



Association Between Fecal Calprotectin Concentration and Mesenteric Lymphadenopathy in Children

Mahnaz Sadeghi Shabestari¹, Mandana Rafeey^{2*}, Maryam Shoaran², Shirin Shirvani²

Abstract

Objectives: Calprotectin is released from neutrophils and monocytes during the inflammatory process. Recently, an increasing number of studies has postulated some possible diagnostic roles of fecal calprotectin (FC), including the inflammatory bowel disease (IBD). Considering that mesenteric lymphadenopathy is due to the inflammation of lymph nodes, it can be assumed that FC can be used as an early diagnostic tool for mesenteric lymphadenopathy. Thus, the aim of this study was to evaluate the association between FC and mesenteric lymphadenopathy in children.

Materials and Methods: Children with abdominal pain admitted to Tabriz Pediatric Hospital were included in this cross-sectional, case-control study during one year. Patients were allocated to two groups based on ultrasonography (US). The case group consisted of children with mesenteric lymphadenopathy revealed by the US while the control group included children without mesenteric lymphadenopathy. Finally, the demographic data and FC concentration, along with other routine laboratory tests were assessed in both groups.

Results: Overall, 119 children were included of whom, 47 and 71 children were in the control and case groups, respectively. The median FC of the case and control groups was 50 and 46.7 $\mu\text{g/g}$ ranging from 6.5-1800 and 5-1000 $\mu\text{g/g}$, respectively, demonstrating a significant difference between the groups ($P=0.001$). The optimal cutoff point of FC was 46.7 $\mu\text{g/g}$ for the diagnosis of mesenteric lymphadenopathy with 61.1% sensitivity and 78.7% specificity.

Conclusions: The results of this study revealed that the level of FC in the case group was significantly higher compared to the control group. Therefore, the FC concentration could be the most useful method for the diagnosis of mesenteric lymphadenopathy.

Keywords: Fecal calprotectin, Mesenteric lymphadenopathy, Children

Introduction

Mesenteric lymphadenopathy is an acute self-limiting inflammatory condition of mesenteric lymph nodes with clinical manifestations such as fever, anorexia, nausea, vomiting, abdominal pain (especially in the right lower quadrant), an important differential diagnosis of appendicitis, intussusception, and rarely pre-lymphoma. This condition typically affects children although 20% of the cases are adults (1-3). Gastroenteritis and upper airway infection are the most frequent causes of mesenteric lymphadenopathy (4). The diagnosis is mainly based on history and ultrasonography (US). Detecting three or more mesenteric lymph nodes with a diameter of 5 mm or more in the US without any identifiable underlying inflammatory process is suggestive for primary mesenteric lymphadenopathy (1). However, routine laboratory studies such as C-reactive protein (CRP) and white blood cell count have limited use for the diagnosis of this disorder (1). US has also its own limitations such as operator-based procedure, difficulties in visualization in obese children, along with the non-adequate preparation of the bowels before US initiation (5).

Calprotectin is a heterodimer protein in the cytosol of

neutrophils and the cell membrane of monocytes which releases when neutrophils are activated or monocytes attach to the endothelium of the vessels during the inflammatory process (6). The released calprotectin can be detected in the blood serum, urine, saliva, and feces (6). A growing number of studies have recently postulated that fecal calprotectin (FC) can be used as a diagnostic test in some inflammatory and non-inflammatory gastrointestinal diseases such as inflammatory bowel disease (IBD) and neoplasms (7-9). Considering that similar inflammatory processes occur within mesenteric lymphadenopathy (1), it can be assumed that FC may be increased in these patients. Given the above-mentioned explanations, this study aimed to evaluate the feasibility of the FC concentration test as a complementary non-invasive test for an early diagnostic method in mesenteric lymphadenopathy, followed by determining the condition of the disease for the follow-up and using the reassurance.

Materials and Methods

This cross-sectional, case-control study prospectively investigated the patients with abdominal pain, who attended the gastroenterology clinic or were admitted to



