



# A Survey of Fecal Calprotectin in Children with Newly Diagnosed Celiac Disease with Villous Atrophy

Iraj Shahramian<sup>1</sup>, Ali Bazi<sup>2,\*</sup>, Negar Shafie-Sabet<sup>3</sup>, Alireza Sargazi<sup>3</sup>, Omolbanin Sargazi Aval<sup>2</sup>, Mojtaba Delaramnasab<sup>2</sup>, Mohadeseh Behzadi<sup>3</sup> and Zahra Zaer-Sabet<sup>3</sup>

<sup>1</sup>Zabol University of Medical Sciences, Zabol, Iran

<sup>2</sup>Faculty of Allied Medical Sciences, Zabol University of Medical Sciences, Zabol, Iran

<sup>3</sup>Student Research Committee, Zabol University of Medical Sciences, Zabol, Iran

\*Corresponding author: Clinical Research Development Unit, Amir-Al-Momenin Hospital, Zabol University of Medical Sciences, Zabol, Iran. Email: m.baziali@gmail.com

Received 2018 September 23; Revised 2019 April 15; Accepted 2019 April 19.

## Abstract

**Background:** Fecal calprotectin (FC) has been used as a diagnostic marker in intestinal inflammatory conditions.

**Objectives:** As a few studies have been dedicated to assess the role of FC in coeliac disease (CD), the current study aimed to address this issue.

**Methods:** This study included 70 newly diagnosed CD (Marsh score 3) and 70 healthy children. The study was performed at the pediatric ward of Amir-Al-Momenin Hospital in Zabol city, the southeast of Iran, during June 2016-September 2017. The FC level was determined using a specific ELISA kit.

**Results:** Women constituted 64.3% (45/70) and 55.1% (38/70) of CD and healthy children, respectively ( $P = 0.1$ ). There was no significant difference in the mean age between children with CD ( $6.3 \pm 3.4$ ) and without CD ( $8.3 \pm 4.5$ ) ( $P = 0.2$ ). The mean level of FC was significantly higher in patients ( $239.1 \pm 177.3 \mu\text{g/g}$ ) than in healthy controls ( $38.5 \pm 34.6 \mu\text{g/g}$ ,  $P < 0.001$ ). The titer of anti-tTG was significantly higher in patients than in healthy children ( $205.9 \pm 156.2 \text{ U/mL}$  vs.  $6.7 \pm 2.1 \text{ U/mL}$ , respectively,  $P < 0.001$ ). There was a significant correlation between the FC level and anti-tTG titer ( $r = 0.611$ ,  $P < 0.001$ ). However, the correlation was not statistically significant between FC and age ( $r = -0.154$ ,  $0.07$ ). The ROC curve analysis revealed an AUC value of 0.893 (95% CI: 0.827 - 0.960,  $P < 0.001$ ). At the level of  $50 \mu\text{g/g}$ , FC rendered the sensitivity and specificity of 90% and 92%, respectively, for the diagnosis of CD. Positive predictive value (PPV) and negative predictive value (NPV) of FC at this cutoff value were 95.5% and 90.5%, respectively.

**Conclusions:** FC can be considered a screening complementary tool for detecting CD with high sensitivity and specificity.

**Keywords:** Fecal Calprotectin, Celiac Disease, Gluten Enteropathy, Marsh Score

## 1. Background

Coeliac disease (CD), known as gluten sensitivity, is an autoimmune condition characterized by the atrophy of small intestine. It has been estimated that CD affects 1% of the western population (1). CD affects a wide age spectrum encompassing pediatrics, young and elderly people. The diagnosis of CD is currently dependent on a combination of clinical, histological, and serological approaches (2). The susceptibility to CD is attributed to the presence of certain HLA alleles (HLA-DQ2 and HLA-DQ8) as the dominant factor in the development of CD. Adherence to a long-term gluten-free diet (GFD) is necessary for the management of CD.

