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Comparative Expression of M1 And M2 Macrophage Markers in Radicular Cysts and Periapical Granulomas: A Study of Immune Polarization

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Abstract

Background: Apical periodontitis (AP) characterized by inflammation and bone destruction in
the periapical tissues due to microbial infection in the dental pulp. This necessitates immunological responses to prevent the spread of infection. Macrophages are a group of heterogeneous cells that have many roles in the development of infections, destruction and reconstruction of bone tissues, and microbe–host interactions

Materials and Methods: Thirty paraffin embedded tissue blocks of radicular neck resected samples of 18 preapical granulomas and 12 radicular cysts were processed histopathological to detected CD68, CD11c and cd163 positive cells. Expression of macrophage was quantitatively assessed.

Results: The study found that M1 macrophage polarization, indicated by CD11c expression, was higher in radicular cysts compared to periapical granulomas (25.45 ± 4.11 vs. 14.66 ± 4.19). There was no significant difference between granulomas and radicular cysts concerning CD68 expression (46.27 ± 5.06 vs. 28.05 ± 5.06; p=0.054). Conversely, M2 macrophage polarization, indicated by CD163 expression, was significantly higher in periapical granulomas compared to radicular cysts (42.12 ± 6.59 vs. 21.03 ± 3.43; p=0.020).

Conclusions: Macrophages play a significant role in the inflammatory response of periapical lesions. The study found that M1 macrophages were more prevalent in radicular cysts, while M2 macrophage expression was significantly higher in periapical granulomas. This suggests distinct macrophage polarization patterns between radicular cysts and periapical granulomas.

التعبير المقارن لعوامل البلاعم من النوع M1 و M2في الأكياس الجذرية والأورام الحبيبية القمية: دراسة عن الاستقطاب المناعي

أحمد هاني مقطوف، بان فاضل الدروبي

الخلاصة

المقدمة: يتميز التهاب اللثة القمي (AP) بالتهاب وتدمير العظام في الأنسجة المحيطة بالقمة بسبب العدوى الميكروبية في لب الأسنان. يتطلب ذلك استجابات مناعية لمنع انتشار العدوى. تعتبر البلاعم مجموعة من الخلايا غير المتجانسة التي لها أدوار عديدة في تطور العدوى، وتدمير وإعادة بناء الأنسجة العظمية، والتفاعلات بين الميكروبات والمضيف.
المواد والطرق: تم معالجة ثلاثين كتلة نسيجية مدمجة في البارافين من عينات تم استئصالها من 18 ورم حبيبي قمي و12 كيس جذري للفحص النسيجي للكشف عن الخلايا الإيجابية لـCD68 ، CD11c وCD163. تم تقييم تعبير البلاعم بشكل كمي.

النتائج: وجدت الدراسة أن استقطاب البلاعم من النوعM1 ، الذي يشير إليه تعبيرCD11c ، كان أعلى في الأكياس الجذرية مقارنة بالأورام الحبيبية القمية (25.45 ± 4.11 مقابل 14.66 ± 4.19). لم يكن هناك فرق كبير بين الأورام الحبيبية والأكياس الجذرية فيما يتعلق بتعبير CD68 (46.27 ± 5.06) مقابل 28.05 ± 5.06؛ p=0.054). وعلى العكس من ذلك، كان استقطاب البلاعم من النوعM2 ، الذي يشير إليه تعبيرCD163 ، أعلى بشكل كبير في الأورام الحبيبية القمية مقارنة بالأكياس الجذرية (42.12 ± 6.59) مقابل 21.03 ± 3.43؛ p=0.020).

الاستنتاجات: تلعب البلاعم دورًا كبيرًا في الاستجابة الالتهابية للآفات حول القمة. وجدت الدراسة أن البلاعم من النوع M1 كانت أكثر انتشارًا في الأكياس الجذرية، بينما كان تعبير البلاعم من النوع M2 أعلى بشكل ملحوظ في الأورام الحبيبية القمية. وهذا يشير إلى أنماط استقطاب متميزة للبلاعم بين الأكياس الجذرية والأورام الحبيبية الفمية.

