The Role of CD68 (+) Histiocytic Macrophages in Nasal Polyp Development

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Abstract

Objectives The aim of this study was to investigate the role of CD68 (+) histiocytic macrophages (H-M) in the nasal polyp pathogenesis.

Materials and Methods The study group consisted of 24 adult patients with nasal polyposis. The control group consisted of 11 adult patients without nasal polyps. A total of 36 nasal polyp samples (10-nasal cavity, 10-maxillary sinus, and 16-ethmoid sinus) from the study group and 11 inferior turbinate samples from the control group were analyzed by immunohistochemical staining, with monoclonal antibodies against CD68 (+) H-M.

Results CD68 positivity was significantly higher than the control group in the subepithelial (SE) layer of the ethmoid sinus, and deep layers of nasal cavity, maxillary, and ethmoid sinuses. In SE and deep layers of ethmoid and maxillary sinuses, CD68 positivity was significantly higher than that of the epithelial layer. In the deep layer, histiocytic macrophages tended to gather around eosinophils.

Conclusion The high numbers of CD68 (+) histiocytic macrophages mainly located in deep layer of lamina propria may be responsible for the phagocytosis of eosinophils within the polyp tissue. Therefore, it may be concluded that increased macrophages in nasal polyps do not trigger the growth of nasal polyps. Instead, they may serve to reduce the number of eosinophils in already-developed nasal polyps.

Keywords

- CD68 (+)
- histiocytic macrophages
- polyp
- ethmoid sinus
- maxillary sinus
- nasal cavity
- pathogenesis

Introduction

Nasal polyposis is a common disorder affecting 1 to 4% of the population.¹ It is a chronic inflammatory disease of the upper airways, characterized by inflammatory cell infiltration, modifications of epithelial differentiation, and tissue remodeling including basement membrane thickening, gland modifications, extracellular matrix (ECM) accumulation, and edema.² Chronic rhinosinusitis with nasal polyps (CRS with NPs) is considered a subgroup of chronic rhinosinusitis (CRS).³

The factors involved in the morphological aspects of NP, such as massive infiltration of inflammatory cells, alterations in the respiratory epithelium, and remodeling of the ECM, are poorly understood. The levels of several cytokines and chemokines have been reported to be elevated in chronic sinusitis and NPs.⁴ Until now, the link between increased levels of cytokines and chemokines and the development of edema and alterations in the ECM has been subject to discussion. Possible factors underlying the development of vascular permeability is

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the secretion of vascular endothelial growth factor by mast cells⁵ and the deposition of toxic mediators, such as eosinophil cationic protein and major basic protein, generated by the degranulation of activated eosinophils that damage the epithelium.⁶

Histomorphological characterization of NP tissue reveals frequent epithelial damage, a thickened basement membrane, and edematous or sometimes fibrotic stromal tissue with a reduced number of vessels and glands.⁷ Macrophages play an important role in the inflammatory process, mainly by antigen processing and secretion of numerous mediators, such as cytokines and growth factors.⁸

The expression of CD68 indicates the activation of macrophages.¹ The CD68 antibody recognizes a 110-kDa glycoprotein that is part of the lysosome, a cytoplasmic organelle. In other words, this marker is organelle-specific rather than lineage-specific. The large numbers of lysosomes present in histiocytes account for the CD68 positivity of these cells.⁹ Even progenitor cells (monoblasts and promonocytes) of the same lineage do not react with the CD68 antibody.¹⁰ Since the other inflammatory cells such as plasma cells and mast cells do not contain abundant lysosomes, they do not react with this marker. Moreover, immunohistochemical studies demonstrate that mature plasma cells are stained with CD138, while mast cells are stained with tryptase.^{10,11}

In immunolabeling studies, CD68-positive cells in the epithelium and in the underlying connective tissue was counted in randomly selected fields. Numerous CD68-positive cells, showing the morphological features of macrophages, were detected in the epithelium and in the lamina propria.⁸

In the present study, we investigated the role of CD68 (+) histiocytic macrophages in the pathogenesis of NPs. CD68 positivity in the epithelium and in the subepithelial and deep layers of lamina propria in the ethmoid sinus, maxillary sinus, and nasal cavity samples of the polyposis group, and in the inferior turbinate samples of the control group was evaluated by immunohistochemical staining.

