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Original Article

Fecal calprotectin and its association with functional dyspepsia in children

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Abstract

Background Calprotectin is a calcium-binding protein found normally in small amounts within the digestive tract. Fecal calprotectin measurement is used as a biomarker to identify digestive tract inflammation. Functional dyspepsia is one of the most common health issues in children, occurring in 3-27%, and accounts for considerable quality of life impairment and health care expenses.

Objective To determine fecal calprotectin concentration in generally healthy children as well as to assess for a possible association between fecal calprotectin and functional dyspepsia.

Methods This cross-sectional study was conducted from February to April 2019 in primary school-aged children in Manado, North Sulawesi. Subjects consisted of 38 children aged 6-12 years. Fecal calprotectin was measured in subjects' stool specimens, and considered to be normal if fecal calprotectin concentration of < 50 μg/g. Diagnosis of functional dyspepsia was defined using the parent-filled Rome IV questionnaire form. Data were analyzed with Chi-square and Phi-coefficient correlation tests.

Results Thirty-eight subjects, 22 boys and 16 girls, were grouped according to fecal calprotectin concentration (normal *vs.* elevated) and functional dyspepsia diagnosis. Mean fecal calprotectin concentration was 312.45 μg/g in the functional dyspepsia group and 20.89 μg/g in the healthy group. Elevated fecal calprotectin was found in 55.6% of the functional dyspepsia group and 10.3% of the healthy group. There was a positive correlation between fecal calprotectin elevation and functional dyspepsia (r=0.471; P=0.004).

Conclusion Current fecal calprotectin physiological cut-off level of 50 μg/g seems valid for children aged 6-12 years. Elevated fecal calprotectin is associated with functional dyspepsia in children. **[Paediatr Indones. 2020;60:71-5; doi: http://dx.doi.org/10.14238/pi60.2.2020.71-5**].

Keywords: *fecal calprotectin; immune system; functional dyspepsia*

Calprotectin is a 36 kDa calcium-binding heterocomplex protein consisting of two heavy chains and one light chain. It belongs to the S-100 protein family and is derived predominantly from neutrophils and monocytes.[1] Being resistant to enzymatic degradation, it can easily be measured in stool with a commercially available ELISA immunoassay. Due to its high sensitivity and specificity, relative simplicity, quick turnaround time, and long stability at room temperature (up to 7 days), it has been used increasingly in the diagnostic process for inflammatory bowel disease (IBD).[2] However, this test is often underappreciated as an immune defense mechanism for intestinal mucosal tissue, as it also has anti-microbial and anti-proliferative activities.[3-5] Calprotectin (*S100A8/S100A9*) has a pro-inflammatory role in innate immunity and is characterized as a damage-associated molecular

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pattern molecule (DAMP), due to its release by activated or damaged cells under conditions of cellular stress. An emerging concept of pattern recognition involves sensing of exogenous pathogen-associated molecular patterns (PAMPs) and endogenous DAMPs via the multi-ligand receptor for advanced glycation end products (RAGE) and toll-like receptors (TLRs), enabling innate immunity to achieve our primary host defense against invading microorganisms and non-specific stress factors.[6]

In accordance with their pro-inflammatory role, calprotectin is significantly overexpressed at sites of inflammation, and there is a strong correlation between its concentration and inflammation.[6] The secretion of calprotectin by phagocytes is induced when phagocytes come into contact with inflamed endothelium. Calprotectin is thought to promote inflammation via induction of pro-inflammatory chemokines, adhesion molecules (e.g., VCAM-1 and ICAM-1) and β2-integrin, thereby mediating leukocyte recruitment, adhesion, and transendothelial migration to inflamed tissue.[7] In previous studies, fecal calprotectin was shown to be a reliable predictor of mucosal healing in patients with IBD.[8,9] This further confirms the role of fecal calprotectin in the innate immune response of intestinal mucosal tissue.

