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Distribution of Macrophages and Plasma Cells in Odontogenic Cysts

Odontojenik Kistlerde Plazma Hücresi ve Makrofajların Dağılımı

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Abstract

Aim: Odontogenic cysts are common and important lesions of the maxillofacial region. Radicular cysts, dentigerous cysts, and odontogenic keratocysts are the most common odontogenic cysts. Macrophages and plasma cells are the main cells of inflammation and play a role in the development of many diseases. This study aimed to compare the presence and distribution of macrophages and plasma cells among the most common odontogenic cysts with clinical data.

Material and Method: Cases diagnosed with odontogenic cysts in our laboratory were included in our study. Hematoxylin-Eosin stained sections of the cases in the archive were re-evaluated. The area that best reflected the inflammation tissue was first marked on the slides and then on the blocks. Then, 2 mm diameter cylindrical-shaped paraffinized tissue samples were taken from donor blocks and transferred to multiple blocks with a manual microarray device. Anti-CD68 and anti-CD138 immunohistochemical stains were applied to multiple blocks. The stained preparations were scored between 0-2 by giving an average score. The scores were then analyzed together with clinical data between the three groups.

Results: Of the 83 odontogenic cysts included in our study, 41 were radicular cysts, 25 were dentigerous cysts, and 17 were keratocysts. The ages of the patients ranged from 17 to 77 years, with a mean of 37.55± 16.42 years. 47% of the patients were male, and 53% were female. There was no significant difference between the odontogenic cyst groups regarding age and gender ($p>0.05$). There was a significant difference between the cyst type and the proportions of CD68+ macrophages and CD138+ plasma cells ($p<0.05$). CD138+ plasma cell density was primarily observed in radicular cysts, while CD68+ macrophages were more intense in odontogenic keratocysts.

Conclusion: There was a significant difference in the distribution of CD68+ macrophages and CD138+ plasma cells in odontogenic cyst type. Therefore, it is important to have more information about the histomorphologic features of odontogenic cysts and to understand their inflammatory processes for correct diagnosis and treatment.

Keywords: Radicular cyst, odontogenic cyst, macrophage, plasma cells

Öz

Amaç: Odontojenik kistler maksillofasiyal bölgenin sık karşılaşılan önemli lezyonlarıdır. Radiküler kistler, dentijeröz kistler ve odontojenik keratokistler en sık karşılaşılan odontojenik kistlerdir. Makrofajlar ve plazma hücreleri inflamasyonun temel hücreleridir ve bir çok hastalığın oluşumunda rol oynarlar. Bu çalışmanın amacı; en sık karşılaşılan odontojenik kistlerde makrofaj ve plazma hücrelerinin varlığını ve dağılımını klinik bilgilerle birlikte karşılaştırmak amaçlanmıştır.

Gereç ve Yöntem: Çalışmamıza laboratuvarımızda Ocak 2013 ile Aralık 2022 tarihleri arasında odontojenik kist tanısı almış, eksizyonel biyopsi uygulanmış olgular dahil edildi. Olgulara ait Hematoksilen-Eozin boyalı kesitler değerlendirildi. İmmünhistokimyasal (İHK) boyama için en uygun olan alanlar işaretlendi. Daha sonra parafin bloklardan 2 mm çapta silindirik şekilli parafinize doku örnekleri manuel mikroarray cihazı ile donör bloklardan alınarak çoklu bloklara aktarıldı. Anti- CD68 ve anti- CD138 immünhistokimyasal boyası çoklu bloklara uygulandı. Boyalı preparatlar ortalama bir skor verilerek 0-2 arasında puanlandı. Daha sonra verilen skorlar 3 grup arasında klinik verilerle birlikte analiz edildi.