Materials and Methods

The study was conducted in the ear, nose, and throat (ENT) department at the Kirikkale University Faculty of Medicine. Immunohistochemical staining and light microscopic examinations were performed in the pathology department of the Kirikkale University Faculty of Medicine. All steps of the study were planned and performed under the approval of the Kirikkale University Faculty of Medicine Local Ethics Committee (date: March 23, 2009, number: 2009/028) and according to the principles outlined in the Declaration of Helsinki.¹² This study was supported by the Kirikkale University Scientific Research Projects Coordination Unit Funds (date: 2009, number: 2009/16).

Subjects

The patients with NPs were selected from the patients examined in the otolaryngology department of the Kirikkale University Faculty of Medicine. They had used topical corticosteroid nasal sprays for at least 6 weeks; after which, if the pathology

remained present, functional endoscopic sinus surgery (FESS) was performed. Total 24 adult patients (21 males and 3 females) with NPs, with a mean age of 45.97 ± 11.60 years, (range = 23–70 years) was included in the study group. They were examined by a questionnaire—ENT examination—endoscopic examination using 0 and 30 degrees endoscopes, Waters' radiography, and axial and coronal computed tomography of the paranasal sinuses.^{13–16}

The control group consisted of 11 adult patients without NPs (six males and five females) who had undergone septoplasty. The mean age of the control group was 29.90 ± 14.22 years (range = 18–56 years). They underwent the same examinations as the study group, described above. All subjects consented to participate in the study and written informed consents were obtained from all participants before any study procedure. No other diseases were present in either of the two groups.^{13–16}

The study and control group were used in the other studies of the authors.^{13–16} The difference of this study was to use different immunohistochemical stains for monoclonal antibodies against CD68 (+) histiocytic macrophages.

Surgery

All patients in the study group underwent FESS for nasal polyposis. Biopsies were performed under general anesthesia. Specimens were obtained from polypoid areas from three regions: ethmoid sinuses (including 16 specimens), maxillary sinus (including 10 specimens), and nasal cavity (including 10 specimens). For the control group, specimens were collected via punch biopsies from inferior turbinates during septoplasty, with a total of 11 specimens analyzed in the control group.^{13–16}

Immunohistochemical Staining

In the study and control groups, surgical specimens were examined by immunohistochemical staining techniques with monoclonal antibodies against CD68 (+) histiocytic macrophages. In each surgical specimen, the numbers of CD68 (+) histiocytic macrophages were evaluated in three to four high magnification fields. In addition, the mean numbers of CD68 (+) cells in the epithelium (E), subepithelial layer of lamina propria (SE), and deep para glandular layer of the mucosa (D) were calculated.

Immunohistochemical Staining Technique

The five-micron thick sections were obtained, transferred onto adhesive slides, and dried in an autoclave at 37°C overnight and 60°C for 20 minutes. They were deparaffinized and dehydrated by immersion in xylene twice for 10 minutes and alcohol twice for 10 minutes. The specimens were then incubated in 3% H₂O₂ for 5 minutes to inhibit endogenous peroxidase activity, and were treated with a citrate-based antigen retrieval solution (Dako; Denmark; pH: 6) for retrieving CD68 (+) histiocytic macrophage antigens (Laboratory Vision Corporation Neomarkers, Fremont, California, United States). All slides were heated in a microwave oven (750 W) twice for 5 minutes. Using a Shandon Sequeza manual staining device for standardization, Streptavidin Avidin-Biotin-Peroxidase (Strept. AB-Peroxidase)

and diaminobenzidine (DAB) chromogen (20 minutes) were applied for immunohistochemical analysis based on three antibodies. Nonimmune mouse serum served as a negative control and Mayer's hematoxylin was used for counterstaining. Cytoplasmic staining was considered as positive for CD68 expression.

