group. This shows that periapical granulomas have a greater inflammatory response, which could help explain the causal mechanisms of periapical lesions and their progression.

The offered research investigates common oral lesions, such as periapical granulomas PG and radicular cysts RC, and emphasizes clinical, morphological, and chemical features of these diseases. The study came to several noteworthy results and observations, including: According to the findings, 65% of cases were classified as periapical granulomas PG, whereas 35% were described as radicular cysts RC. This shows that the patients in the research had a higher prevalence of granulomas [10].

The percentage of women in the cysts group was greater, at 71.4%, compared to 50% in the granulomas group. While the proportions of males and females in the granulomas group were close [11].

Human periapical lesions were most common in patients aged 13 to 20 years, accounting for 42.3% of granuloma cases and 35.7% of radicular cyst cases. This suggests that young men and women are the most prone to the disease.

Periapical lesions, such as radicular cysts and granulomas, are prevalent disorders in teenagers aged 13 to 20 years old. Unhealthy diets high in sugars and carbohydrates, as well as a lack of interest in cleaning teeth or visiting the dentist regularly, are among the most prominent reasons causing the disease and alterations. Hormonal conditions [12, 13] .73.1 %of granulomas and 50% of radicular cysts were found in the upper jaw teeth's front section. One of the reasons for this is that the front of the mouth is generally damaged by bruising and injuries, as well as orthodontic procedures that endure for long periods and make cleaning the teeth difficult. In addition to not cleaning one's teeth or attending to regular dental examinations [14, 15, .16]

It was discovered that the expression rate of CD68 and TNF proteins was higher in PGs than in RCs, implying that PGs have a higher rate of immune response than RCs. The cause for this could be granulomas' immunological reaction to pulp infection. This immune response consists of phagocytic and lymphocyte inflammatory cells, whereas the cysts in radicular cysts are a row of epithelial cells. Granulomas frequently cause chronic inflammation that extends to the tooth's root. This finding is consistent with the presence of high levels of the cytokine TNF, which signals the severity of the immunological response [17, 18,19].

The researchers discovered differences in the expression of CD68 and TNF between the granuloma, radicular cyst, and control groups. Individual injuries, genetic predispositions, or immunological responses may all contribute to these lesions [20, 21,22] . The incidence of granulomas was found to be higher than the prevalence of root cysts, which is consistent with earlier studies [23,24.]

These findings contribute to the diagnosis and treatment of the condition, offering insights into investigating the origins of infections within periapical lesions. This opens up avenues for research into the causes of these lesions. Future studies could explore how this increased expression impacts health and strategies to manage these diseases, and focusing on genetic and immune response through multiple approaches like cytokine and chemokines analysis, work on specific gene connected to immune inflammation, regulation and bone metabolism.

The importance of examining these lesions from various perspectives, including clinical, anatomical, and chemical aspects, cannot be emphasized enough based on these results. The study revealed levels of protein markers in the analyzed tissues.

The differences in CD68 and TNF expression between granulomas (PGs) and radicular cysts (RCs) indicate immunopathogenesis pathways for these lesions. High CD68 expression in PGs suggests a macrophage-mediated response that may facilitate tissue repair and remodeling.

Conversely, lower levels of CD68 and TNF in RCs suggest an immune response potentially explaining the delayed growth and encapsulation observed in cysts.

This immunohistochemistry study enhances our understanding of the immunopathogenesis involved in lesions. It highlights differing roles played by TNF. CD68 in the response seen in periapical granulomas versus radicular cysts. More study is needed to determine the precise mechanisms underlying these variances and to identify possible treatment targets for these prevalent oral lesions. Future study opportunities can be identified by broadening the temporal and geographical scope to include larger populations and numerous geographic regions.

Clinical research may help to explain the molecular and clinical mechanisms behind the found connections.