Bulgular: Çalışmamıza dahil edilen 83 odontojenik kistin 41 tanesi radiküler kist, 25 tanesi dentijeröz kist ve 17 tanesi keratokistti. Hastaların yaşları 17 ile 77 arasında değişiyordu ve ortalama 37,55± 16,42' idi. Hastaların %47'si erkek iken % 53 kadındı. Radiküler kist, dentijeröz kist ve keratokist grupları arasında yaş ve cinsiyet açısından anlamlı fark yoktu ($p>0.05$). Kist tipi ile CD68+ makrofajların ve CD138+ plasma hücrelerinin oranları arasında anlamlı fark saptandı. ($p<0.05$). CD138+ plazma hücre yoğunluğu özellikle radiküler kistlerde gözlemlenirken, CD68+ makrofajlar odontojenik keratokistlerde daha yüksek yoğunlukta bulunmuştur.

Sonuç: Odontojenik kist tipleri arasında CD68+ makrofajların ve CD138 + plasma hücrelerinin dağılımında anlamlı fark saptanmıştır. Bu nedenle odontojenik kistlerin histomorfolojik özellikleri hakkında daha fazla bilgi sahip olmak ve inflamatuar süreçlerini anlamak doğru tanı ve tedavi için önem arz etmektedir.

Anahtar Kelimeler: Radiküler kist, odontojenik kist, makrofaj, plazma hücresi

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INTRODUCTION

Odontogenic cysts arise from the epithelium of dental structures in the head and neck zone. Odontogenic cysts are divided into inflammatory and developmental cysts.[1]

The epithelium of odontogenic cysts originates from malassez epithelial remnants, dental lamina, enamel organ, dental papillae, and basal layer cells of the oral mucosa.[1]

The most common odontogenic cysts observed in studies are radicular cysts, dentigerous cysts, and odontogenic keratocysts.[2] Radicular cysts are in the inflammatory cyst group, while dentigerous and keratocysts are in the developmental cyst group.[2] Radicular cysts are formed in the tooth apex due to a possible inflammation stimulated by malassez residues.[3] Radicular cysts are the most common cysts in the jawbone. The cavity of radicular cysts is usually lined with stratified squamous epithelium, and there is a chronic inflammatory infiltrate in the fibrous cyst wall. Foamy macrophages, russel's bodies, cholesterol clefts, and glandular odontogenic epithelial remnants are frequently encountered in histologic findings.[4] Keratocysts are developmental cysts and usually draw attention with their aggressive clinical behavior. The recurrence rate of keratocysts is 10-30%.[5] The histologically prominent feature is parakeratosis and basal palisading of the laying epithelium.[5] The formation of odontogenic cysts is usually dominated by acute inflammation accompanied by mononuclear cells and macrophages.[6]

Macrophages and plasma cells are important cells of the inflammatory response and initiate the chronic inflammatory process with the cytokines they secrete and cause the surrounding epithelium and tissues to be affected.[4]

The formation and pathophysiology of odontogenic cysts in the maxilla have not been clearly elucidated. However, the presence of inflammatory cells detected in the cyst wall and subepithelial area in most cases reveals that inflammation is an important finding in cyst formation.[6] This study aims to demonstrate macrophage and plasma cell distributions of developmental and inflammatory odontogenic cysts using anti-CD68 and anti-CD138 autoantibodies.

MATERIAL AND METHOD

Our study included cases diagnosed as odontogenic cysts and underwent an excisional biopsy in our laboratory between January 2013 and December 2022. Accordingly, 41 of 83 odontogenic cysts were radicular cysts, 25 were dentigerous cysts, and 17 were keratocysts. Hematoxylin-Eosin (H&E) stained sections of the cases in the archive were re-evaluated. Clinical information of the patients was retrieved from the our hospital's system. Our study was approved by the ethics committee of our university (Ethics Committee Reference Number: 2022/186)

The area that best reflected the inflammation tissue and was most suitable for immunohistochemical (IHC) staining was first marked on slides and then on blocks. Biopsies that were not suitable enough for staining and sampling were excluded from the study. Then, paraffinized tissue samples of 2 mm diameter cylindrical-shaped paraffin blocks were taken from donor blocks and transferred to multiple blocks by mapping technique with a manual microarray device.