The slides were reviewed by an expert pathologist. In each slide, the numbers of CD68 (+) histiocytic macrophages and inflammatory cells (plasma cells, granulocytes, and other mononuclear cells) per 100 cells were counted using a light microscope (Leica, Germany). The mean of these counts in three to four high magnification fields was then calculated. CD68 positivity was evaluated on a scale of 0 to 3 in epithelial (E), subepithelial (SE), and deep (D).

Positivity Index

For the quantitative assessment of CD68 (+) histiocytic macrophage expression, staining in the E, SE, and (D) in endothelial cells and inflammatory cells (plasma cells, granulocytes, and other mononuclear cells) was assessed by counting the number of CD68 (+) cells out of a total of 100 mainly inflammatory cells in three to four high magnification fields and calculating the means for these totals. The mean number of CD68 (+) histiocytic macrophages per high magnification field ($\times 400$) was calculated for the E, SE, and deep layers of the lamina propria. Scoring was performed on a scale of 0 to 3, where 0 represented negative staining; 1, weakly positive; 2, positive; and 3, strongly positive.^{13–17} More specifically:

Positivity Index (PI) 0 meant that antigen (CD68) (+) cell count was 0% (no stained cells).

PI 1 meant that antigen (CD68) (+) cell count was <5%.

PI 2 meant that antigen (CD68) (+) cell count was 5 to 50%.

PI 3 meant that antigen (CD68) (+) cell count was >50%.

Assessment was performed for CD68 (+) histiocytic macrophages at three levels: (1) E, (2) SE, and (3) D.

Statistical Analysis

The statistical package for SPSS (Version 16.0) was used for statistical evaluation. For all four subgroups; namely the nasal cavity, maxillary sinus, ethmoid sinus, and control, differences in CD68 (+) histiocytic macrophages between groups were analyzed by Kruskal–Wallis one-way analysis of

Fig. 1 CD68 histiocytic macrophage positivity in epithelial, subepithelial, and deep layers of the ethmoid sinus, maxillary sinus, nasal cavity, and control group specimens.

variance. A p -value of <0.05 was considered statistically significant.

Statistically significant results were further analyzed using the Mann–Whitney U test with Bonferroni correction for *post hoc* pairwise comparisons. An adjusted p -value of <0.0125 was considered statistically significant.

In each of the subgroups (nasal cavity, maxillary sinus, ethmoid sinus, and control), the difference between E, SE, and D values for CD68 (+) histiocytic macrophages were analyzed by the Kruskal–Wallis one-way analysis of variance. A p -value of <0.05 was considered statistically significant.

Statistically significant results were further analyzed using the Mann–Whitney U test with Bonferroni correction for *post hoc* pairwise comparisons. A p -value of <0.0175 was considered statistically significant.

Results

CD68 histiocytic macrophage positivity in the E, SE, and D layers of the ethmoid sinus, maxillary sinus, and nasal cavity samples obtained from the study group and inferior turbinate samples obtained from the control group is presented as mean $\pm$ standard deviation in ►Table 1 and ►Fig. 1.

The differences between CD68 histiocytic macrophage positivity, in each of the three layers (E, SE, and D) of four subgroups of samples (ethmoid sinus, maxillary sinus, nasal

Table 1 Levels of CD68 positive histiocytic macrophages (scored from 0 to 3, as described in the text) in the ethmoid sinus, maxillary sinus, and nasal cavity specimens of the study group, and inferior turbinate specimens of the control group

	Groups								
	Control		Nasal cavity		Maxillary sinus		Ethmoid sinus		
CD68 positivity	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	<i>p</i> -Value ^a
Epithelial	0.18	0.40	0.60	0.51	0.30	0.48	0.62	0.61	0.120
Subepithelial	0.77	0.83	1.90	0.99	1.10	0.73	1.75	0.68	0.015
Deep	0.50	0.84	2.00	0.94	2.00	1.05	1.62	0.95	0.007
<i>p</i> -Value ^a	0.194		0.003		0.001		<0.001		

^a p -values shows the results of Kruskal–Wallis one-way analysis of variance.

