Correlation between the Eosinophil Colonic Mucosa Density and Severity of Ulcerative Colitis

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ABSTRACT

Background: *The inflammatory process of ulcerative colitis (UC) involves eosinophils. Eosinophils are not only related to the severity of UC but also to clinical improvement. The role of eosinophils in colonic mucosa has not been fully understood. This study aims to determine the correlation between the density of eosinophils in the colonic mucosa and the severity of ulcerative colitis.*

Method: *This is a prospective cross-sectional study with consecutive sampling between August 2020 and July 2021. The subjects were UC inpatients/outpatients at Dr. Sardjito Hospital who met the inclusion and exclusion criteria. The number and location of the biopsies are determined based on a colonoscopy performed by a Gastroenterology-Hepatology Consultant. An Anatomic Pathology Specialist examined the eosinophil colonic tissue. The severity of UC was classified using the Truelove Witts score. Data analysis using a computer program with a p-value < 0.05 was considered statistically significant.*

Results: *There are 3 UC severity categories according to the Truelove Witts score: 22 mild (45.8%), 9 moderate (18.8%), and 17 severe (35.4%). Seven participants (14.6%) had eosinophilia, with a median eosinophilia score of 203.33 cells/microL (0–1470 cells/microL). Five participants (10.4%) with colonic mucosal eosinophilia, with a median value of 14.13 cells/HPF (0–172 cells/HPF). The density of colonic mucosal eosinophils and the severity of UC had a moderate connection (r = 0.396; p = 0.005).*

Conclusion: *There is a significant correlation between the density of eosinophils in the colonic mucosa and the severity of UC.*

Keywords: *ulcerative colitis, colonic mucosal eosinophils, eosinophilia, Truelove Witts score*

ABSTRAK

Latar belakang: *Proses inflamasi kolitis ulseratif (KU) melibatkan eosinofil. Eosinofil tidak hanya berhubungan dengan keparahan KU tetapi juga berhubungan dengan perbaikan klinis. Sehingga peran eosinofil di mukosa kolon pada penderita KU belum sepenuhnya dipahami. Tujuan dari penelitian ini adalah untuk mengetahui korelasi antara kepadatan eosinofil di mukosa kolon dan keparah KU.*

Metode: *Penelitian ini adalah studi prospektif, cross sectional dengan consecutive sampling dalam kurun waktu Agustus 2020–Juli 2021. Subjek penelitian adalah penderita KU yang menjalani rawat inap atau rawat jalan di RSUP Dr. Sardjito yang memenuhi kriteria inklusi dan kriteria eksklusi. Jumlah biopsi dan lokasi pengambilan biopsi ditentukan berdasarkan kolonoskopi yang dilakukan oleh Konsultan Gastroentero-Hepatologi. Perhitungan eosinofil dalam jaringan mukosa kolon dilakukan oleh Spesialis Patologi Anatomi.*

Penilaian keparahan penyakit KU menggunakan skor Truelove Witts. Analisis menggunakan program komputer dengan nilai p < 0,05 dianggap bermakna secara statistik.

Hasil: *Terdapat tiga katergori keparahan berdasarkan skor Truelove Witts: keparahan ringan 22 orang (45,8 %), sedang 9 orang (18,8 %), dan berat 17 orang (35,4 %). Tujuh orang mengalami eosinofilia (14,6 %), median nilai eosinofilia adalah 203,33 sel/microL (0–1470 sel/micro L). Lima orang (10,4 %) mengalami eosinofilia mukosa kolon dengan median 14,13 sel/HPF (0–172 sel/HPF). Terdapat korelasi sedang antara kepadatan eosinofil mukosa kolon dengan keparahan KU (r = 0,396; p = 0,005).*

Simpulan: *Terdapat korelasi bermakna antara kepadatan eosinofil di mukosa kolon dan keparahan KU.*

Kata kunci: *kolitis ulseratif, eosinofil mukosa kolon, eosinofilia, skor Truelove Witts*

INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD). UC is an idiopathic chronic disease, mediated by immune processes in the body against the digestive tract.[1] The inflammatory process of the disease involves various types of inflammatory cells. The role of neutrophils in IBD, especially UC, has been confirmed, namely their role in active conditions.[2] In contrast with neutrophils in IBD activity, the role of eosinophils is still not fully understood.