1. Introduction

Granulomas are aggregates of macrophages and other immune cells forming in response to persistent stimuli that cannot be eradicated by individual macrophages (De Rosa *et al.*, 2020). Periapical granuloma (PG) comprises chronically inflamed granulation tissue and is the most common periapical lesion (Holzer-Geissler *et al.*, 2022). Histologically, it includes granulation tissue with mixed inflammatory infiltrates, newly developed blood vessels, and nerve fibers (Ruth *et al.*, 2020). Bacterial toxins from necrotic pulp initiate local inflammatory responses, leading to the release of cytokines and bone destruction (Kong *et al.*, 2020). Radicular cysts, inflammatory odontogenic cysts, make up a significant portion of cystic lesions in the jaw. They develop from cystic degeneration of cell rests of Malassez due to inflammatory stimulation from necrotic pulp (Ye *et al.*, 2023). Histologically, these cysts are lined by nonkeratinized stratified squamous epithelium, often infiltrated with inflammatory cells (Keerthana, Sindhu and Deepak, 2020; Islam *et al.*, 2023). Macrophages play crucial roles in the immune response against chronic periapical lesions, participating in phagocytosis, inflammatory mediator production, and lesion repair. They interact with cyst epithelium through cytokines like TNF-α, regulating bone growth and resorption (Duque and Descoteaux, 2014; Shapouri-Moghaddam *et al.*, 2018). Macrophages are classified into M1 and M2 phenotypes. M1 macrophages produce reactive oxygen species and inflammatory cytokines, while M2 macrophages release growth factors and immunosuppressive cytokines (Pérez and Rius-Pérez, 2022; Strizova *et al.*, 2023; Wang *et al.*, 2023). The activation of classical and alternative complement pathways leads to M1 and M2 polarization, respectively (Pérez and Rius-Pérez, 2022). TNF-α, primarily released by M1 macrophages, regulates cystic epithelium and is a bone resorption modulator (Parameswaran and Patial, 2010; Parameswaran and Sonika, 2010). The initial phase of periapical inflammatory disease, known as acute periapical periodontitis, is characterized by a persistent dull and throbbing pain. The affected tooth typically exhibits a negative response to vitality testing, or in some cases, a delayed positive response (Igna *et al.*, 2022). Chronic periapical inflammatory disease is detected without any prior recollection of an acute phase. Most periapical granulomas are asymptomatic, although pain and sensitivity can develop if there is an acute exacerbation (MARTON and KISS, 1993; França *et al.*, 2019). Periapical granulomas are characterized by inflamed granulation tissue that is encased in a fibrous connective tissue wall. This granulation tissue typically exhibits a variably dense infiltrate of lymphocytes, which is often intermixed with other cell types including neutrophils, plasma cells, histiocytes, and less frequently, mast cells and eosinophils (Cilmiaty *et al.*, 2020; Cilmiaty, 2023). When a significant number of plasma cells are present, one may observe scattered eosinophilic globules of gamma globulin, known as Russell bodies. Additionally, clusters of lightly basophilic particles, referred to as pyronine bodies, may also be seen in association with the plasmacytic infiltrate. It is important to note that these plasma cell products are not specific to periapical granulomas and can be found in any tissue accumulation involving plasma cells (Sun *et al.*, 2023). Within the granulation tissue, epithelial rests of Malassez may be identified. These are remnants of the Hertwig epithelial root sheath that can become involved in periapical lesions. Furthermore, collections of cholesterol clefts, which are often accompanied by multinucleated giant cells, as well as areas of red blood cell extravasation with hemosiderin pigmentation, may be present. Such features indicate previous hemorrhage and subsequent breakdown of red blood cells (De Santa *et al.*, 2019; Xue *et al.*, 2024). Occasionally, small foci of acute inflammation with focal abscess formation can be observed within the granulation tissue. However, the presence of these foci does not necessarily warrant a diagnosis of a periapical abscess, as they may not be sufficiently extensive to meet the criteria for such a diagnosis.