Table 2 Pairwise comparisons for the ethmoid sinus, maxillary sinus, and nasal cavity specimens of the study group and inferior turbinate specimens of the control group, in subepithelial and deep layers, calculated using the Mann–Whitney U Test with Bonferroni correction

CD68 positivity	Ethmoid sinus-maxillary sinus		Ethmoid sinus-nasal cavity		Ethmoid sinus-control		Maxillary sinus-nasal cavity		Maxillary sinus-control		Nasal cavity-control	
	z	p-Value	z	p-Value	z	p-Value	z	p-Value	z	p-Value	z	p-Value
Subepithelial	−2.002	0.045	−0.623	0.533	−2.586	0.010	−1.902	0.057	−0.915	0.360	−2.285	0.022
Deep	−0.989	0.322	−1.056	0.291	−2.669	0.008	−0.080	0.936	−2.811	0.005	−2.883	0.004

cavity, and control tissue) were analyzed by the Kruskal–Wallis one-way analysis of variance (►Table 1). Statistically significant differences were found in the SE ($p = 0.015$) and D layers ($p = 0.007$; ►Table 1). To find the values which caused a difference, pairwise comparisons were performed using the Mann–Whitney U test with Bonferroni correction (►Table 2):

The CD68 positivity for the ethmoid sinus-SE (mean: 1.75 ± 0.68) was significantly higher than that for the control-SE (mean: 0.77 ± 0.83 ; $p = 0.010$).

The CD68 positivity for the ethmoid sinus-D (mean: 1.62 ± 0.95) was significantly higher than that for the control-D (mean: 0.50 ± 0.84 ; $p = 0.008$).

The CD68 positivity for the maxillary sinus-D (mean: 2.00 ± 1.05) was significantly higher than that for the control-D (mean: 0.50 ± 0.84 ; $p = 0.005$).

The CD68 positivity for the nasal cavity-D (mean: 2.00 ± 0.94) was significantly higher than that for the control-D (mean: 0.50 ± 0.84 ; $p = 0.004$).

The intragroup comparisons in each subgroup of samples (ethmoid sinus, maxillary sinus, nasal cavity, and control group), for the differences in CD68 positivity between the E, SE, and D layers were analyzed by the Kruskal–Wallis one way analysis of variance (►Table 1).

The differences were statistically significant in the ethmoid sinus ($p < 0.001$), maxillary sinus ($p = 0.001$), and nasal cavity ($p = 0.003$; ►Table 1).

►Table 3 shows the results of *post hoc* pairwise comparisons performed using the Mann–Whitney U test with Bonferroni correction (►Table 3).

For the Ethmoid Sinus

The CD68 positivity for the SE layer (mean: 1.75 ± 0.68) was significantly higher than that for the E layer (mean: 0.62 ± 0.61 ; $p < 0.001$).

The CD68 positivity for the D layer (mean: 1.62 ± 0.95) was significantly higher than that for the E layer (mean: 0.62 ± 0.61 ; $p = 0.003$).

For the Maxillary Sinus

The CD68 positivity for the SE layer (mean: 1.10 ± 0.73) was significantly higher than that for the E layer (mean: 0.30 ± 0.48 ; $p = 0.015$).

The CD68 positivity for the D layer (mean: 2.00 ± 1.05) was significantly higher than that for the E layer (mean: 0.30 ± 0.48 ; $p = 0.001$).