Three µm tissue sections taken from paraffin-embedded blocks were placed on 3-aminopropyltriethoxysilane-coated glass slides for immunohistochemical analysis. Anti-CD68 (MS-397-R7, Thermo Scientific) and anti-CD138 (MS- 1793-R7, Thermo Scientific) primary Mouse monoclonal antibody immunohistochemical stain was applied to multiple blocks in Leicia Bond- Max device. (**Figures 1**, **2** and **3**) Stained slides were examined under light microscopy, and the expression of each marker was classified based on the following scores: 0 (negative/focal) if there were no positive cells or less than 5% of the cells were positively stained; 1 (weak to moderate) if between 5% and 50% of the cells were positively stained; and 2 (strong) if more than 50% of the cells were positive. Three groups were formed based on the given scores, and the groups were analyzed together with clinical data.

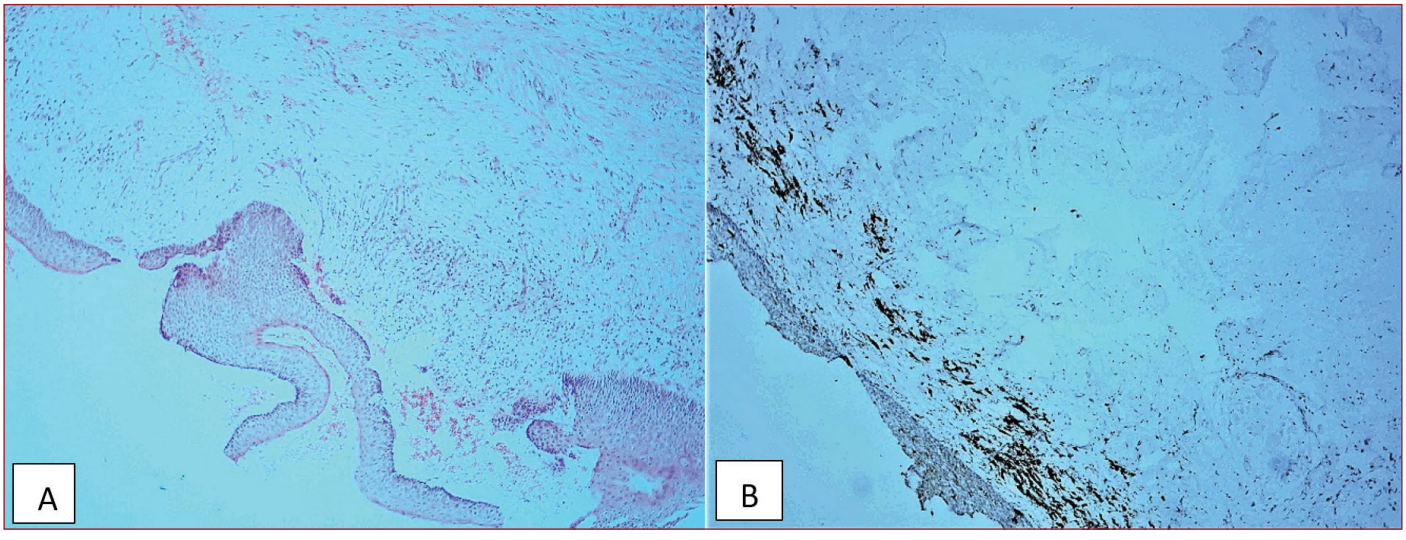

Figure 1: Dentigerous cyst, A: Hemotoxylen& Eosin section B: CD68+ macrophages (immunoperoxidase, original magnification, x200)