Periapical cysts, also known as radicular or apical periodontal cysts, are true epithelium-lined cysts that typically form at the apex of a nonvital tooth. These cysts develop when inflammation stimulates the epithelium at the apex. The primary source of this epithelium is usually the epithelial rests of Malassez, but it can also originate from crevicular epithelium, sinus lining, or the epithelial lining of sinus tracts (Barbosa *et al.*, 2015; Gordon and Plüddemann, 2018). Periapical pocket cysts are characterized by an incomplete epithelial lining, where the apical portion of the tooth extends into the cyst lumen. In contrast, periapical true cysts form a complete epithelium-lined structure that is adjacent to, but separate from, the tooth apex. The lumen of the cyst is often filled with fluid and cellular debris. The wall of the cyst is composed of dense fibrous connective tissue. The innate immune system comprises both cellular and humoral components, with macrophages and neutrophils playing significant roles as innate effectors and producers of humoral fluid-phase pattern recognition molecules. Macrophages are key players in the innate immune response, responsible for the recognition and clearance of exogenous pathogens in infectious diseases, and are integral to the regulation of the inflammatory response and the reparative process in many diseases. They exhibit significant heterogeneity and plasticity, allowing them to adapt their phenotypes in response to their microenvironment, which is illustrated by their classification into two polarized states: classically activated (M1) and alternatively activated (M2) macrophages (Stashenko *et al.*, 1994; Mantovani *et al.*, 2013; Jamiyan *et al.*, 2020; Sari, Özdemir and Çilekar, 2022).

M1 macrophages, stimulated by interferon-gamma (IFN-γ) and lipopolysaccharides (LPS), produce high levels of inflammatory cytokines like interleukin-12 (IL-12) and interleukin-23 (IL-23) while releasing low levels of interleukin-10 (IL-10), thereby promoting Th1 responses and contributing to inflammatory autoimmune pathologies. M1 macrophages also utilize inducible nitric oxide synthase (iNOS) to catabolize L-arginine into nitric oxide (NO) and citrulline, enhancing their microbicidal capabilities. Conversely, M2 macrophages, which are induced by interleukins IL-4 and IL-13, secrete high levels of IL-10 and low levels of IL-12 and IL-23, facilitating wound healing, immune regulation, and promoting Th2 responses. M2 macrophages also induce arginase 1, which metabolizes arginine into ornithine and polyamines essential for collagen synthesis and cellular proliferation (Kong *et al.*, 2013; Labonte, Tosello-Trampont and Hahn, 2014; Parisi *et al.*, 2018; Xue, Wang and Han, 2023).

M2 macrophages, known for their anti-inflammatory roles, control the progress of efferocytosis and are involved in tissue repair, fibrosis, and angiogenesis. Their ability to repolarize in response to changing local environments enables them to shape the inflammatory milieu, contributing to their dual roles in both promoting and resolving inflammation. This adaptability is crucial in chronic periapical lesions, where macrophages participate in phagocytosis, inflammatory mediator production, and lesion repair. They interact directly with cyst epithelium through cytokines like tumor necrosis factor-alpha (TNF-α), which regulate bone growth and resorption, potentially explaining the immunological theory of radicular cyst formation. CD68 is a commonly used marker for macrophages, with CD11c identifying M1 macrophages and CD163 marking M2 macrophages. The complex interplay of immune signals, tissue-derived factors, and macrophage activation states underscores the multifaceted roles of macrophages in health and disease, highlighting their significant contributions to both the inflammatory response and tissue repair in periapical lesions (Bolego *et al.*, 2013; Watanabe *et al.*, 2019; Song *et al.*, 2022; Guha Ray *et al.*, 2023; Papotti *et al.*, 2023).

