Research Article

Fecal Calprotectin Associated with Spondyloarthritis Disease Activity

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ABSTRACT
Background: Fecal calprotectin (FC) is one of fecal biomarkers that has validation to distinguish the organic and functional bowel disease but the relationship with disease activity in Spondyloarthritis (SpA) is still questionable. We determined the correlation of FC to the degree of disease activity on SpA.
Material and Method: This cross sectional analytic study involved thirty-two subjects diagnosed with SpA. FC levels were analyzed using ELISA method. The Spondyloarthritis disease activities were assessed by ASDAS- CRP scores.
Results: The median age was 52.5 (23.0-71.0) years with the average ASDAS-CRP score of 2.53 ± 0.61. The median level of FC increased by 58.15 (5.20-269.20) µg/g and was found to be positive (≥50 µg/g) in 56.3% subjects. FC levels was significantly correlated with the degree of SpA activity according to ASDAS-CRP scores ($r = 0.563$; $P = 0.001$)
Conclusion: Fecal Calprotectin associated with the degree of activity in the SpA measured by ASDAS-CRP scores.

Keywords: Spondyloarthritis, Fecal Calprotectin, Disease activity, ASDAS-CRP

INTRODUCTION

The intestine is an organ with a complex immune system. The intestinal lumen consist of trillions of microbes, food protein that can potentially become allergens. The intestinal defense system includes the mucosal barrier, epithelial layer, natural, and adaptive immune system. In normal condition, the tolerance and immunity to non-self-antigen stays in equilibrium. However, when immune system dysregulation occurs, especially in genetically susceptible individuals, bacterial dysbiosis can trigger autoimmune reactions, including spondyloarthritis (SpA) [1–3].

Progressive SpA can cause morbidity in the form of motion limitation, pain, and depression, increased risk of fracture, radiculopathy and decreased quality of life. SpA patients also have a mortality rate 1.5 times higher than the normal population.[4–8] Other studies presented that chronic arthritis is associated with other diseases, including Lyme disease and risk factor for TB [9–12].

The relationship between the intestines and joints or commonly called the gut-joint axis has been proven over the past few decades. Some studies explored intestinal inflammation with physical exercise [13]. Besides, according to a prospective study, intestinal inflammation is independently associated with edema in the sacroiliac joint, higher progression to the US, and conversion to Crohn's disease. However, the evidence that intestinal inflammation is the initiator and systemic and local inflammatory modulator that influences the severity of SpA is still inconclusive and controversial [1,2,7,14].

Inflammation is a response to eliminate various pathogens and preserve host integrity [15]. Intestinal inflammation in SpA is mostly subclinical, asymptomatic and difficult to detect clinically but appears macroscopically and microscopically through biopsy colonoscopy. However, this examination is invasive and expensive. Pro-inflammatory action increases when there is infection [16]. Fecal Calprotectin (FC) is a biomarker that has high sensitivity and specificity to assess intestinal inflammation so that it has the potential as a modality for inflammatory bowel disease (IBD) screening purpose. Similar research is expected to be a consideration for therapeutic decision making with targets, as well as gut-joint at an early stage to prevent further progression [14,17]. We determined the correlation of FC to the degree of disease activity on SpA.

MATERIALS AND METHODS

This analytic observational study used cross-sectional design and conducted at Rheumatology Outpatient Rheumatology Clinic at dr. Soetomo General Tertiary Hospital in Surabaya. The population was all axial or peripheral SpA patients who came to this clinic.

The sample was thirty-two SpA consecutive patients who were classified based on ASAS criteria for axial or peripheral SpA type. The inclusion criteria were men or women aged ≥ 18 years who were willing to join the study and signed the informed consent form. The patients with previously diagnosed with proven IBD (Crohn's disease or Ulcerative Colitis), colorectal cancer, gastric cancer, infected gastroenteritis, Gastro-esophageal disease (GERD) and hepatic cirrhosis were excluded from study. This study was approved by local ethical committee of dr. Soetomo Hospital. All subjects underwent the same examination, including history taking, physical examination, and laboratory data. The SpA disease activity was assessed with Ankylosing Spondylitis Disease Activity Score with CRP (ASDAS-CRP) score, involving subjective assessment, such as peripheral pain/swelling, duration of morning stiffness, back pain patient global assessment of disease activity, and objective measurement (CRP).

Sample Collection and Assay

Stool preparations were taken by the patients or laboratory employees. The minimal amount of 15 mg stool was analyzed by PhiCal © enzyme- linked Calprotectin immunosorbent assay (ELISA) kit (Immunodiagnostic AG, Stubenwald-Allee 8a, D-64625 Bensheim) in Prodia Laboratory, Surabaya. Calprotectin was stable in stool for 6 days, but it is recommended that it is stored for 48 hours at a temperature of 2-8ºC or more than 48 hours at -20ºC with a normal reference value <50 μg /g. Before the examination performed, the Non-Steroid Anti-Inflammatory Drug (NSAID) was previously stopped for 2 days according to the kit manufacturer's instructions. The ASDAS- CRP scores of subjects was classified into four degrees, inactive disease state (score 0-1.2), moderate disease activity (score 1.3-2.0), high disease activity (score 2.1-3.4), and very high disease activity (score >3.5).