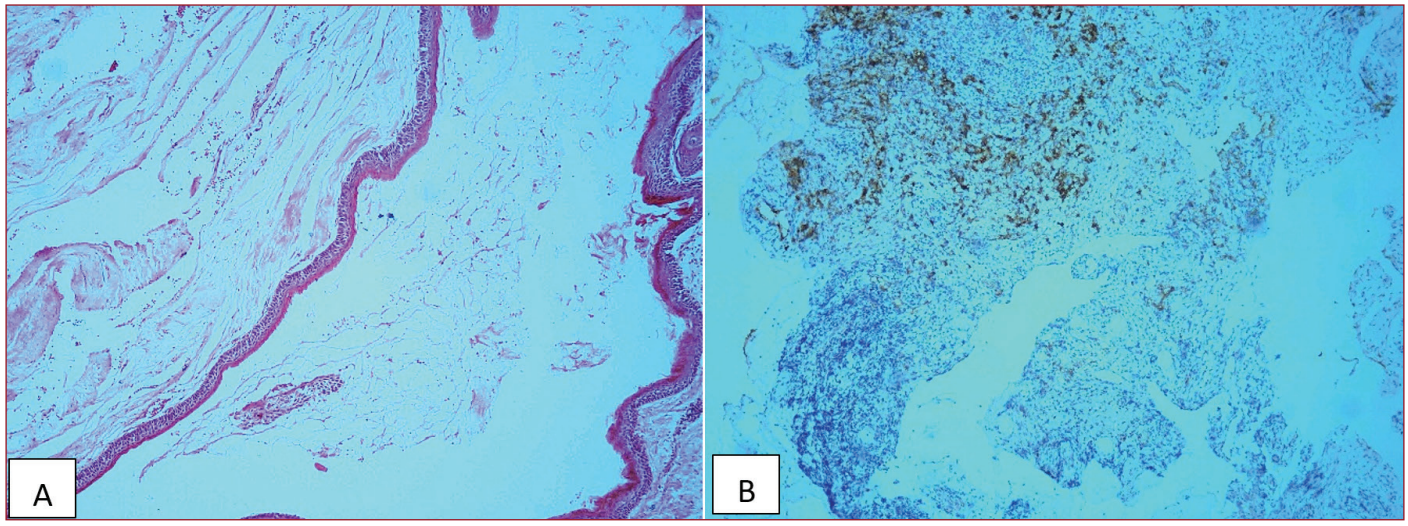

Figure 2: Odontogenic keratocyst A: Hemotoxylen & Eosin section B: CD38+ plasma cells (immunoperoxidase, original magnification, x200)

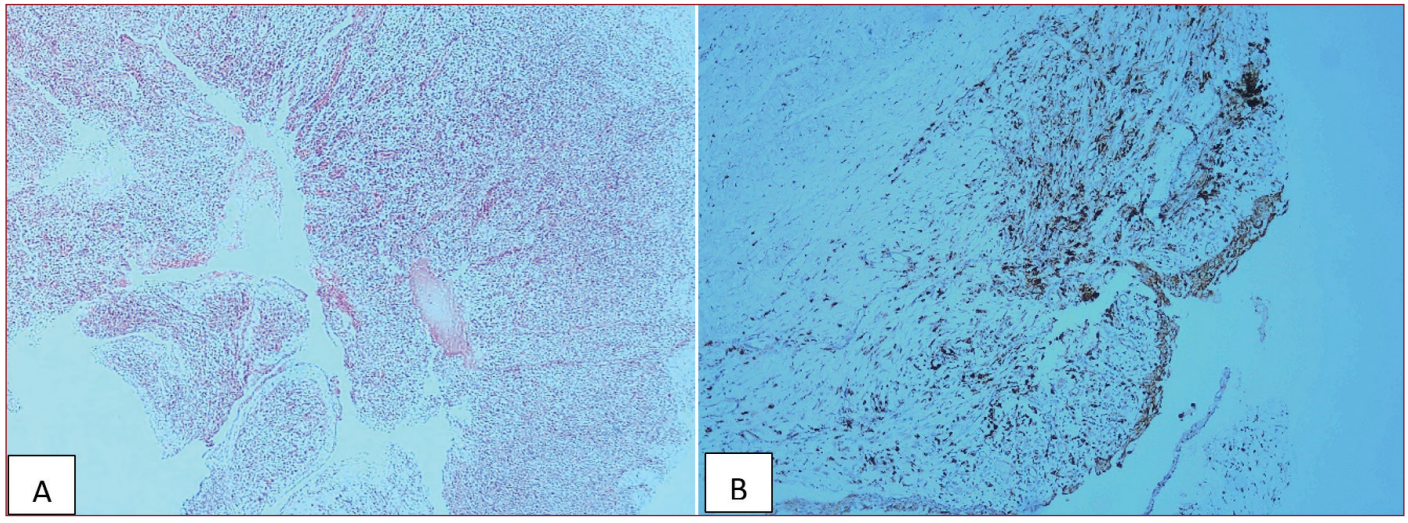

Figure 3: Radicular cyst A: Hemotoxylen & Eosin section B: CD 68+ macrophages (immunoperoxidase, original magnification, x200)

Statistical Method

The chi-square test was used to examine any relationships between categorical variables. The likelihood ratio test statistic was used instead of Pearson's test statistic when the expected counts were <5. Spearman's rank correlation coefficient was calculated to determine any relationships between the score variables. One-way anova test was used for descriptive statistical information. A $p<0.05$ value was accepted as statistically significant. All statistical analyses were performed using IBM SPSS v28 (IBM, Armonk, NY, USA).