Evaluating the disease activity in CD patients requires performing the screening tests routinely. In addition, the efficiency of new therapeutic strategies (such as gluten

proteases and immunomodulators) for CD can be validated by using sensitive screening markers (3). Being highly invasive in nature, using sensitive screening markers is not amenable by intestinal biopsies and therefore, it necessitates the application of non-invasive markers. On the other hand, available serological markers of CD can be useful in the diagnosis phase; however, these markers have limitations for predicting relapse or remission during the course of CD (1, 4).

Fecal calprotectin (FC) is a relatively new inflammatory marker for intestinal pathological changes. It has been used for monitoring common intestinal inflammatory diseases including inflammatory bowel disease (IBD), Ulcerative colitis (UC), and Crohn's disease (CD) (5-7). FC also elevates in colitis and gastrointestinal neoplasms (8, 9). Accordingly, FC has been superior to traditionally available markers of inflammation such as C-reactive protein for re-

flecting intestinal atrophy (10). Furthermore, FC has been suggested as a reliable marker for predicting the relapse of intestinal inflammation in IBD (8). FC also has the potential to be used as a point of care test by patients in their homes (5).

## 2. Objectives

The role of FC as a disease indicator in CD is uncertain. There are a few studies on this issue representing inconclusive remarks. We aimed to assess the diagnostic capacity of FC in newly diagnosed CD children.

## 3. Methods

### 3.1. Study Population

This study included 70 newly diagnosed CD children recruited from the pediatric ward of Amir-Al-Momenin Hospital in Zabol city, the southeast of Iran. As controls, 70 healthy age and sex-matched children were recruited from the same geographical region. The sample size was determined based on the availability of newly diagnosed CD children and the report of Biskou et al. (11). The individuals with systemic disorders, family history of intestinal inflammatory disease, and history of taking a gluten-free diet (GFD) were excluded. The study was performed during June 2016 - September 2017. It was approved by the Ethics Committee of Zabol University of Medical Sciences. We followed the principals of the Declaration of Helsinki.

### 3.2. Serological Assessment

Blood samples were drawn (5 mL) from each participant in the morning. The samples were immediately transferred to the laboratory of the hospital where sera were separated by centrifuging at 5000 rpm. The serum samples were kept at -20°C until use. The levels of IgA anti-tTG were determined using specific ELISA kits (AESKULISA tIgA New generation, Germany). The titers of higher than 20 U/mL were considered positive (12, 13).

### 3.3. Intestinal Biopsy

Upper endoscopy was performed to obtain biopsy samples. Histological diagnosis was made based on the observation of villous atrophy in at least one biopsy from the bulb and four biopsies from the distal duodenum. The biopsies were observed by the same experienced pathologist. Only were those children with a Marsh score of 3 included in the study.

### 3.4. FC Measurement

Stool samples were obtained in the morning in sterile containers and stored in a freezer (-20°C) until use. A specific ELISA kit (Calprotectin ELISA, EuroImmuno, Germany) was purchased. The protocol was followed as noted in the manufacturer's instructions.

### 3.5. Statistical Analysis

Statistical methods were performed in SPSS 19 software. Shapiro-Wilk test was used to determine the normality of data distribution. Descriptive measures (means, standard deviations, and frequencies) were deployed to present the data. Independent sample *t*-test and Fisher's exact test were considered to assess any association between intended variables. Receiver operating characteristic (ROC) analysis was used to ascertain the validity of FC levels for the diagnosis of CD. The significance level was considered at  $P < 0.05$ .

## 4. Results

Overall, women constituted 83 out of 140 participants (59.7%). In the sample of children with CD, females and males constituted the ratios of 64.3% (45/70) and 35.7% (27/70), respectively. In healthy children, there were 38 (55.1%) and 32 (44.9%) females and males, respectively. The gender distribution showed no significant difference between the two groups ( $P = 0.1$ ). The mean age of the participants was  $7.3 \pm 4.1$  (range 1 to 18 years old). There was no significant difference in the mean age between children with CD ( $6.3 \pm 3.4$ ) and without CD ( $8.3 \pm 4.5$ ,  $P = 0.2$ ).