Eosinophils are multifunctional leukocytes involved in the pathogenesis of various inflammatory processes.[3] The physiological mechanism of eosinophils in the gastrointestinal tract are maintenance of the barrier function of the gastrointestinal mucosa, provision of an immune response against pathogens in the intestinal lumen, interaction with the enteric nervous system, and linking natural and adaptive immune responses.[4] In the gastrointestinal tract, there is a role for eotaxin-1 modulating the accumulation of eosinophils in the gastrointestinal tract. Serum eotaxin-1 was reported increase in active IBD.[4,5] Active eosinophils will last longer, eosinophils will degranulate resulting in the release of cytotoxic granules and proinflammatory cytokines. Then active eosinophils will cause damage to enterocytes and colonocytes.[4] However, eosinophils can also cause repair effects.[6] The role of eosinophils in the tissue repair process is associated with the activation function of fibroblasts, in which fibroblasts act as the main target cells for healing and tissue remodeling.[7,8] This study aims to determine the correlation between eosinophil density in the colonic mucosa and the severity of UC.

METHOD

This research method is a prospective study using the cross-sectional method with consecutive sampling within one year (August 2020 to July 2021). The research subjects were UC sufferers who were inpatients or outpatients at Dr. Sardjito General Hospital and who met the inclusion and exclusion criteria. Inclusion criteria were: adult > 18 years old, diagnosed with UC based on clinical criteria, colonoscopy, and histological findings, complete medical record clinical data based on the case report form used, underwent colonoscopy at Dr. Sardjito General Hospital, and signed a research agreement. The exclusion criteria were: having comorbid malignancy (colorectal, gynecological), comorbid disease (tuberculosis/TB, human immunodeficiency virus/HIV, and autoimmune disease), and comorbid gastrointestinal infection (worms and fungi), and history of radiation.

Researchers assessed data on absolute eosinophil levels in peripheral blood and eosinophil density in the colonic mucosa. Eosinophilia is absolute eosinophils > 350 cells/microL in venous blood. The definition of colonic mucosal tissue eosinophilia is eosinophils > 25 eosinophils/HPF covering 0.2 mm^2 of tissue. The calculation of mucosal eosinophil density is the most eosinophil result when more than one tissue sample obtain. The severity of UC disease was classified using the Truelove Witts score and anatomical classification by Montreal criteria. The number and the location of taking the biopsies were determined based on a colonoscopy performed by a Gastroenterology-Hepatology Consultant. An Anatomic Pathology Specialist examined the eosinophil colonic tissue. Blood tests and colonoscopy were performed 3–-7 days after cessation of steroid therapy in patients on steroid therapy. Bivariate data analysis using Mann Whitney test, multivariate data analysis using Kruskal Wallis test, and correlation using Spearman's Rho test with a *p*-value < 0.05 was considered statistically significant. Statistical analysis using SPSS program. Research has obtained the research permit from the Research Ethics Committee of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada (Ref. Number: KE/FK/0974/EC/2020).

RESULTS

The research subjects were 48 people with a median age of 53.92 years (minimum 24 years; maximum 77 years), the distribution of men (26 people; 54.2%) more than women (22 people; 45.8%). The severity of UC was classified using Truelove Witts scores criteria. Twenty-two mild UC (45.8%), moderate UC 9 people (18.8%), and severe UC 17 people (35.4%). Most of the UC patients (52.1%) had pancolitis lesions. Seven patients have blood eosinophilia (14.6%) and five patients (10.4%) have tissue eosinophilia. The median peripheral blood eosinophil value was 203.33 cells/microL (0–1470 cells/microL). The median colonic mucosal eosinophil value was 14.13 cells/HPF (0–172 cells/HPF). The complete data are presented in Table 1.

Table 1. Subject characteristic

Subject characteristic	N (%)	Median (min–max)
Age		53.92 (24–77)
Sex		
- Male	26 (54.2)	
- Female	22 (45.8)	
UC severity		
- Mild	22 (45.8)	
- Moderate	9 (18.8)	
- Severe	17 (35.4)	
Lesion location		
- Proctitis	5 (10.4)	
- Left sided	18 (37.5)	
- Pancolitis	25 (52.1)	
Absolute eosinophil levels		203.33 (0–1470)
Blood eosinophilia (Absolute eosinophil levels > 350 cell/microL)	7 (14.6)	
Colonic mucosal eosinophil		14.13 (0–172)
Tissue eosinophilia (eosinophil > 25 cell/HPF)	5 (10.4)	

Table 2. Analysis of differences in blood absolute eosinophil levels and eosinophil density in the colonic mucosa based on the severity of ulcerative colitis