RESULTS

Of the 83 odontogenic cysts included in our study, 41 were radicular cysts, 25 were dentigerous cysts, and 17 were keratocysts. Patients' ages ranged from 17 to 77 years, with a mean of 37.55± 16.42 years. While 47% of the patients were male, 53% were female. There was no significant difference between the radicular cyst, dentigerous cyst, and keratocyst groups regarding age and gender ($p>0.05$). (**Table 1** and **Table 2**)

Table 1: Distribution of the relationship between cyst type and gender

Crosstab

		Gender		Total
		male	female	
Cyst type				
Radicular cyst	Count	21	20	41
	% within Cyst type	51.2%	48.8%	100.0%
Keratocyst	Count	7	10	17
	% within Cyst type	41.2%	58.8%	100.0%
Dentigerous cyst	Count	11	14	25
	% within Cyst type	44.0%	56.0%	100.0%
Total	Count	39	44	83
	% within Cyst type	47.0%	53.0%	100.0%

Table 2: Distribution of the relationship between cyst type and age

Age

	N	Mean	Std. Deviation	Minimum	Maximum
Radicular cyst	41	39.39	15.349	15	77
Keratocyst	17	42.12	16.718	13	77
Dentigerous cyst	25	31.44	16.835	14	65
Total	83	37.55	16.426	13	77

There was a significant difference between the cyst type and the proportions of CD68+ macrophages and CD138+ plasma cells (p<0.05). While 51.2% of radicular cysts had more than 50% CD138+ plasma cell positivity, this rate was 12% in dentigerous cysts. (**Table 3**)

Table 3: Distribution of the immunoscore of CD138+ plasma cells in odontogenic cysts.

Crosstab

		CD138 ratio			Total
		< %5	%5-%50	≥%50	
Cyst type					
Radicular cyst	Count	9	11	21	41
	% within Cyst type	22.0%	26.8%	51.2%	100.0%
Keratocyst	Count	9	3	5	17
	% within Cyst type	52.9%	17.6%	29.4%	100.0%
Dentigerous cyst	Count	17	5	3	25
	% within Cyst type	68.0%	20.0%	12.0%	100.0%
Total	Count	35	19	29	83
	% within Cyst type	42.2%	22.9%	34.9%	100.0%

In 41.2% of keratocysts, the proportion of CD68+ macrophages was above 50%, whereas, in dentigerous and radicular cysts, it was 8% and 34.1%, respectively. (**Table 4**) There was no significant difference between the mean scores of CD68+ macrophages and CD38+ plasma cells and the age and gender of the patients (p>0.05).

Table 4: Distribution of the immunoscore of CD68+ macrophages in odontogenic cysts.

Crosstab

		cd68 ratio			Total
		<5%	5%-50%	≥50%	
Cyst type					
Radicular cyst	Count	6	21	14	41
	% within Cyst type	14.6%	51.2%	34.1%	100.0%
Keratocyst	Count	3	7	7	17
	% within Cyst type	17.6%	41.2%	41.2%	100.0%
Dentigerous cyst	Count	9	14	2	25
	% within Cyst type	36.0%	56.0%	8.0%	100.0%
Total	Count	18	42	23	83
	% within Cyst type	21.7%	50.6%	27.7%	100.0%

DISCUSSION

Due to the similar etiology and histomorphologic features of odontogenic cysts, diagnosis is sometimes challenging. Radicular cysts constitute the inflammatory cyst group, while dentigerous cysts and keratocysts belong to the developmental cyst group.[7]

The clinical manifestations of these cysts, such as aggressiveness, recurrence, and malignant transformation, vary depending on the cyst type.[2] Odontogenic keratocysts have a more aggressive course and high recurrence rates, while other cysts have a very good prognosis after treatment.[2] The different distribution of inflammatory cells involved in the histopathogenesis of these cysts and the cytokines secreted by these cells may play a role in this situation.