the gastroenterology ward of Tabriz Children Hospital from November 2017 to November 2018. Mesenteric lymphadenopathy was defined as three or more mesenteric lymph nodes with a short-axis diameter of 5 mm in the ultrasonographic examination without any identifiable underlying inflammatory disorder (1, 2). The case group consisted of all children with mesenteric lymphadenopathy revealed by the US. Further, the control group included the children with abdominal pain but without mesenteric lymphadenopathy in the US. The inclusion criteria were being within the age range of 2-14 years old and having access to US investigation. On the other hand, the exclusion criteria encompassed the presence of neoplastic and IBDs, as well as other known disorders and parental non-consent for US and laboratory investigation. After a complete medical history and physical examination, transabdominal US was performed for all children. All the US were done by an expert sonographer with the built-in Aixus ACQ software on the Siemens US device (Siemens Medical Solutions, Issaquah, WA). The transducer frequency varied between 3.5 and 5 MHz. The case and control groups were matched based on their age and gender. In addition, all patients underwent routine hematology and chemistry tests such as complete blood cell (CBC) count, erythrocyte sedimentation rate (ESR), and serum CRP. Furthermore, FC concentration was measured in all patients using a single stool sample collected on the first day after the initial visit. The specimens were stored at -20°C and assayed for calprotectin within 4 weeks. Moreover, calprotectin concentration was measured using the ELISA kit manufactured by Bühlmann Laboratories (Schönenbuch, Switzerland) according to the method of Tøn et al (10), followed by determining the level of ESR by the standard modified Westergren sedimentation method. Additionally, the serum CRP level and the CBC were checked by the ENISON kit, Iran and the cell counter (SYSMEX-KX-21 & SYSMEX-XF-800, Japan), respectively. The mean of the two measurements on each sample was reported as the concentration of FC. Similarly, the normal range of FC concentrations provided by the manufacturer was <50 µg/g, 50-100 µg/g, and >100 µg/g indicating negative, borderline, and positive values,

respectively.

Statistical Analysis

The median and ranges of all variables were reported and the Mann-Whitney test was used to compare the values between case and control groups. In addition, the Spearman rank-order correlation coefficient was calculated to verify the correlation between individuals' FC concentration and the size of the lymph nodes. Further, the Kruskal-Wallis test was used to compare the values among three sub-groups of the case group. The optimal cutoff point of the FC for distinguishing case and control groups was determined using the receiver operating characteristic (ROC) curve analysis and Youden's index. The sensitivity and specificity, as well as the positive and negative predictive values of the FC were reported for the optimal cutoff point based on the manufacturer's explanations. Eventually, the data were analyzed using SPSS, version 23 and $P < 0.05$ was considered statistically significant.

Results

In general, 119 consecutive children were included in this study (median age = 6 years, range = 2-12 years). Overall, 55 patients (45.8%) were males and 64 (54.2%) were females. The case group consisted of 71 patients and 47 patients were included in the control group. Based on the data in Table 1, no statistical difference was observed between the case and control groups regarding their age and gender ($P = 0.351$ & 0.848 for age and gender, respectively). The median FC was 50 µg/g and 25 µg/g ranging from 6.5 to 1800 µg/g and from 5-1000 µg/g in case and control groups, respectively, which represented a significant difference between the two groups in this regard control group ($P < 0.001$, Table 1 and Figure 1). The median WBC was 9735 cells/µL (within the range of 7000-24900 cells/µL) and 13800 cells/µL (in the range of 7200-27600 cells/µL) in case and control groups, respectively. However, the difference was not statistically significant ($P < 0.298$). In addition, the median ESR in the case group was less than the control group but the difference was not significant (control group, 11 mm/h within the range of 3-79 vs. case

Table 1. Demographic and Laboratory Tests Results

Variable	Total	Case	Control	P-value
Age (y)	6 (2-12)	6 (2-12)	6 (2-12)	0.351
Male (n, %)	55 (45.8%)	33 (46%)	21 (44%)	0.848
Female (n, %)	64 (54.2%)	38 (54%)	26 (56%)	
FC (µg/g)	44 (5-1800)	50 (6.5-1800)	25 (5-1000)	0.001
WBC (cell/µL)	9900 (7000-27600)	9,735 (7000-24900)	13800 (7200-27600)	0.298
HB (g/dL)	24 (20-14.2)	12.5 (10-14.2)	12.1 (11.2-12.9)	0.484
PLT (cell/µL)	277000 (216000-409000)	246,500 (216000-277000)	322000 (240000-409000)	0.248
ESR (mm/h)	10 (1-79)	11 (3-79)	9.5 (1-58)	0.541
CRP (mg/L)	2.6 (0-10)	2.3 (0-10)	3.1 (0-3.5)	0.473

Note. FC: Fecal calprotectin; WBC: White blood cells; HB: Hemoglobin; PLT: Platelets; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein.

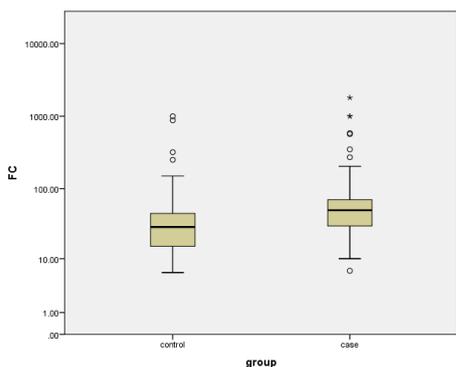


Figure 1. Fecal Calprotectin in Case and Control Groups.