The mean level of FC was significantly higher in patients ( $239.1 \pm 177.3 \mu\text{g/g}$ ) than in healthy controls ( $38.5 \pm 34.6 \mu\text{g/g}$ ,  $P < 0.001$ ). Accordingly, 90% of children with CD had the FC levels of higher than  $50 \mu\text{g/g}$  while only 4.3% of the healthy controls showed values above this cutoff (Table 1). In addition, the titer of anti-tTG was significantly higher in patients than in healthy children ( $205.9 \pm 156.2 \text{ U/mL}$  vs.  $6.7 \pm 2.1 \text{ U/mL}$ , respectively,  $P < 0.001$ , Figure 1).

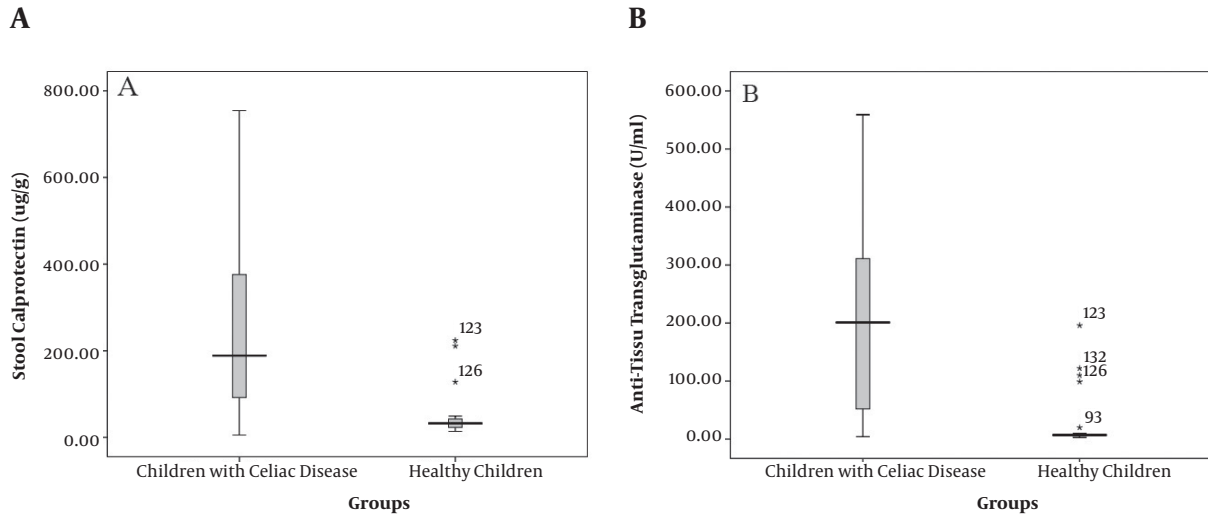
There was a significant correlation between the FC level and anti-tTG titer (Figure 2A). However, the correlation was not statistically significant between FC and age (Figure 2B).

ROC curve analysis revealed a high AUC value for the FC level regarding the diagnosis of CD (AUC = 0.893, 95% CI: 0.827 - 0.960,  $P < 0.001$ , Figure 3). At the level of  $50 \mu\text{g/g}$ , FC rendered the sensitivity and specificity of 90% and 92%, respectively, for the diagnosis of CD. Positive predictive value (PPV) and negative predictive value (NPV) of FC at this cutoff value were 95.5% and 90.5%, respectively.