2. Materials Subjects and Methods

The investigation was conducted on thirty formalin-fixed, paraffin-embedded blocks of 18 periapical granulomas and 12 radicular cysts. These blocks were obtained from the archives of the Oral Pathology laboratory at the College of Dentistry, Baghdad University. The samples were collected between 2012 and 2019. The clinicopathological data, including age, gender, and lesion size, were extracted from the case sheets accompanying the lesion specimens. The tissue specimens were fixed in a 10% neutral formalin solution and then processed into normal paraffin blocks. Every specimen that was fixed in formalin and embedded in paraffin had consecutive sections. For histopathological re-examination, a section measuring four micrometers in thickness was put on clean glass slides from each block of the examined sample and the control group. The sections were then subjected to routine Hematoxylin and Eosin staining (H&E). Three further sections, each measuring 4mm in thickness, were affixed to positively charged microscope slides (AFCO) for immunohistochemical staining. immunohistochemistry specificity is deemed affirmative when the immunohistochemistry brown stain is only present in the selectively positive control tissue slides, as indicated by the manufacturer's datasheets, and is absent in the negative control tissue slides. Each tissue section was examined under a 400X objective microscope. Five fields were selected and visually assessed. The mean positive percentage for each case was then recorded. Both pathologists blindly scanned all of the slides.

2.1.Statistical Analysis

The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 25. The data is presented in terms of the mean, standard deviation, and ranges. The data is categorized and given in the form of frequencies and percentages. The continuous variables were compared using the Independent t-test and Analysis of Variance (ANOVA) (two-tailed). The Receiver Operating Characteristic (ROC) curve analysis was employed to predict the differentiation of preapical granuloma. A P-value below 0.05 was considered significant.

3. Results

3.1.Macrophage Marker Expression in Granulomas and Radicular Cysts

Thirty patients were enrolled in the present cross-sectional study; the results were expressed in mean plus minus standard error of the mean (Mean ± SE). Seventeen patients (56.7%) were males and 13 patients (43.3%) were females, the mean patients' age was 34.37 ± 2.95 years; fifteen patients were aged bellow 30 years and 15 patients were equal or above 30 years as shown in Fig.1.

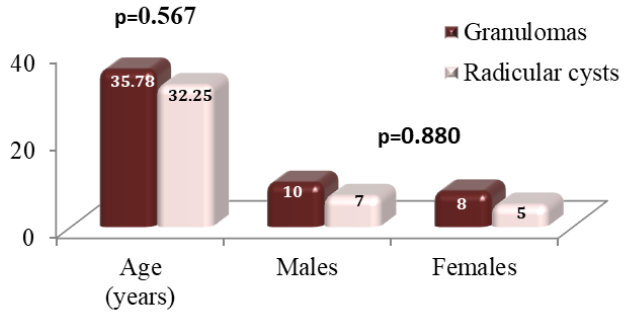

Figure 1: Comparison of Demographic Features Between Patients with Granulomas and Radicular Cysts

There is no significant difference between granulomas and radicular cysts concerning CD68 (46.27 ± vs. 28.05 ± 5.06; *p*=0.054) as presented in Table 1

Table 1: Comparison of CD68 Markers Between Granulomas and Radicular Cyst Lesions

Parameters (Mean ± SE)	Granulomas lesion N.=18	Radicular cyst lesion N.=12	p value
CD 68	46.27 ± 6.54	28.05 ± 5.06	0.054, NS
SE: Standard error; NS: Not significant (p> 0.05); S: Significant (p≤0.05)			

There were M1 (CD11c) higher in radicular cyst as compare to preapical granuloma but there were no significant differences (25.45± 4.11vs. 14.66 ± 4.19; *p*=0.149) as presented in Table 2 and Fig.2.

Table 2: Comparison of CD11c Between Granulomas and Radicular Cyst Lesions

Parameters (Mean ± SE)	Granulomas lesion N.=18	Radicular cyst lesion N.=12	p value
CD 11C	14.66 ± 4.19	25.45 ± 4.11	0.149 NS
SE: Standard error; NS: Not significant (p> 0.05); S: Significant (p≤0.05); Ŧ: Independent sample t test			