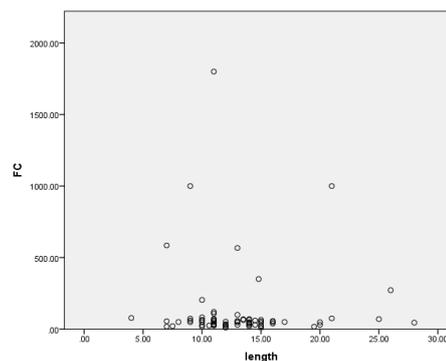


Figure 2. Scatter Plot, Fecal Calprotectin in the Case Group Based on the Size of Hypertrophic Lymph Nodes.

group, 9.5 mm/h in the range of 1-58, $P < 0.541$, Table 1).

The number of hypertrophic lymph nodes in the case group was recorded as follows.

Overall, 64 (88.9%) had one hypertrophic lymph node, 7 patients (9.7%) had two hypertrophic lymph nodes, and one patient (1.4%) had three hypertrophic lymph nodes. Median FCs in these three sub-groups were 75 μg in patients with three hypertrophic lymph nodes, 60 μg in patients with two hypertrophic lymph nodes, and 50 μg in the patient with one hypertrophic lymph node. However, the difference was not statistically significant ($P < 0.572$, Table 2). No statistically significant difference was found among these three sub-groups in terms of WBC, ESR, and CRP (Table 2).

The median size of hypertrophic lymph nodes was 12.5 mm ranging from 5 to 28 mm. There was no significant correlation between the size of hypertrophic lymph nodes and the FC concentration ($P < 0.529$, Figure 2).

The ROC analysis showed a good performance of FC in differentiation between case and control groups with the area under the curve of 0.719 (95% CI: 0.620-0.819, $P < 0.001$, Figure 3). The sensitivity and specificity of FC in cutoff points provided by the manufacturer are summarized in Table 3. The optimal cutoff point was calculated as 46.7 (Youden's index = 0.398, Table 3).

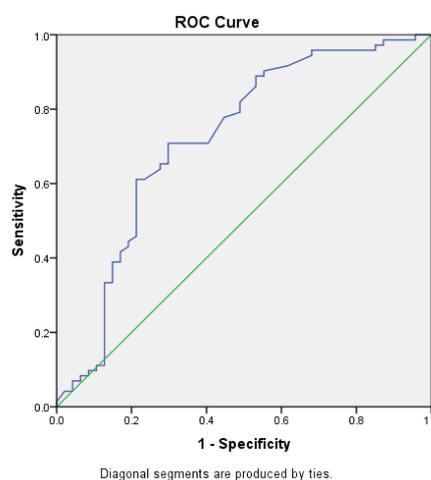


Figure 3. ROC Curve for Fecal Calprotectin for Differentiation Between Patients With and Without Primary Mesenteric Lymphadenopathy.

Discussion

Mesenteric lymphadenopathy is an acute self-limiting inflammatory condition of mesenteric lymph nodes. Lymph nodes are located in a membrane and attach the intestine to the abdominal wall. The most clinical

Table 2. Laboratory Test Results in the Case Group Based on the Number of Hypertrophic Lymph Nodes

	Number of Hypertrophic Lymph Nodes			P Value
	One	Two	Three	
No. (%)	64 (88.9%)	7 (9.7%)	1 (1.4%)	
FC ($\mu\text{g/g}$)	50 (6.6-1800)	60 (20-204)	75	0.572
WBC (cell/ μL)	12700 (7000-24900)	9735 (9670-9800)	10400	0.231
ESR (mm/h)	12 (3-79)	8 (6-10)	26	0.087
CRP (mg/L)	2.4 (0-10)	0 (0-0)	2.5	0.421

Table 3. The Sensitivity and Specificity of Fecal Calprotectin in Different Cutoff Points

Cutoff Point of Fecal Calprotectin	Number of Patients With Positive Results	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
46.7 $\mu\text{g/g}$	65	61.1%	78.7%	81.5%	56.9%
50 $\mu\text{g/g}$	76	52.8%	78.7%	79.2%	52.1%
100 $\mu\text{g/g}$	103	15.3%	87.2%	64.7%	40.2%

manifestations of mesenteric lymphadenopathy are fever, anorexia, nausea, vomiting, and abdominal pain which is an important differential diagnosis of appendicitis, intussusception, and rarely pre-lymphoma. This condition mainly affects children while it is found in 20% of adults as well.