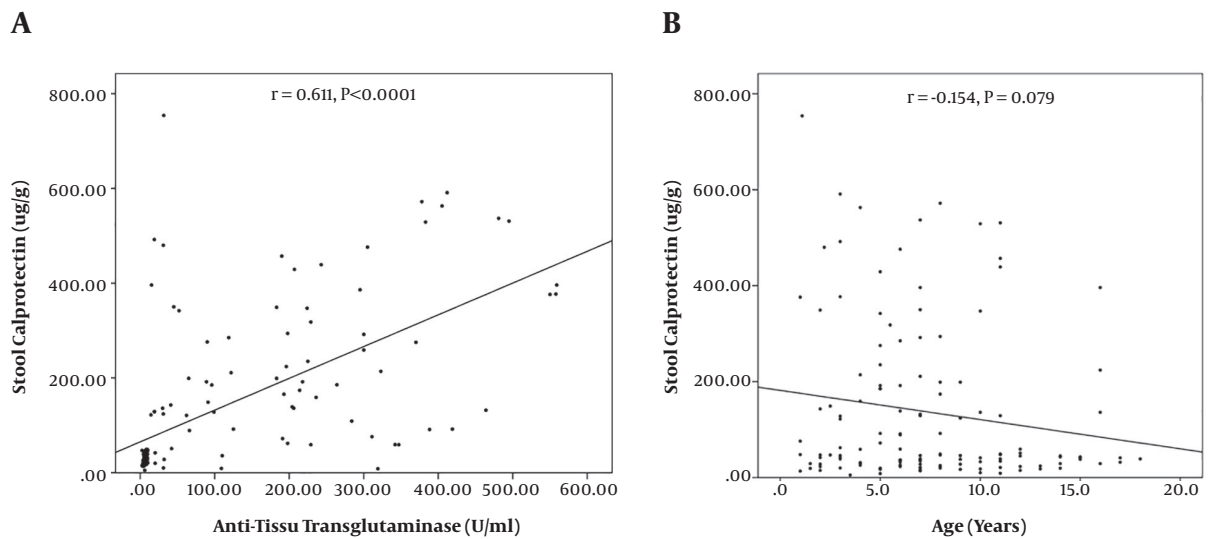
**Table 1.** The Ratios of Normal and Abnormal Fecal Calprotectin Levels in Children with CD and Healthy Children

Fecal Calprotectin Level	Celiac Disease (N = 70), No. (%)	Healthy Controls (N = 70), No. (%)	P Value
< 50 µg/g	7 (10)	67 (95.7)	< 0.001 <sup>a</sup>
> 50 µg/g	63 (90)	3 (4.3)	

<sup>a</sup>Fisher's exact test.



**Figure 1.** Comparison of fecal calprotectin levels (A) and IgA tissue transglutaminase titer (B) between newly diagnosed CD (Marsh score 3) and healthy children. Differences were statistically significant for both variables ( $P < 0.001$ ).

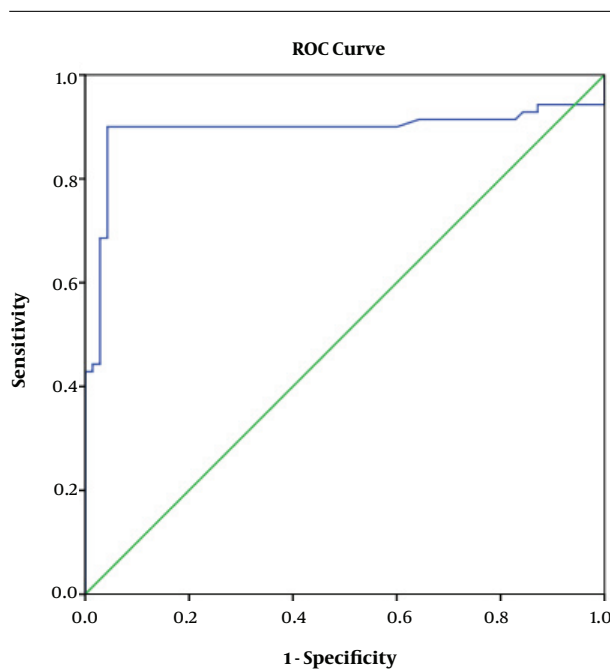


**Figure 2.** Correlation of fecal calprotectin levels with IgA tissue transglutaminase (A) and age (B) in newly diagnosed children with CD (Marsh score 3)

### 5. Discussion

FC has been suggested as a potential biomarker for the diagnosis and evaluation of a variety of intestinal in-

flammatory conditions. FC promotes important biological activities encompassing anti-microbial, antiproliferative, and immunomodulation functions (8). In the present