Statistical analysis

All data collected in the data collection sheet was arranged in table form and processed statistically using the SPSS 21.0 program. The data distribution was analyzed by the Shapiro-Wilk normality test. Association between FC with the degree of SpA activity was analyzed using the Pearson correlation test if the data were normally distributed or the non-parametric Spearman correlation test if the data were not normally distributed. Strength of relationships was based on correlation coefficients.

RESULTS

The observation results from thirty-two subjects including 12 male (37.5%) and 20 (62.5%) female obtained the subject characteristics summarized in Table 1. The patients' age in this study was quite heterogeneous with an average of 51.78 ± 12.68 years with a minimum age of 23 years and a maximum of 71 years. The SpA type was dominated by axial types of 22 subjects (68.75%), and the rest were peripheral types of 10 subjects (31.25%). The median of SpA onset was 2.5 years (0.25-18) years.

According to the investigation, the mean value of LED was 46.00 ± 26.53 mm/hour, and the median CRP was 3.50 with the minimum and maximum value of 1.00-25.00 mg/L.

According to observations, Table 2 shows that the FC levels in the study subjects are described as having a median of 58.15 μg/g. The minimum value was 5.2 μg/g, and maximum was 269.2 μg/g. As shown in Figure 1, positive results of FC (levels ≥50 μg/g) are obtained in 18 persons (56.3%), while negative results of FC (<50 μg/g) are obtained in 14 persons (43.80%). In the two axial and peripheral SpA groups seen in Table 2, FC levels in 22 axial SpA types have a higher median of 79.70 (5.20-269.2) μg/g than 10 peripheral SpA types with levels of 36.65 (5.20-105.20).

The degree of SpA activity as shown in Table 3 is measured by ASDAS-CRP. The mean ASDAS-CRP scores was 2.53 ± 0.61. The ASDAS-CRP scores of subjects in moderate disease activity was 8 subjects (25%), high disease activity was 23 subjects (71.87%), very high disease activity was 1 subject (3%), and no subjects with inactive disease state.

To find out the correlation between FC and disease activity, the ASDAS-CRP score was used. Because FC data were not evenly distributed, a non-parametric correlation test from Spearman was conducted with the results as shown in Table 4 and Figure 1, with r value of 0.563 (P = 0.001) and linearity of 0.006. It showed a statistically significant and linear correlation between FC and ASDAS-CRP with medium correlation strength.

DISCUSSION

Findings of this study revealed that FC levels were positively correlated with disease activity of SpA according to ASDAS-CRP score with moderate strength. The positive results of FC levels affected the high disease activity of ASDAS-CRP scores. This result was consistent with a new study which examined 130 persons with axial SpA. Each patient was divided into four groups according to the ASDAS-CRP scale, and a significant difference from the whole between groups and third compared between groups of patients with positive FC (≥50 mg/kg) and negative FC (<50 mg/kg) was found which showed a significant difference only in global VAS, BASFI index,

Table 1: Demographic and clinical characteristic of subjects

Characteristic	Frequency (%)	Mean ± SD	Median (min-max)
Sex			
Male	12 (37.50)		
Female	20 (62.50)		
Age			52.50 (23.00-71.00)
SpA Type			
Axial SpA	22 (68.75)		
Peripheral SpA	10 (31.25)		
LED (mm/jam)		46.00 ± 26.53	
CRP (mg/L)			3.50 (1.00-25.00)
SpA onset (year)			2.50 (0.25-18.00)

Table 2: The Fecal Calprotectin level of subjects

Characteristic	Frequency (%)	Median (min-max)
FC levels (μg/g)		58.15 (5.20-269.20)
Positive FC (≥50 μg/g)	18 (56.30%)	
Negative FC (<50 μg/g)	14 (43.80%)	
FC levels in axial SpA (μg/g)		79.70 (5.20-269.2)
FC levels in peripheral SpA (μg/g)		36.65 (5.20-105.20)

Table 3: Degree of spondyloarthritis disease activity of research subjects

ASDAS-CRP	Frequency (%)
Mean±SD	2.53 ± 0.61
Inactive disease	0 (0)
Moderate disease activity	8 (25.00)
High disease activity	23 (71.87)
Very High disease activity	1 (3.00)

Table 4: The correlation of fecal calprotectin level with degree of SpA disease activity according to ASDAS-CRP score

	ASDAS-CRP		
	r Spearman	p-value	Linearity test
Fecal Calprotectin	0.563	0.001	0.006

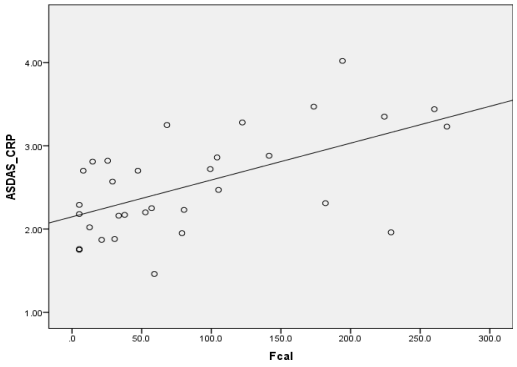

Fig.1: The composition graph between positive result and negative result group of fecal calprotectin in research subjects.

