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Tumor Necrosis Factor-α, Fecal Calprotectin, and Disease Activity in Inflammatory Bowel Disease Patients

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Abstract

INTRODUCTION: Inflammatory bowel disease (IBD) has increased incidence and the lack of effective long-term therapeutic options has resulted in mortality from intestinal complications and also significant costs to the country's health-care system. Evaluation of disease activity in IBD patients is beneficial for establishing clinical judgments, especially in giving therapy and knowing the response of the given therapy. Tumor necrosis factor (TNF)-α, fecal calprotectin (FC), Lichtiger index, and Crohn's Disease Endoscopic Index of Severity (CDEIS) are useful tools for evaluating disease activity. This study wants to know the relationship between biomarkers with disease activity.

METHODS: A cross-sectional retrospective study was conducted on IBD patients. The diagnosis of IBD was based on findings from the gastrointestinal tract during colonoscopy and biopsy that showed features of IBD. TNF-α was taken from the patient's serum, while FC was from the patient's stool sample. Lichtiger index was taken from anamneses, while CDEIS from endoscopy. The data will be analyzed using SPSS 22.0.

RESULTS: A total of 35 patients with IBD met the inclusion criteria. This study found a 0.7-fold risk in IBD patients to have moderate-severe activity if they had TNF-α levels of ≥ 1.14 pg/mL, although it was not significant (p = 0.581; OR:0.68; 95%CI 0.18-2.66). A significant relationship was found in the value of FC with disease activity in IBD patients, with a six-fold risk of having IBD with moderate-to-severe activity if FC levels ≥254 μg/g (p=0,011, OR:6,24, 95%CI 1,44-27,06).

CONCLUSION: Fecal calprotectin levels have significant relationship with disease activity in IBD patients, both clinically and endoscopically. Fecal calprotectin is a decent marker for assessing disease activity in IBD patients.

Introduction

Inflammatory bowel disease (IBD) is an umbrella term for chronic or relapsing inflammatory diseases of the gastrointestinal tract, and it most commonly refers to ulcerative colitis (UC) and Crohn's disease (CD), both of which were first described in 1859 [1], [2]. The incidence and prevalence of IBD vary in different countries. In the 21st century, the incidence of IBD is globally increasing, in line with the development of industrialized countries such as Asia, South America, and Africa, as well as the western world [3].

Changes and injury of the mucosal lining wall in patients with IBD appear to be related to the result of chronic inflammation and increased intestinal permeability, due to various risk factors such as environment, genetics, medication, and resultant perturbations of the intestinal microbiota [4]. IBD causes substantial morbidity and productivity losses. The increasing incidence and prevalence of IBD, as well as the lack of effective long-term therapeutic options, have caused mortality from intestinal complications and significant costs to the country's health-care system [3], [5], [6].

Evaluation of disease activity in IBD patients is beneficial for establishing clinical judgments, especially in giving therapy and knowing the response of the given therapy. The assessment refers to the actual condition which includes clinical symptoms, endoscopy results, histopathology, biomarkers, and the quality of life of the patient. Tumor necrosis factor (TNF)-α is a proinflammatory cytokine that both maintain intestinal integrity and are a cause of intestinal inflammation. Meanwhile, fecal calprotectin (FC) is a protein granule secreted in feces by neutrophils that reflect IBD disease activity [7], [8], [9].

Assessment of IBD disease activity can also be done using a scoring system. The Lichtiger index is a scoring system to evaluate disease activity in UC patients that consists of eight assessment variables, while The CDEIS examines disease activity in CD patients from endoscopy [10], [11]. This study wants to know the relationship between biomarkers with disease activity.

Methods

This was a cross-sectional retrospective study of IBD patients conducted at the RSUP Prof. dr. R. D. Kandou Manado, from December 2021 to June 2022. A total of 35 patients, aged ≥18 years, were newly diagnosed with IBD. After a thorough explanation of the study, all patients provided informed consent. The health research ethics committee of RSUP Prof. Dr. R.D. Kandou Manado Hospital approved this study (126/EC/KEPK-Kandou/VIII/202).