Fecal calprotectin concentrations in healthy individuals have been established in several studies. In the original study, the median stool calprotectin concentration in healthy adults was 2 mg/L, and the suggested cut-off for a positive test was 10 mg/L.[10] In the newer assay, the suggested upper limit of normal was increased by a factor of five, to 50 μg/g.[11] The pediatric reference range for fecal calprotectin was established in a study of 117 healthy children (median 13.6 μg/g; 95%CI 9.9 to 19.5 μg/g). The adult cut-off level for intestinal inflammation of 50 μg/g was suggested to apply to children as well.[12]

Functional dyspepsia presents with various signs and symptoms, which are often concerning. Functional dyspepsia occurs in 3-27% of children and accounts for a considerable impact on quality of life and health care costs. It is defined by the Rome IV classification as persistent upper abdominal pain or discomfort, not related to bowel movements, and without an organic cause, that is present for at least 2 months prior to diagnosis.[13] However, these manifestations have no organic cause and require extensive diagnostics based on clinical criteria, results of physical examination, and key diagnostic studies. In practice, diagnostics of functional disorders are frequently based on exclusion of organic causes, which implies a more or less invasive approach including radiological and endoscopic studies.[14] In children, diagnosis of functional dyspepsia can be made using the validated Rome-IV questionnaire. This type of questionnaire is preferred as rapid diagnosis can be obtained without any invasive testing needed.[15]

This study aimed to determine fecal calprotectin concentration in generally healthy children as well as to assess for a possible association between fecal calprotectin and functional dyspepsia.

Methods

Children aged 6-12 years were recruited between February and April 2019. One elementary school in Manado (#30), North Sulawesi, was chosen by simple random sampling. The minimum required sample size of 38 subjects was determined with statistical power. A total of 38 subjects were collected by consecutive sampling. Inclusion criteria were children aged 6-12 years who attended elementary school #30 in Manado. Exclusion criteria were children with any medical condition which prohibited them from attending class, other known gastrointestinal and digestive system disorders, malnutrition status (body mass index < 15), taking any anti-inflammatory medication (including NSAID and corticosteroids) within the last 7 days, and children whose parents did not consent for stool collection and filling the questionnaire.

Stool specimens were collected using a spatula/stool collector and placed in clean stool containers. Specimens were sent to *ISO-9001* certified laboratory within two hours of collection. During transport, direct sunlight and sudden change in temperature were avoided. In the laboratory, fecal calprotectin was measured using an enzyme-linked immunosorbent assay/ELISA (*fCAL*® ELISA kit, BÜHLMANN Labs). Fecal calprotectin concentration < 50 μg/g were considered to be normal, and > 50 μg/g considered to be elevated.

All 38 subjects' parents filled the Rome-IV diagnostic questionnaire parent-report form for

children and adolescents (4 years of age and older) in the Indonesian language. The questionnaire was translated from English to Indonesian by a certified medical translator based in Jakarta. A functional dyspepsia diagnosis was determined using the scoring instructions that came with the questionnaire.[15]

This study was approved by the Ethics and Research Committee at Sam Ratulangi University and subject sampling was approved by the school principal and subjects' parents. Data were analyzed with *SPSS software V. 22, IBM*. Statistical correlation between groups was analyzed using Chi-square test. A P value of < 0.05 was considered to be statistically significant. Phi-coefficient correlation analysis was performed to investigate a possible association between fecal calprotectin and functional dyspepsia.

Results

A total of 38 subjects, 22 boys and 16 girls, had a mean age of 8.73 years, ranging from 6-12 years. Subjects were grouped according to their fecal calprotectin results (normal vs. elevated) and functional dyspepsia diagnoses (functional dyspepsia *vs.* healthy).

Table 1 showed fecal calprotectin concentration between these groups. Fecal calprotectin was found to be elevated in 8 subjects (21.1%), and functional dyspepsia was found in 9 subjects (23.7%). In the functional dyspepsia group, the mean and median fecal calprotectin levels were 312.45 μg/g and 167.7 μg/g, respectively. In the healthy group, the mean and median fecal calprotectin levels were 20.89 μg/g and 7.2 μg/g, respectively.