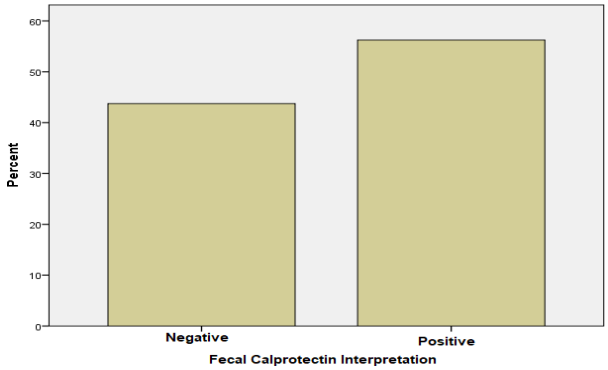

Fig.2: The scatter plot graph of correlation between fecal calprotectin levels and Spondyloarthritis disease activity according to ASDAS-CRP scores.

and ASDAS-CRP. There was no significant difference with the BASDAI index.[18] Different results were indicated by small-scale studies that reported no significant differences in the BASDAI and ASDAS indices in the group of SpA patients with FC <50 μg/g and FC> 100 μg/g [19].

The link between bowel lesions and joint abnormalities has been known for the past three decades. Several animal experiments have shown that transgenic mice with HLA-B27 stimulated by the administration of nutrients that contain bacteria cause spontaneous and multisystem inflammatory disorders, such as bowel inflammation, peripheral arthritis, and spine. The exact pathogenesis is still unclear but generally it can be caused by genetic vulnerability in this case the presence of HLA-B27, which is influenced by environmental factors, such as dysbiosis or imbalance of the intestinal bacterial ecosystem. HLA-B27 itself is a MHC class 1 molecule which functions to present antigenic, both self and non- self. HLA-B27 is strongly associated with SpA because it is involved in the aberrant antigen presentation process, clearance of intracellular bacteria, and the process of misfolding the heavy chain immunogenic homodimers [20–22].

There is what is known as the "leaky gut" concept in SpA, which is increased intestinal permeability due to the direct effect of bacterial toxins on zonula occluden, increased zonulin (prehaptoglobin-2) related to HLA-B27 and the inflammatory process of several signaling pathways, such as NFκβ, MAP kinase and inflammasome, which affects the tight junction protein [7,23,24].

Infiltration of bacteria in the lamina propria will activate the innate immune response. Macrophages and neutrophils will activate signaling pathways, epithelial proliferation and differentiation, and increased epithelial permeability. Dysbiosis also stimulates Pannteh Cells to secrete IL-17 and IL-23 cytokines as a differentiation from Innate Limphoid Cells-3 (ILC-3) which further secretes pro-inflammatory cytokines IL-22, IL-17 which together with TNF alpha and IFN gamma cause intestinal inflammation. The Gut Iteropathy concept is one hypothesis that can explain the relationship of inflammatory bowel and joints to SpA. In general, normal T cells that have been exposed to antigens in the peer's patch will recirculate to systemic to function as immune surveillance. T cells have homing capacity to return to their original place of differentiation. However, in SpA, T cells are pathogenic, extravasation in synovial high endothelial Venule (HEV) through molecules and adhesive ligands, namely Vascular adhesion protein1 (VAP1), CD18-ICAM-1, α4β7/α4β1-integrin-VCAM-1, L-selectin- peripheral lymph node addressins, and CD44. Aberrant homing is stronger than intestinal α4β7-MAdCAM-1 interactions. In addition, macrophages involved in intestinal inflammation can also be directly attached to synovial HEV through PSGL-138 and P-Selectin adhesion molecules [25–28].

Calprotectin is a calcium binding protein contained in the cytosol of phagocytic cells (macrophages, monocytes) and intestinal epithelium having antimicrobial activity by binding to calcium as one of the ingredients needed by microbes. Calprotectin which is involved in intestinal inflammation will be attached to the stool homogeneously and is one of the recommended biomarkers of intestinal inflammation [14,24,29,30].

Some limitations exist in this study. FC levels were not compared with controls (healthy subjects). Non-steroidal anti-inflammatory drugs (NSAIDs) are confounding factors that are difficult to control. In the study, at the time of FC examination, the subjects stopped taking NSAIDs for at least 48 hours without an analysis of the duration and frequency of NSAID use. In this study, there were no endoscopic/colonoscopy biopsy, complete fecal examination, fecal culture to exclude other factors causing FC increase, such as IBD, infection, malignancy, GERD. This study was conducted with a cross sectional design, so the dynamics of FC levels could not be evaluated with different degrees of activity conditions, such as after obtaining certain treatments.

CONCLUSION

The results of this study revealed that fecal calprotectin were positively correlated with disease activity of SpA according to ASDAS-CRP score. This findings supported the gut-joint involvement in SpA that might the future treatment can target both for better outcome.

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