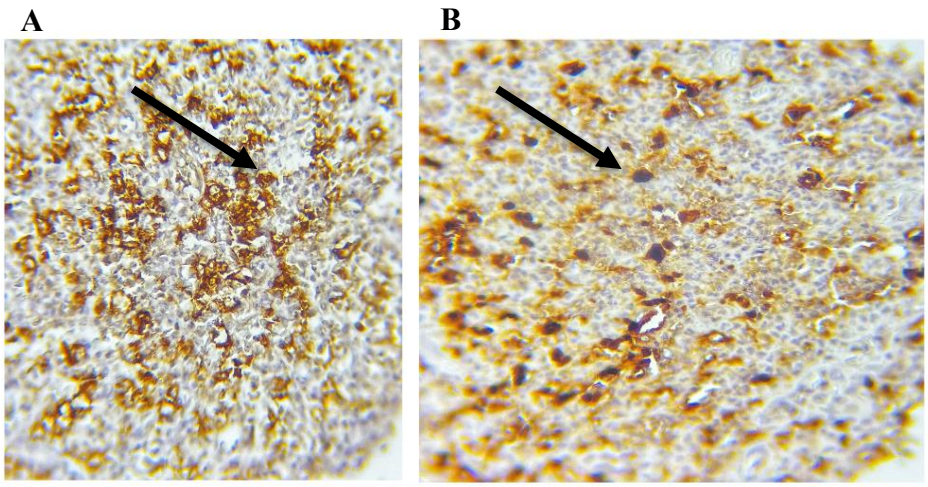

Figure 2: Membranous Expression of CD11c Immunohistochemical Marker (The Black Arrow). **A)** With 10X, **B)** With 20X.

CD 163 expression was significantly higher among patients with granulomas as compare to patients with radicular cyst (42.12 ± 6.59 vs. 21.03 ± 3.43; *p*=0.020) as presented in Table 4 and Fig3.

Table 3: Comparison of CD163 Between Granulomas and Radicular Cyst Lesions

Parameters (Mean ± SE)	Granulomas lesion N.=18	Radicular cyst lesion N.=12	p value
CD 163	42.12 ± 6.59	21.03 ± 3.43	0.020 S
SE: Standard error; NS: Not significant (p> 0.05); S: Significant (p≤0.05).			

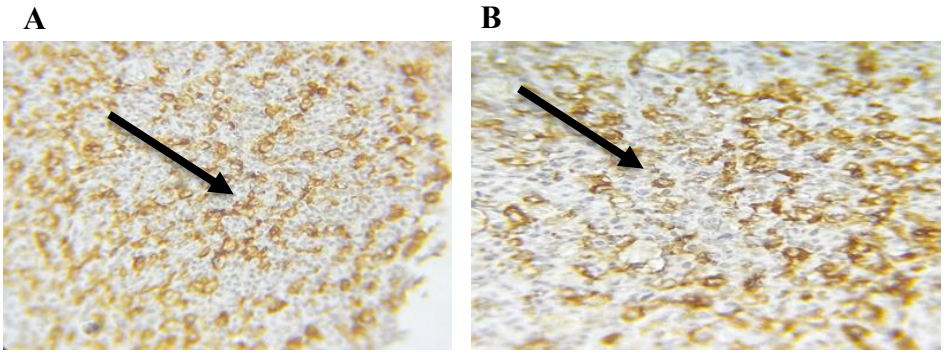

Figure 3: Cytoplasmic and Membranous Expression of CD136 Immunohistochemical Marker (The Black Arrow). **A)** With 10X, **B)** With 20X.

3.2.CD 163 Expression as Predictor for Granulomas Diagnosis

Receiver Operative Characteristic curve (ROC curve) of CD163 had been used as a predictor of granulomas lesions diagnosis, according to the results, the cut off value of CD 163 expression was ≥ 26.5 with sensitivity = 72.2%, specificity = 75.0% and accepted area under the curve (AUC =0.731) as demonstrated in Table 4 and Fig.4.

Table 4: ROC of CD163 Discriminatory Ability for Granulomas Diagnosis

ROC curve characteristics	CD 163 expression
Cut off value (ng/ml)	≥ 26.5
Sensitivity %	72.2
Specificity %	75.0
Area under curve (AUC)	0.731
p value	0.034 S
S: Significant (p ≤ 0.05)	