Correlation Tests

In the study group, the correlations between patient age, polyp duration, Brinkman Index, and CD68 positivity were analyzed separately in the ethmoid sinus, maxillary sinus, and nasal cavity samples by the Pearson Correlation test. Correlations between gender, smoking, and CD68 positivity were analyzed separately in the ethmoid sinus, maxillary sinus, and nasal cavity samples using the Spearman's rho correlation coefficient. With increasing age of the polyps, CD68 (+) histiocytic macrophages decreased in the deep layer of the mucosa in the maxillary sinus and nasal cavity ($p = 0.012$, $r = -0.864$). In patients who smoked, CD68 (+) histiocytic macrophages increased in the maxillary sinus and nasal cavity. In males, CD68 (+) histiocytic macrophages increased in the nasal cavity. CD68 (+) histiocytic macrophages decreased with increasing age of the subjects (►Table 4).

Histopathologic Findings

Light microscopic examination revealed a high number of CD68 (+) histiocytic macrophages, mainly localized in the deep layer of the mucosa in the ethmoid sinus, maxillary sinus, and nasal cavity (►Fig. 2). In ethmoid sinus samples, macrophages were also located at the subepithelial layer.

Table 3 Pairwise comparisons of the epithelial, subepithelial, and deep layers in the ethmoid sinus, maxillary sinus, and nasal cavity by Mann–Whitney U test with Bonferroni correction

	Ethmoid sinus		Maxillary sinus		Nasal cavity	
	z	p-Value	z	p-Value	z	p-Value
Epithelial-subepithelial	−3.768	<0.001	−2.428	0.015	−1.352	0.176
Epithelial-deep	−2.979	0.003	−3.207	0.001	0.000	1.000
Subepithelial-deep	−0.323	0.747	−2.009	0.045	−1.314	0.189

Fig. 2 Immunohistochemical staining of a polyp sample demonstrating that CD68 (+) histiocytic macrophages are mainly localized in the deep layer of the lamina propria ($\times 100$).

Fig. 4 Immunohistochemical staining of the polyp sample demonstrating that CD68 (+) histiocytic macrophages tended to gather around eosinophils ($\times 200$).

Fig. 3 Immunohistochemical staining of the polyp pedicle demonstrating the presence of thick-walled blood vessels surrounded by CD68 (+) histiocytic macrophage accumulation ($\times 100$).

In most cases, CD68 (+) histiocytic macrophages were observed in edematous and highly vascularized areas. In one case, thick-walled blood vessels, surrounded by a dense histiocyte accumulation were seen at the pedicle of the polyp (**Fig. 3**). Additionally, histiocytic macrophages tended to gather around eosinophils in the deep layers of lamina propria (**Fig. 4**).

Discussion

Nasal polyposis is a multifactorial disease (3) and represents the final stage of chronic nasal inflammation.¹¹ NP patients with or without chronic rhinosinusitis has been reported to have significantly increased levels of CD3 +, CD4 +, and CD8+ T cells, as well as B cells positive for CD20 antigen and CD68-positive macrophages.¹⁸

The pattern of immune cell infiltration is different in nonallergic and allergic patients who have chronic rhinosinusitis with NPs. Nonallergic patients have fewer CD4 (+) cells in the epithelium and more CD8 (+) cells in the lamina propria compared with that of patients with allergies. The number of macrophages is also increased in NPs. These cells are immune cells derived from monocytes¹⁹ and have enhanced mannose receptor expression, which enables phagocytosis and signal transduction for pro-inflammatory mechanisms.²⁰ Macrophages participate in inflammation and adaptive immunity, promote cell proliferation by producing growth factors and products of the arginase pathway, scavenge debris by expressing scavenger receptors, promote angiogenesis and tissue remodeling and repair, suppress adaptive immunity, and act as tumor promoters.¹⁹ Patients with NP alone had higher numbers of CD68 (+) macrophages in the periglacial areas of both polyp tissue and bronchial mucosa.²¹