Future research can also be done by combining ocular and clinical data to better understand the links between biological variables and clinical problems. Completing experimental investigations, conducting long-term studies, and increasing the sample size to get better statistical representation and more solid results. The study's limitations include environmental and behavioral factors. Uncontrolled environmental and behavioral factors may influence the outcomes, and they may be impossible to entirely control. Molecular mechanisms can be used to explore PGs and RCs depending on the target like Transcriptomic Analysis, Proteomic and Flow cytometry.

CONCLUSIONS

The patients in the study have a higher prevalence of granulomas, and gender and age differences play a role in the kind of infection. High CD68 expression in PGs indicates a robust immunological response mediated by macrophages, which may aid in tissue repair and remodeling. Lower expression of CD68 and TNF- in RCs, on the other hand, may imply a less aggressive immune response, which may explain the cysts' delayed growth and encapsulation. Understanding these responsibilities is critical for better diagnosis and therapy of various oral diseases.

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Ethical statement: The Research Ethics Committee at the University of Mosul, specifically the College of Dentistry, approved the research investigation. This study's unique permission number is UoM.Dent. 23/28.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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دراسة كيميائية مناعية للعلامات المؤيدة للالتهابات في الآفات المحيطة بالذروة

هبة فائز حمودات

الملخص

الاهداف: . تهدف هذه الدراسة إلى تشخيص الأورام الحبيبية والكيسات الجذرية ومدى اختلاف الاصابة حسب االجنس و العمر, فضلا عن دراسة تاثير تعبير البروتينات CD68 و TNF-α , دورها في تشخيص وإدارة هذه الآفات الفموية .**المواد وطرائق العمل**: تم فحص ما مجموعه 40 حالة، كانت 26 PGs و14 RCs و10 عينات مراقبة (سيطرة). تم جمع البيانات السريرية مثل عمر المريض والجنس والموقع التشريحي لربطها بالنتائج الكيميائية المناعية. شخصت 65% من الحالات على أنها أورام حبيبية محيطة بالذروية (PG)، بينما بلغت نسبة الحالات التي تم تشخيصها ككيس جذري 35% (RC). هذا يشير إلى انتشار أكبر للأورام الحبيبية بين المرضى في الدراسة. كما أن الاناث كانت نسبتهن أعلى في مجموعة الكيسات بنسبة 71.4% مقارنة بنسبة 57.5% في مجموعة الأورام الحبيبية. بينما كانت النسب متقاربة بين الذكور والإناث في مجموعة الأورام الحبيبية.**النتائج**: اظهرت النتائج أن حالات الآفات المحيطة بالذروية البشرية الكرونية كانت أكثر في الاعمار 13 و20 عامًا بنسبة 42.3% من حالات الورم الحبيبي و35.7% من حالات الكيس الجذري. لوحظ أن تعبير البروتين CD68 كان أعلى بشكل ملحوظ في مجموعة الورم الحبيبي المحيطي (PG) بمعدل متوسط قدره 3.27 بالمقارنة بمجموعة الكيس الجذري (RC) التي كان معدلها المتوسط 2.36. تعبير البروتين TNF-α ، كان أعلى بشكل ملحوظ في مجموعة (PG) بمعدل متوسط قدره 3.65 مقارنة بمجموعة (RC) حيث كان معدلها المتوسط 2.21. وكما في الحالة السابقة. **الاستنتاجات**: نستنتج ان هناك انتشار أكبر للأورام الحبيبية بين المرضى في الدراسة , كما ان اختلاف الجنس والعمر له دور في نوع الإصابة, يشير التعبير العالي لـ CD68 في PGs إلى استجابة مناعية قوية بوساطة البلاعم، مما قد يساهم في إصلاح الأنسجة وإعادة تشكيلها. على العكس من ذلك، قد يشير انخفاض تعبير CD68 وTNF-α في RCs إلى استجابة مناعية أقل عدوانية، مما قد يفسر التقدم الأبطأ والطبيعة المغلفة للخراجات, يعد فهم هذه الأدوار أمرًا بالغ الأهمية لتحسين تشخيص وإدارة هذه الآفات الفموي