Table 1. Fecal calprotectin concentration in the functional dyspepsia and healthy groups

Fecal calprotectin concentration	Functional dyspepsia group (n=9)	Healthy group (n=29)
Elevated	5	3
Normal	4	26
Mean (SD), ug/g	312.45 (354.6)	20.89 (51.9)
Median (range), ug/g	167.7 (11.4-993.2)	7.2 (0.6-273)

In the functional dyspepsia group, fecal calprotectin was elevated in 5/9 subjects (55.6%), in comparison with only 3/29 subjects (10.3%) in the healthy group (**Figure 1**). Data analysis using Phi-coefficient correlation showed a positive correlation between elevated fecal calprotectin and functional dyspepsia (r=0.471; P=0.004).

Discussion

The aim of this study was to assess fecal calprotectin concentration in generally healthy children. Mean fecal calprotectin concentration in the healthy group was 20.89 μg/g and median was 7.2 μg/g. A previous study reported a median fecal calprotectin of 13.6 μg/g in healthy children.12 The slight difference in results might be explained by differences in ethnicity, age groups, sample handling, or laboratory reagent calibration. However, from this study result, a fecal calprotectin physiological cut-off level of 50 μg/g seems reasonable and valid for this subject group.

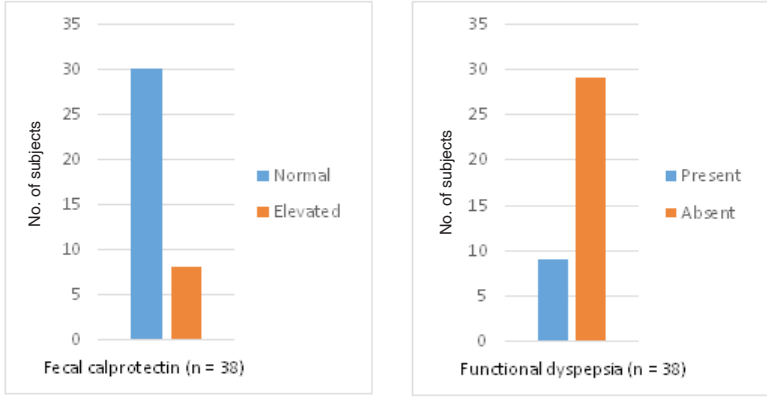

Figure 1. Fecal calprotectin and functional dyspepsia distribution of subjects

We also assessed for a possible association between fecal calprotectin and functional dyspepsia in children. Subjects with functional dyspepsia had elevated fecal calprotectin levels (mean 312.45 μg/g and median 167.7 μg/g). Moreover, Phi-coefficient correlation revealed a positive correlation between elevated fecal calprotectin and functional dyspepsia ($r=0.471$; $P=0.004$).

To our knowledge, no other studies have assessed for a possible correlation between elevated fecal calprotectin and functional dyspepsia in children. However, a study reported a significantly higher fecal calprotectin level in children with functional constipation, as compared to healthy children.[16] Our finding suggests some indications of ongoing inflammation in subjects with functional dyspepsia. Misdiagnosis is also possible, as this study determined a diagnosis of functional dyspepsia only using the parent-filled questionnaire form, without further any confirmatory tests, such as endoscopy, to rule out any organic/inflammatory causes.

Limitations of this study were the small sample size and limited diagnostic tools for diagnosing functional dyspepsia in children. Due to the fact that all of our subjects appeared to be generally healthy children, any invasive procedures to rule-out organic/inflammatory causes were not performed.

In conclusion, this study shows promising results of fecal calprotectin as a clinical test for detection of gut mucosal inflammation, as it has a significant correlation with functional dyspepsia symptoms in children. Current fecal calprotectin physiological cut-off level seems valid for children aged 6-12 years old. Elevated fecal calprotectin is associated with functional dyspepsia in children. Further studies are required to evaluate and confirm the clinical importance of fecal calprotectin as a mucosal barrier immune defense.

Conflicts of Interest

None declared.

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