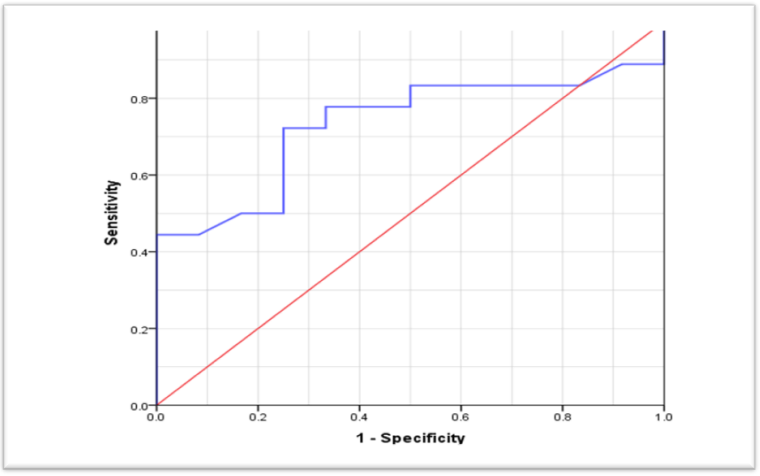

Figure 4: ROC Curve of CD163 Discriminatory Ability for Granulomas Diagnosis

3.3.Correlations Among Immunohistochemistry Markers Among Patients with Granulomas Lesions

There was a positive significant correlation between CD 68 with both CD11C (r= 0.519 & *p* =0.027) and CD 163 expression (r= 0.966 & *p*<0.001), in addition to a positive significant correlation between CD 11C and CD 163 expression (r= 0.546 & *p*=0.019) as shown in Table 5.

Table 5: Correlations Between Patient's Age, Lesion Size & Immunohistochemistry Markers Among Patients with Granulomas Lesions

Parameters	Statistics	CD 68	CD 11C	CD 163
CD 68	r	1	0.519	0.966
	p value		0.027 S	< 0.001 S
CD 11C	r	0.519	1	0.546
	p value	0.027 S		0.019 S
CD163	r	0.966	0.546	1
	p value	< 0.001 S	0.019 S	
r: Pearson's correlation coefficient; NS: Not significant (p>0.05); S: Significant (p≤0.05)				

3.4.Correlations Between Immunohistochemistry Markers Among Patients with Radicular Cyst Lesions

Patients with radicular cysts revealed a positive significant correlation between CD 68 and CD 11C (r=0.968 & p<0.001), CD 68 and CD 163 (r=0.931 & p<0.001) and between CD 163 and CD 11C (r=0.914 & p<0.001) as shown in Table 6.

Table 6: Correlations Between Patient's Age, Lesions Size & Immunohistochemistry Markers Among Patients with Radicular Cysts Lesions

Parameters	Statistics	CD 68	CD 11C	CD 163
CD 68	r	1	0.968	0.931
	p value		< 0.001 S	< 0.001 S
CD 11C	r	0.968	1	0.914
	p value	<0.001 S		<0.001 S
CD 163	r	0.931	0.914	1
	p value	<0.001 S	<0.001 S	
r: Pearson's correlation coefficient; NS: Not significant (p>0.05); S: Significant (p≤0.05)				

4. Discussion

Chronic periapical lesions, such as periapical granuloma and radicular cyst, are characterized by the presence of a plethora of immune cells, including macrophages, which are distributed throughout the connective tissue stroma of these lesions. Macrophages are key immune cells that play a crucial role in modulating T cell responses. They interact with stem cells and are involved in the control of tissue homeostasis, making them vital components in the immune response to periapical lesions (Guo *et al.*, 2018). Macrophages exhibit remarkable plasticity, enabling them to polarize into distinct phenotypes such as M1 and M2, each contributing differently to the pathogenesis and progression of periapical lesions. M1 macrophages, identified by CD11c markers, produce pro-inflammatory cytokine, which are crucial for initiating and maintaining the inflammatory response. These cytokines effectively destroy pathogens but also contribute to tissue damage (Čolić *et al.*, 2009; Cavalla *et al.*, 2021; Farahi, Sinha and Lusis, 2021).

In current study There were no significant differences between patients with granulomas and radicular cysts concerning mean ages (35.78 ± 3.93 vs. 32.25 ± 4.58; p=0.567) and their gender (p=0.880). That analog to the result obtains by Pinheiro who found the mean age Odontogenic cysts was 31 years, there is a predominance of cases affecting individuals in their third and fourth decades of life. These findings are possibly associated with the

highest prevalence of untreated dental caries in young adults, leading to the development of periapical disease. On the other hand, as observed by other authors, these lesions are rare among adults older than 60 years. This probably occurs because tooth extraction, as an alternative to conventional endodontic treatment, is carried out more frequently in the elderly than in younger patients (De Souza *et al.*, 2010; Giovacchini *et al.*, 2020).