Researchers collected identity, patient's anamneses, diagnosis, and TNF-a and fecal calprotectin from 35 patients. Patient severity is categorized as mild and moderate-severe. Patients with gastrointestinal disorders or autoimmune diseases other than IBD will be excluded from the study. The diagnosis of IBD was based on findings from the gastrointestinal tract during colonoscopy and biopsy that showed features of IBD. Patients that have been diagnosed with IBD were classified into CD and UC based on its key features. Patients with CD had transmural inflammation and presence of granulomas (histologic findings); discontinuous lesions, strictures, and linear ulcerations (endoscopic findings). Ulcerative colitis patients have mucosal, submucosal inflammation, and polymorphonuclear cells aggregate (histologic findings); and continuous lesions, presence of crypts, and formation of residual mucosal tissue (endoscopic findings) [12].

TNF-α was taken from the patient's serum and measured with the quantitative sandwich Enzyme-Linked Immunosorbent-Assay (ELISA) method using the kit from Quantikine® ELISA Human TNF-α Immunoassay R&D Systems USA. Fecal calprotectin examination is conducted by taking the patient's stool sample and measured using ELISA method with fCAL® ELISA kit, BÜHLMANN Labs. The cutoff of TNF-α and fecal calprotectin was determined from the median value in this study.

Lichtiger index is defined by eight variables, consisting of the frequency of daily bowel openings, night-time stools, blood in the stool, fecal incontinence, abdominal discomfort, overall well-being, abdominal tenderness, and the need for antidiarrheals [13]. In each of the following locations, CDEIS (Crohn's Disease Endoscopic Index of Severity) identifies four endoscopic parameters (the existence of deep ulceration, superficial ulceration, the length of ulcerated mucosa, and the length of diseased mucosa): the rectum, the sigmoid and the left colon, the transverse colon, the right colon, and the ileum [11], [14]. Patients with mild activity had a Lichtiger index of 4-8 or a CDEIS of 3-8. Meanwhile, IBD patients with moderate-severe activity have a Lichtiger index or CDEIS with a value of >8 [11], [13], [14].

The collected data will be analyzed using SPSS 22.0 (SPSS Inc., Chicago) with a confidence interval of 95%. Descriptive analysis was done using the univariate method to obtain the median, minimum, and maximum values. Chi-square analysis was used because there were no cells with an expected count <5, with $p < 0.05$.

Results

A total of 35 patients met the inclusion criteria, with a median age of 42 (18–81) years, with female patients more than male patients (57.14% vs. 42.86%). The group with patients aged 31–40 years is the largest age group (31.43%), followed by 41–50 years (25.71%) and 51–60 (14.29%). TNF-α in this study had a median of 1.14 (0.08–20.4) pg/mL and 20 people (57.14%) of them had TNF-α values ≥ 1.14 pg/mL. The median fecal calprotectin in this study was 254 (6.8–2100) μg/g and 18 people (51.43%) of them had fecal calprotectin values ≥ 254 μg/g (Table 1).

Table 1: Characteristics of research subjects (n=35)

Characteristics	Parameter	Data distribution (%)
Age (year)	Median	42
	Range (min-max)	18–81
Sex	Male	15 (42.86)
	Female	20 (57.14)
Diagnosis	UC	15 (42.86)
	CD	20 (57.14)
Disease Activity of IBD	Mild	17 (48.58)
	Moderate-severe	18 (51.42)
TNF-a (pg/mL)	Median	1.14
	Range (min-max)	0.08–20.40
Fecal calprotectin (μg/g)	Median	254
	Range (min-max)	6.80–2100.00

This study found a 0.7-fold risk in IBD patients to have moderate-severe activity if they had TNF-α levels of ≥1.14 pg/mL, although there was no significant relationship between TNF-α levels and disease activity of IBD patients (p = 0.581, OR: 0.68, 95%CI 0.18–2.66). A significant relationship was found in the value of FC with disease activity in IBD patients (p = 0.011, OR: 6.24, 95%CI 1.44–27.06). Patients with FC levels ≥254 μg/g have a six-fold risk of having IBD with moderate-to-severe activity (Table 2).

Table 2: Relationship of biomarker levels with IBD disease activity

Disease activity of IBD				
Biomarker	Moderate-severe	Mild	p	OR (95% CI)
	N-18	N-17		
TNF-a				
≥1,14 pg/ml	10	11	0.58	0.68 (0.18–2.66)
<1,14 pg/ml	8	6		
Fecal calprotectin				
≥254 μg/g	13	5	0.01	6,24 (1.44–27.06)
<254 μg/g	5	12		

Discussion

Inflammatory bowel disease is a chronic intestinal inflammatory disease, where environmental and genetic factors were believed to play important

role in the pathogenesis of this disease. More women were found in this study. This was consistent with the condition of IBD patients from studies in Colombia in 2010 and Spain in 2018, which found that women with IBD were found more often than men [15], [16], [17]. The median age of the study sample in this study was 42 years. A similar median age (42 years) was also found in a study by Rocio *et al*. in Spain [17].