	UC severity (mean rank)			*P*
	Mild (22)	Moderate (9)	Severe (17)	
Absolute eosinophil levels	20.66	26.06	28.65	0.195*
	14.86	18.78	–	0.276**
	–	12.28	14.15	0.553**
	17.30	–	23.50	0.092**
Colonic mucosal eosinophils	18.57	27.61	30.53	0.022*
	14.14	20.56	–	0.073**
	–	12.06	14.26	0.483**
	15.93	–	25.26	0.011**

*) *Kruskal Wallis* test (for three groups); **) *Mann Whitney* (for two groups)

Table 3. Correlation between the density of colonic mucosal eosinophils and the severity of ulcerative colitis

	UC severity	
	r	*P**
Absolute eosinophil levels	0.262	0.072
Colonic mucosal eosinophils	0.396	0.005

*) Spearman's Rho test; significant p < 0.05

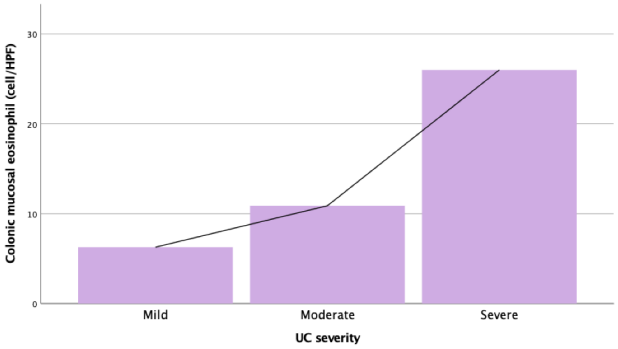

Figure 1. Correlation between the density of colonic mucosal eosinophils and the severity of ulcerative colitis

Analysis of differences in blood absolute eosinophil levels and eosinophil density in the colonic mucosa based on the severity of UC (Table 2) was analyzed using the Kruskal Wallis test (for three groups) and Mann Whitney (for two groups). There was a significant difference in the levels of colonic mucosal eosinophils based on the severity of UC ($p = 0.022$).

This study shows a correlation between the density of colonic mucosal eosinophils and the severity of UC. Table 3 shows Spearman's Rho correlation test with a correlation coefficient (r) of 0.396. This correlation is a moderate/moderate correlation which is statistically significant ($p = 0.005$).

DISCUSSION

The 48 patients who were the subjects of the study had a median age of 53.92 years old. The comparison of the number of male and female patients in this study was not much different, namely 26 people (54.2%) and 22 women (45.8%). The prevalence of UC in 2010–2014 at Dr. Saiful Anwar Hospital is 8.2%, 53.4% male vs. 46.6% female. The disease can occur at any age, but the incidence of onset according to age occurs in a bimodal pattern, with the first peak at age 15–25 years and the second peak at age 55–65 years old.[9] The prevalence of IBD in Asia is lower than the prevalence in Europe but the incidence in Asia is increasing rapidly. The prevalence of UC in Taiwan in 2015 was higher than Crohn's disease (CD). There are similar studies reported in Hong Kong, South Korea, and Japan.[10]

This study obtained an average blood eosinophil level in UC patients is 203.33 cells/microL. It found that 14.6% of UC patients had eosinophilia, absolute eosinophil levels > 350 cells/microL. These results showed a smaller proportion of eosinophilia compared to previous studies. The previous studies found that the proportion of IBD subjects who experienced an

increase in absolute eosinophils in the peripheral blood was 19.2%.[11] In the pediatric population study, the UC patients who had eosinophilia on blood tests was 27%.[12] The incidence of peripheral blood eosinophilia was more common in cases of pediatric-onset IBD than adult-onset IBD.[13]

Several previous studies have shown differences in the relationship between absolute blood eosinophil levels and the severity of IBD. In a retrospective study of 142 subjects, there was no difference in peripheral blood eosinophil levels in UC and CD patients and no relation with disease severity based on histology. Blood absolute eosinophils were not associated with disease recurrence and hospitalization rates.[14] However, other studies convey different results. In a large prospective cohort study (2,066 subjects), the incidence of peripheral blood eosinophilia in IBD patients was seen only in a minority of patients. However, it was associated with inflammation and severity of IBD patients followed over a multi-year period compared with patients without peripheral blood eosinophilia.[11] In another study with a population of children, peripheral blood eosinophilia was more common in UC than in CD (61.3% vs. 36.3%, $p < 0.05$). Significantly related to disease activity of UC and CD.[15] The incidence of peripheral blood eosinophilia was more common in cases of pediatric-onset IBD than adult-onset IBD.[13] This difference in results could be due to differences in study design or current medical therapies that are more effective, thereby controlling inflammation and reducing eosinophilic signaling. The lower prevalence of peripheral blood eosinophilia in adults may be related to age-mediated differences.[11]