In vitro studies have shown that apoptosis of eosinophils is activated in a sequential manner²² and that eosinophils are phagocytosed as intact cells by macrophages.²³ Phagocytosis of eosinophils by macrophages in humans have recently been described in an *in vivo* study in allergen-induced cutaneous late-phase reactions.²⁴ In a different study, it was demonstrated that eosinophils were phagocytosed by macrophage-like cells (CD68 [+]) cells within the polypoid tissue.¹⁸

In the present study, CD68 positivity in the ethmoid sinus-SE and ethmoid sinus-deep were significantly higher than that in the control-SE and control-deep, respectively. CD68 positivity in the maxillary sinus-deep and nasal cavity-deep were significantly higher than that in the control-deep. CD68 positivity in the epithelial, SE and deep layers of the mucosa were significantly different between the ethmoid sinus, maxillary sinus, and nasal cavity. For the ethmoid sinus, CD68 positivity in the SE and deep layers was significantly higher than that in the epithelial layer. For the maxillary sinus, CD68 positivity in the SE and deep layers was significantly higher than that in the epithelial layer.

Table 4 Correlations between CD68 positivity and patient age, gender, polyp age, smoking status, and Brinkman Index in the ethmoid sinus, maxillary sinus, and nasal cavity samples

		CD68 positivity levels					
		Epithelial		SE		Deep	
		r	p-Value	r	p-Value	r	p-Value
Ethmoid sinus	Age ^a	0.460	0.073	−0.440	0.088	0.138	0.611
	Gender ^b	0.069	0.800	−0.407	0.118	−0.129	0.634
	Duration ^a	0.389	0.212	−0.194	0.545	0.169	0.599
	Smoking ^b	0.501	0.081	0.099	0.747	0.381	0.199
	Brinkman Index ^a	0.523	0.067	−0.084	0.784	0.379	0.202
Maxillary sinus	Age ^a	−0.689	0.028	−0.173	0.633	0.259	0.470
	Gender ^b						
	Duration ^a	−0.440	0.323	−0.094	0.842	−0.036	0.939
	Smoking ^b	0.167	0.721	0.000	1.000	−0.428	0.338
	Brinkman Index ^a	−0.196	0.674	−0.211	0.649	0.274	0.552
Nasal cavity	Age ^a	0.193	0.594	−0.248	0.490	−0.444	0.199
	Gender ^b	0.612	0.060	0.091	0.802	0.094	0.091
	Duration ^a	0.339	0.456	−0.503	0.250	−0.864	0.012
	Smoking ^b	−0.091	0.846	0.418	0.350	0.796	0.846
	Brinkman Index ^a	−0.269	0.560	0.513	0.239	0.269	0.560

^ap-value shows the results of the Pearson correlation test.^bp-value shows the results of the Spearman's rho correlation coefficient.

Our results showed that CD68 (+) histiocytic macrophages were mainly localized in the deep layer of the lamina propria in the ethmoid sinus, maxillary sinus, and nasal cavity. In the ethmoid sinus, macrophages were also localized in the subepithelial-layer. In most cases, CD68 (+) histiocytic macrophages were observed in the edematous and highly vascularized areas. In one case, thick-walled blood vessels surrounded by a dense histiocyte accumulation were seen at the pedicle of the polyp, and histiocytic macrophages had a tendency to gather around eosinophils also in the deep layers of lamina propria.

In the NP patient group of the present study, older polyps showed a decrease in CD68 (+) histiocytic macrophages at the deep layer of lamina propria in the maxillary sinus and nasal cavity. In older polyps over time, edema decreases and fibrosis increases. Along with this, the inflammatory cell count also decreases, including the number of macrophages, which are mainly the phagocytosing eosinophils. In patients who smoked, CD68 (+) histiocytic macrophages increased in the maxillary sinus and nasal cavity. This may be related to an increased inflammatory response to cigarette smoke, resulting in an increase in macrophages and phagocytosing eosinophils.