An objective simple non-invasive test for differentiating a self-limiting condition such as mesenteric lymphadenopathy from its critical differential diagnoses can be of great importance for clinicians and can guarantee an early and reliable diagnosis. Moreover, routine laboratory tests are carried out for evaluating the possible causes of abdominal pain such as WBC, ESR, and CRP. In their study, Moustaki et al demonstrated that WBC and neutrophils were in a normal range but CRP levels (significantly associated with abdominal pain) indicated an increase (11). Additionally, Fagerberg et al reported that platelet count, ESR, and CRP were normal (12), which is in line with the results of the present study. In our study, the above-mentioned parameters had limited values for the diagnosis of patients with mesenteric lymphadenopathy.

Furthermore, Manz et al considered a value of equal or greater than 50 µg/g as the cutoff level for calprotectin (13). They also found the sensitivity of 73% and specificity of 93% with good positive and negative likelihood ratios (10.8 and 0.29) whereas our optimal cutoff point of FC was 46.7 µg/g for the diagnose of mesenteric lymphadenopathy with 61.1% sensitivity and 78.7% specificity.

Previous studies considered FC as an appropriate diagnostic test for some gastrointestinal pathologic conditions (7,8). For instance, Summerton et al evaluated the usefulness of FC as a screening test for alimentary inflammation and neoplasm and postulated that the elevated FC in IBD and combined colorectal carcinoma have a sensitivity of 81.8% and a specificity of 73.2%. The sensitivity of the elevated FC for merely IBD was 78.6% (7). Its sensitivity and specificity in identifying the organic causes of chronic diarrhea in children were 70% and 93%, respectively (8). However, to the best of our knowledge, no previous study has evaluated the level of FC in patients with mesenteric lymphadenopathy. The result of our study demonstrated a significant difference in FC concentration in patients with mesenteric lymphadenopathy versus the control group. The median FC of the control group in our study was comparable to those reported for the healthy control groups in some previous studies (12, 14). Fagerberg et al reported that the median FC was 16.5 µg/g (within the range of 5.0-65 µg/g) in children with gastrointestinal symptoms but without inflammation (12). Further, Berni Canani et al found that the median FC was 28.0 µg/g (in an interquartile range of 15-57) in healthy children (14).

The case and the control groups in our study obtained similar results regarding the routine laboratory tests (i.e., WBC, HB, PLT, ESR, and CRP). Therefore, FC was the only useful test for differentiating these two groups.

The elevated FC concentration demonstrated

good performance in the diagnosis of mesenteric lymphadenopathy. Furthermore, the sensitivity and specificity in the FC concentration of 46.7 µg/g were appropriate and comparable with those previously postulated for the elevated FC in other alimentary inflammatory and neoplastic diseases (7,8,15). However, the concentration of FC was lower in comparison to the reported median FC in more severe inflammatory diseases such as IBD and neoplastic disorders. Correspondingly, the estimated optimal cutoff in our study was close to the cutoff concentration provided by the manufacturer. However, it was lower than the cutoff concentration of previously reported FC for IBD (102.9266 µg/g or neoplastic disease (10 mg/L) in studies conducted by Berni Canani et al and Tibble et al (14,16). The specificity of FC in the level of 100 µg/g was higher (87.2%) but the sensitivity was markedly low in this cutoff level. Therefore, the false-negative can be increased at this cutoff level.

The size of the lymph nodes does not always correlate with the severity of the underlying disease although the number and distribution of the lymph nodes are substantial in this regard (17). Normal mesenteric lymph nodes may be commonly detected at the mesenteric root and throughout the mesentery, especially in the right iliac fossa in children (18) and at the mesenteric root in adults (19). Most of our patients had only one hypertrophic lymph node but no evident association was found between the size and number of hypertrophic lymph nodes and the concentration of FC. One possible explanation could be the scarcity of patients with more than one hypertrophic lymph node. Therefore, identifying a meaningful correlation between the numbers of lymph nodes with FC concentration may be possible through studies with greater sample size.

Conclusions

In general, the FC concentration was an appropriate objective simple diagnostic test for mesenteric lymphadenopathy with acceptable sensitivity and specificity. Contrarily, other routine laboratory tests have limited value for the diagnosis of this condition. No significant association was found between FC concentration, as well as the size and number of hypertrophic lymph nodes. The high cost of the calprotectin kit was the limitation of our study and led to the selection of fewer patients for this study.

Conflict of Interests

The authors declared that they have no conflict of interests.

Ethical Issues

This study was approved by the Ethical Committee of Tabriz University of Medical Sciences under the ethical code of 94/3-9/17.

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