In this study, patients with CD were found to be more than those with UC. It has similar results to the research of Siew *et al*. in African countries which show that CD had more prevalence and incidence compared with UC [3]. More IBD patients with moderate-severe activity were found in this study. Unlike the research by Wang *et al*. in 2007 in England, who found that patients with mild activity were found to be more common [18]. Wang's study involved outpatients with mild activity, whereas this study involved patients who were diagnosed for the first time, with various complaints, and had not received therapy.

TNF-α is a critical mediator of the inflammatory process. Under physiological conditions, activation and production of TNF-α result in inflammatory responses and apoptosis, and also help in the defense against infection and local cell damage. TNF-α tissue elevation in the mucosa and lamina propria of IBD patients, on the other hand, results in a distorted proinflammatory response associated with dysregulation of mucosal immune cells or tissue damage [19]. TNF-α levels in the intestinal mucosa are linked to disease activity, the magnitude of intestinal inflammation, and can predict IBD reoccurrence. TNF-α plays an important role in the pathogenesis of IBD so therapies that reduce TNF-α levels with anti-TNF are widely studied [20], [21].

No significant relationship was found between TNF-α levels and IBD patients' disease activity in this study ($p > 0.05$). Different findings were found in several other studies conducted by Olsen *et al*., Doszhan *et al*., and Rismo *et al*., which reported a significant relationship and difference between levels of TNF-α and disease activity in IBD patients who had received therapy. Another study was done on UC and CD patients who received anti-TNF therapy. These patients experienced decreased TNF-α levels so patients have an improvement in their disease activity. This condition is marked by the healing of the intestinal mucosa known from endoscopy or using an index of assessment. In addition, there is no cutoff value of TNF-α in IBD patients in Indonesia at this time.

Fecal calprotectin is a biomarker of neutrophil activation and is known to be one of the potential, reliable, and accurate non-invasive tests for IBD patients. Not only useful for assessing disease activity but FC can also assess prognosis, mucosal repair, histologic remission, and predict recurrence so FC examination is beneficial in monitoring therapy [22], [23]. The correlation coefficient for FC levels in UC patients is 0.51–0.83, while it is 0.48–0.73 in CD patients. The sensitivity and specificity of fecal calprotectin in IBD ranged from 81 to 91% and 58 to 100%, respectively. Fecal calprotectin levels also have higher sensitivity and specificity compared to other inflammatory markers from the blood such as CRP or stool-like lactoferrin [22], [24].

There was a significant relationship between FC levels and disease activity in IBD patients in this study ($p < 0.05$). Sipponen *et al*., D'Inca *et al*., and Schoepfer *et al*. also reported an association between FC levels with disease activity in IBD patients. Research by Rϕseth *et al*. showed that normal histological examination in IBD patients also had normal fecal calprotectin levels [25], [26], [27]. Pathological conditions in IBD cause inflammation of the intestinal mucosa and distortion of the mucosal permeability that will increase the migration of neutrophils and monocytes. Fecal calprotectin is an important inflammatory biomarker, produced by neutrophils and monocytes, which can induce migration, adhesion, and phagocytosis of neutrophils that are associated with tissue damage. The more severe inflammation and damage that occurs in the intestinal mucosa will cause more migration of neutrophils and monocytes so that levels of fecal calprotectin production will also increase [28], [29].

The limitation of this study is the measurement of TNF-α and fecal calprotectin levels which were not carried out in serial examination when the patient was first diagnosed and after the patient received treatment. The degree of inflammation and disease activity in IBD patients can also be affected by the duration of the disease in patients, but disease duration was not a concern in this study. The absence of a cutoff value of TNF-α and fecal calprotectin was also a confounding factor in this study.

Conclusion

Fecal calprotectin levels have significant relationship with disease activity in IBD patients. Fecal calprotectin is a non-invasive, inexpensive, and efficient parameter for assessing disease activity in IBD patients, both clinically and endoscopically. The correlation between fecal calprotectin and the disease activity allows an easier assessment of the disease activity; therefore, it has the potential to replace colonoscopy for the serial assessment of the gastrointestinal tract condition in IBD patients.

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