In males who were exposed to noisy machines and equipment in workplaces, CD68 (+) histiocytic macrophages increased in smokers and in nasal cavity polyps.²⁵ In older patients, the number of CD68 (+) histiocytic macrophages usually decreases. This may be related to decreased inflammatory and immune responses due to aging, since decreased inflammatory responses lead to decreased macrophage counts.

CD68-positive cells (macrophages) were identified in both the stroma and epithelium of the NPs. The number of scattered cells in the stroma ranged from 0 to 3 in a high-power field (HPF). The number of inflammatory cells was high, and the number of CD68-positive cells increased to 8 to 11 per HPF in the edematous stroma of the NP. Overall, the number of macrophages was significantly higher in the tissue of eosinophilic NPs than that of lymphocytic NPs.²⁰

In a study by Wu et al,²⁶ CD68 (+) cell expression was higher in eosinophil dominated NPs than in neutrophil dominated polyps. They concluded that inflammatory cell (eosinophils, CD45RO-, CD20-, and CD68-positive cells) infiltration and cell proliferation in epithelial cells, glandular cells, and fibroblasts were strongly correlated with NP formation. NPs are characterized not only by eosinophilia but also by the infiltration of lymphocytes, dominated by CD45RO- and CD68-positive cells. CD68-positive cells may be the stem cells of NP.

CD68 (+) histiocytic macrophages play important roles in various conditions. For example, prolactin derived from macrophages in the nasal mucosa may participate in the formation of NPs by local immune modulation.²⁷

Davidsson et al²⁸ have demonstrated that eosinophils are phagocytosed by macrophage-like cells (CD68 [±] cells) within the polypoid tissues. Macrophage-like cells were found in substantial numbers in most polyps. Further, a double-staining technique clearly showed the phagocytosis of eosinophils within the polyp tissue by the macrophage-like cells.²⁸

In the present study, histiocytic macrophages in the deep layers of lamina propria had a tendency to gather around

eosinophils. The high number of CD68 (+) histiocytic macrophages, mainly localized in the deep layer of lamina propria, may act in the phagocytosis of eosinophils within the polyp tissue. Whereas there were no subgroups based on neutrophilic dominant versus eosinophilia dominant, we did not categorize NPs as eosinophilic NPs and lymphocytic NPs. This is a limitation of our study and we plan to do this research in the future.

The high numbers of CD68 (+) histiocytic macrophages mainly located in deep layer of lamina propria may be responsible for the phagocytosis of eosinophils within the polyp tissue. Therefore, it may be concluded that increased macrophages in NPs do not trigger the growth of NPs. Instead, they may serve to reduce the number of eosinophils in already-developed NPs.

Our study showed that the numbers of CD68 (+) histiocytic macrophages were increased in NPs. CD68 antibody is an organelle-specific marker rather than a lineage-specific one. The large numbers of lysosomes that are present in the histiocytes account for CD68 positivity. In NPs, phagocytosis requires an intense lysosomal activity and an increase in the numbers of CD68 (+) histiocytic macrophages, which contain lysosomes. In the future, other mediators, which affect the number and distribution of CD68 (+) histiocytic macrophages, should be investigated to evaluate their effects on the pathogenesis of NPs. Such investigations would enable more detailed comments.

Authors' Contributions

N.B.M. supported in planning, data collection, statistical analysis, interpretation of results, literature survey, writing the manuscript, and submission. P.A., R.K., E.T.Y., and O.K.A. performed planning, data collection, and literature survey

Ethics Committee Approval

Kirikkale University Faculty of Medicine Local Ethics Committee (date: March 23, 2009, number: 2009/028).

Informed Consent

The polyp tissues were obtained retrospectively. Therefore, no informed consent were obtained.

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Conflict of Interest

None declared.

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