Odontogenic cysts are one of the most common osseous-destructive lesions affecting the jaws. These cysts arise from the epithelial components of the odontogenic apparatus or its remnants that lie entrapped within bone or in the gingival tissue. Commonly, Odontogenic cysts exhibit slow growth and a tendency towards expansion (42)

In current study Lesions size was significantly higher among patients with radicular cysts than that of preapical granuloma (5.07 ± 1.35 vs. 0.94 ± 0.21; p=0.048). That analoge to the result obtains by Laux, M. who found that radicular cysts could be separated from periapical granulomas based on the high size of radicular cysts (more than 5.9 mm) (De Rosa *et al.*, 2020).

Assessment of CD68 marker:

CD 68 is a highly glycosylated type I trans membrane glycoprotein that belong to the lysosome -associated membrane protein family.CD 68 mostly expressed by monocyte linage and widely used to identify macrophage in tissue sections as a pan-macrophage marker. CD68, the most extensively used macrophage marker, is expressed on all macrophages, which does not allow for the discrimination between M1 and M2 macrophage subsets. In crurrent study there is no significant difference between granulomas and radicular cysts concerning CD68 (46.27± 6.54 vs.28.05 ± 5.06; p=0.054). Yao Song et al (2022) also observed similar patterns of CD68 expression across different periapical lesions, indicating that macrophages, marked by CD68, are present in both granulomas and cysts but do not vary significantly in their levels. They noted that CD68 is crucial for phagocytosis and initial inflammatory responses and these results could suggest that all macrophages found in RCs and PGs have similar expression characteristics (De Souza *et al.*, 2010; Labonte, Tosello-Trampont and Hahn, 2014; Malysheva *et al.*, 2021; Song *et al.*, 2022).

Assessment of CD11c marker:

CD11c is a type I trans membrane protein that is expressed on monocytes, granulocytes, a subset of B cells, dendritic cells, and macrophages. CD11c is abundantly expressed in monocytes and macrophages. CD11c is marker that commonly used for Ml macrophage phenotype. However, some authors have also used CD11c as a marker for dendritic cells. In current study the CD11C highest in radicular cyst than periapical granuloma (25.45 ± 4.11vs. 14.66 ± 4.19). this result is concurrent to the result found by Weber who found the radicular cysts showed higher degree of M1 macrophage polarization than apical granulomas (Teixeira-Salum *et al.*, 2010; De Carvalho Fraga *et al.*, 2013; Rigamonti *et al.*, 2014; Lee *et al.*, 2021; Malysheva *et al.*, 2021).

The current study analyzes macrophage infiltration and putative markers of macrophage polarization (proinflammatory M1) in apical granulomas, radicular cysts. The expression ratios of macrophage markers as indicators of macrophage polarization in preapical granulomas, radicular cysts. Ratio of CD11c and CD68 cell count as an indicator of M1 polarization. In current study M1 polarization higher in radicular cyst. These results are consistent with previous studies regarding cytokine secretion. radicular cysts were characterized by high IFN-gamma- and TNF-alpha expression which are M1-associated cytokines. Accordingly, another study revealed high expression of the M1-associated cytokine IFN-gamma in radicular cysts and a predominance of the M2-associated cytokine IL-4 in apical granulomas (Shabo and Svanvik, 2011; Yilmaz *et al.*, 2022).

A shift towards M1 polarization in the periapical inflammation could be the reason for the formation of radicular cysts. Macrophages are known to execute their role in tissue homeostasis and remodeling by interacting with stem cells in several ways. Macrophages can induce epithelial proliferation, remodel extracellular matrix, and vascularization during organ development. One-way macrophages interact with stem cells is via the secretion of cytokines and might contribute to the formation of radicular cysts in apical periodontitis. The higher levels of infiltration of M1-like macrophages in RCs indicate that an aggressive state of RCs could be observed, more proinflammatory cytokines might be secreted to the lesion area, and the activated osteoclasts in the apical region of the affected teeth (Lin, Huang and Rosenberg, 2007; Shabo and Svanvik, 2011; Schmidt *et al.*, 2016; França *et al.*, 2019).