Peripheral blood eosinophilia in IBD is associated with younger age, asthma, rheumatic disease, adrenal insufficiency, and primary sclerosing cholangitis.[11] Smoking can increase the incidence of UC and CD.[1,15] However, smoking status alone is not associated with peripheral blood eosinophilia in IBD.[11]

This study found that the average density of eosinophils in the mucosa of the colonic tissue in IBD patients was 14.13 eosinophils/HPF. It found that 10.4% of patients had colonic mucosal eosinophilia, namely with found eosinophils > 25 cells/HPF. These results are lower when compared with the results of previous studies. The results of previous studies showed an increase in eosinophils in the tissue by 50%.[14] In another study, with child subjects, the results showed an increase in eosinophils in the tissues by > 50% of the study subjects.[12] Differences in absolute eosinophil levels in the peripheral blood did not show significant differences based on the severity of UC. But significant differences in the density of colonic mucosal eosinophils based on the severity of UC.[16]

In this study, the mean rank of colonic mucosal eosinophil density increased based on the severity of UC. So, the researchers conducted additional analysis to determine the correlation between the two. The result showed a correlation between the density of colonic mucosal eosinophils and the severity of UC. Spearman's Rho correlation test between colonic mucosal eosinophil density and UC severity showed a correlation with a moderate/moderate strength relationship which was statistically significant ($r = 0.396$; $p = 0.005$).

Several previous studies have shown different results. In a 1987 study, a prospective study concluded that there was no correlation between tissue eosinophilia and the severity of UC.[17] However, a retrospective study involving the pediatric population found that tissue eosinophilia was associated with the severity of IBD ($p = 0.02$). The study also concluded that tissue and peripheral blood eosinophilia could serve as diagnostic markers for disease activity, severity, and short-term outcome in the pediatric population.[12] The existence of a correlation between colonic mucosal eosinophils and the severity of IBD patients is still widely debated. Colonic mucosal eosinophils and serum eotaxin-1 increase in IBD patients who recovered after vedolizumab therapy. They were associated with the reflection of accelerated eosinophilic trafficking to the intestine in treated IBD.[18] The colonic mucosa of UC patients during the remission period may show a leakage of the mucosal barrier and an increase in the number and activity of eosinophils compared to irritable colonic syndrome patients.[19]

The difference in these studies' results may base on the existence of several roles for eosinophils in the gastrointestinal tract. The role of eosinophils as a pro-inflammatory promotility agent can cause diarrhea, inflammation, tissue destruction, fibrosis, and stricture formation however it has a repair effect.[6] Eosinophils can communicate directly with T cells and mast cells in a two-way manner. Eosinophils activate T cells by acting as antigen-presenting cells and major basic protein (MBP) which trigger mast cells. The presence of a stimulus (in the form of tissue damage, viral/bacterial infection, allergen, allograft, or tumor) will stimulate the degranulation of eosinophils.[20] After recruitment into inflammatory tissues, eosinophils cause tissue damage by producing oxidative stress through EPO. Eosinophil peroxidase will damage the

architectural organization of the extracellular matrix, encouraging cell cytotoxicity.[8] Eosinophils play a role in intestinal barrier dysfunction and dysregulation of the immune system in the gastrointestinal tract.[4]

However, the opposite has been concluded in other studies. In IBD, a predominance of eosinophils can reduce the risk of recurrence and treatment.[14] The role of eosinophils in the tissue repair process is associated with the function of fibroblast activation. Fibroblasts are the primary target cells for tissue healing and remodeling.[7] Eosinophils directly promote fibrosis by releasing transforming growth factors β (TGF-β), IL4, and IL13. Indirectly, eosinophils can trigger fibrosis by stimulating epithelial cells in tissues via MBP or EPO to express pro-fibrotic mediators.[8]

In inflammatory conditions, the number of eosinophils increases. Morphological and immunohistochemical studies have revealed eosinophil activation in inflammatory diseases. However, eosinophil activity was higher during the inactive phase than during the active/inflammatory phase. These observations suggest that eosinophils may be involved in the resolution of inflammation and reparation of damaged intestinal tissue.[2] Other studies have reported that eosinophilia occurs more frequently in UC than in CD. Eosinophilia occurs more frequently in males and at a higher severity.[21]

Even though this study has obtained a significant moderate correlation, there are still differences with previous studies' results. For this reason, it is necessary to carry out further research with a larger sample size.

CONCLUSION

There is a significant correlation between the density of eosinophils in the colonic mucosa and the severity of UC. Multicenter follow-up research is needed by involving a larger number of research samples.

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