There are three stages in the etiopathogenesis of cyst formation. These are initiation, formation, and growth.[8] In the formation of periapical lesions and radicular cysts, pulp necrosis is usually initiated by a multibacterial infection.[9] This triggers a local humoral response, leading to the activation of B cells and plasma cells.[9] Plasma cells produce Ig G, Ig A, and Ig M in order from more to less.[10]

Macrophages are the primary cells of chronic inflammation and acquired immunity. Macrophages interact directly with the cyst epithelium and are involved in releasing cytokines such as IL-1, IL-6, and TNF-a.[8] They prepare antigen and provide it to T-helper cells.[11]

Our study showed a significant difference between cyst type and the distribution of CD68+ macrophages and CD138+ plasma cells (p<0.05). CD138+ plasma cell density was primarily observed in radicular cysts, while CD68+ macrophages were more intense in odontogenic keratocysts.

Unlike us, Azeredo et al.[12] found no significant difference between macrophage and plasma cell distributions in their study on periapical cysts and granulomas.

Gazivodo et al.[13] compared the cytokines produced in periapical lesions and found a significant difference between the amount of cytokines produced, whether the lesion was symptomatic or not, and the lesion's size. We did not include these criteria in our study, but we did not find a significant difference between demographic data such as age and gender and plasma cell-macrophage distributions.

Kouhsoltani et al.[14] found no significant difference between cyst types and the distribution of CD68+ macrophages in their study but found that macrophage density was lower in keratocyst odontogenic tumors compared to dentigerous cysts. Radicular cysts were considered the lesion with the highest macrophage density. In our study, odontogenic keratocysts had the highest macrophage distribution. This may be because we sampled from the area with the highest inflammation using the microarray method.

Many studies have investigated whether there is a relationship between the amount of macrophages in lesions and clinical course. Some studies suggest that macrophages are directly involved in the development and prognosis of the disease.[14]

In their review on macrophage distribution in periapical lesions, Song et al.[15] reported that macrophages were evaluated as M1-like and M2-like macrophages. In this article, it was suggested that M1-like macrophages are pro-inflammatory and M2-like macrophages are anti-inflammatory. For this reason, they emphasized that immunohistochemical markers and macrophage polarization should also be indicated when evaluating macrophage distribution in studies.

Weber et al.[16] conducted a study on apical granulomas, radicular cysts, and dentigerous cysts and evaluated macrophage polarization and revealed that M1-like macrophages were predominant in radicular cysts while M2-

like macrophages were predominant in apical granulomas. This may be due to the progression of apical granulomas to radicular cysts.[17]

It is very difficult to diagnose odontogenic cysts clinically, and one study reported that the initial clinical diagnosis accuracy rate was 36%.[18] Therefore, it is important to determine the distribution of inflammatory cells histologically. Marçal et al.[19] argued that mononuclear infiltration is more frequent than mixed-type infiltration in periapical cysts. Our study found more than 50% CD138+ plasma cell positivity in 51.2% of radicular cysts.

CONCLUSION

Our study showed a significant difference between cyst type and the ratios of CD68+ macrophages and CD138+ plasma cells ($p<0.05$). Although odontogenic cysts have different etiologies, it is difficult to determine the cyst type clinically and histopathologically. In addition, treatment approaches, and clinical course after treatment differ with cyst types. New information about the subtypes of inflammatory cells and their different polarizations is being revealed daily. Clinical, radiologic, and histologic evaluation of this information and new studies will allow new approaches to emerge in the diagnostic and therapeutic process.

ETHICAL DECLARATIONS

Ethics Committee Approval: Our study was approved by the Ordu University Clinical Researches ethics committee (Date: 05.08.2022, Decision No: 2022/186).

Informed Consent: For this type of study informed consent is not required (Retrospective study).

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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