Assessment of CD163 marker:

CD163 is a trans membrane receptor. It is a scavenger receptor for the haptoglobin-hemoglobin (Hp-Hb) complex and is expressed by monocytes/macrophages. CD163 is expressed in the M2 macrophages and in tumor associated macrophages. CD163 can also produce anti-inflammatory cytokines, scavenge the tumor necrosis factor-like weak inducer of apoptosis, and recognize bacteria. The expression of CD163 is up regulated by the acute phase mediator IL-6, glucocorticoids and IL-10 and is down regulated by IL-4, TGF-beta, interferone-gamma and by the proinflammatory liposaccharide. It has been suggested that CD163 is a differentiation antigen for monocyte/macrophage. Macrophages have a higher expression of CD163 than monocytes indicating a maturation process to phagocytic macrophages. CD163 is expressed in the M2 macrophages and in tumor associated macrophages (Song *et al.*, 2022; Yin, Li and Hou, 2022).

In current study there was significantly higher CD 163 expression among patients with granulomas (42.12 ± 6.59 vs. 21.03 ± 3.43; p=0.020) this result similar to the result found by Weber who found that CD163-positive macrophages were significantly more prevalent in periapical granulomas compared to radicular cysts. The study emphasized that M2 macrophages (CD163-positive) play a crucial role in anti-inflammatory responses and tissue repair, which aligns with the current study's findings. Weber found that apical granulomas showed high levels of M2 cytokines TGF-beta and IL-10 and low quantities of pro-inflammatory cytokines, while radicular cysts were characterized by high IFN-gamma- and TNF-alpha expression, which are M1-associated cytokines. Other study found increased M1 macrophage polarization in RCs, whereas M2 polarization was more frequent in PGs, concluded that the periapical tissue microenvironment may define the type of lesion to be developed, leading to a specific macrophage subpopulation. As M2 macrophages are known to induce Treg development in a TGF-beta dependent manner, this goes in line with the finding of current study that in apical granuloma, M2 macrophages were more prevalent than in radicular cysts (Mantovani *et al.*, 2013; Shapouri-Moghaddam *et al.*, 2018).

It is assumed that apical periodontitis can progress from apical granulomas to radicular cysts. The proliferation of epithelial cell rests of Malassez (ERM) is the essential process in formal pathogenesis. M2 macrophages, on the other hand, are characterized by their ability to inhibit the cytotoxic and inflammatory functions of M1 macrophages, while also being involved in angiogenesis, anti-inflammatory effects, tissue repair and remodeling, and fibrosis. As M2 macrophages are known to induce Treg development in a TGF-beta dependent manner, this goes in line with the finding of current study that in periapical granuloma, M2 macrophages were more prevalent than in radicular cysts. It is assumed that apical periodontitis can progress from apical granulomas to radicular cysts. In this context, the proliferation of epithelial cell rests of Malassez (ERM) is the essential process in formal pathogenesis (Stashenko *et al.*, 1994; Mantovani *et al.*, 2013; Gordon, Plüddemann and Martinez Estrada, 2014;

Kierdorf *et al.*, 2015; Gordon and Martinez-Pomares, 2017; Gordon and Plüddemann, 2017; Parisi *et al.*, 2018; Wu and Hirschi, 2021).

5. Conclusions

The expression of M1 macrophage higher in radicular cyst as compare to periapical granuloma using cd11c markers as an indicator of M1 polarization. M1 polarization higher in radicular cyst. A shift towards M1 polarization in the periapical inflammation could be the reason for the formation of radicular cysts. The progression of an periapical granuloma to a radicular cyst is associated with an increased M1 polarization. The proliferation of epithelial cell rests of Malassezia to form cystic lesions in radicular cysts might be induced by M1 macrophages. The expression of M2 macrophages in periapical granuloma significantly higher than that in radicular cyst using CD136 markers as an indicator of M2 polarization.

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