**Figure 3.** Receiver operating characteristic (ROC) analysis of fecal calprotectin levels for diagnosis of children with newly diagnosed CD. Area under curve = 0.893, 95% CI: 0.827 - 0.960

study, we found that the mean FC level was significantly higher in children newly diagnosed with CD ( $239.1 \pm 177.3 \mu\text{g/g}$ ) than in healthy children ( $38.5 \pm 34.6 \mu\text{g/g}$ ,  $P < 0.001$ ). At the cutoff value of  $50 \mu\text{g/g}$ , FC showed high diagnostic validity for CD (AUC = 0.893, 95% CI: 0.827 - 0.960,  $P < 0.001$ ). It has been asserted that the elevated levels of FC could appropriately distinguish between intestinal inflammatory conditions from non-pathological conditions (14). In a Canadian study, the mean level of FC in children with CD (Marsh score II/III) was  $67.5 \mu\text{g/g}$  with a wide range ( $4.9 - 3068 \mu\text{g/g}$ ) at diagnosis. This value fell within the range of  $1.11 - 736.5 \mu\text{g/g}$  (mean  $33 \mu\text{g/g}$ ) after one year of GFD administration (4). In another report of 29 newly diagnosed CD children, it was revealed that the FC levels were significantly higher in patients than in controls (15). In a recent report, children with total villous atrophy showed higher FC levels ( $13.8 \pm 9.3 \text{ mg/L}$ ) than those with partial atrophy ( $3.7 \pm 1.8 \text{ mg/L}$ ) (15). Similarly, 17 children newly identified with CD had higher FC levels than healthy children (11). According to the report by Tola et al. (16), the mean value of FC was significantly higher in adults with CD than in healthy ones. Nevertheless, the elevated FC levels were observed mainly in those patients with active CD (55.6%) compared to individuals with treated CD (13.6%) (16). In two reports in adult patients with CD, the FC levels were not significantly different between newly diagnosed cases and

healthy counterparts (17, 18). In general, these observations highlight the potential applicability of FC for the monitoring and diagnosis of CD.

As another finding, we detected a strong significant correlation ( $r = 0.611$ ,  $P < 0.001$ ) between the FC level and IgA anti-tTG titer in the patients. Anti-tTG antibodies of IgA class are the most common and validated serological markers for diagnosis of CD. In comparison, no significant association was detected between FC and Marsh score, clinical symptoms, or anti-tTG titer in adults with CD (18). The levels of FC were higher in serologically diagnosed children ( $89.6 \mu\text{g/g}$ ) than in histologically diagnosed children ( $51.4 \mu\text{g/g}$ ), indicating a potential correlation between the FC levels and IgA anti-tTG titer (4). The levels of FC can be influenced by the clinical picture of CD as symptomatic children may show higher FC levels than children with no signs and symptoms (19). Accordingly, the FC levels were higher in newly diagnosed children in comparison with those under GFD (19). Intestinal atrophy seems to be a dominant feature influencing the FC levels in CD patients (15). Here, we detected markedly higher FC levels in the patients that all had villous atrophy (Marsh score 3) while previous reports incorporated children with lower Marsh scores (4, 11, 15). However, FC was associated with neither the grade of intestinal inflammation nor with the clinical picture of CD in a report by Montalto et al. (17). This may be due to the impact of some other individual, physiological, or environmental factors modulating the FC levels in patients with CD. Nevertheless, the incorporation of FC with serological findings can provide high diagnostic accuracy.

A point of concern regarding the use of FC in the monitoring of pediatric inflammatory diseases is uncertainty regarding a valid and consensus cutoff value. Some have suggested a cutoff value of  $50 \mu\text{g/g}$ ; nevertheless, the range of FC could be very wide that limits the FC diagnostic potential (7, 20). In the current study, we found that the  $50 \mu\text{g/g}$  threshold resulted in high sensitivity, specificity, PPV, and NPV (90%, 92%, 95.5%, 90.5%, respectively) for CD diagnosis. However, the elevated levels of FC may be diagnostic for intestinal inflammation, but its normal value may not necessarily exclude a pathological condition (21). Accordingly, it is suggested that the FC levels be interpreted taking into consideration other available non-invasive markers such as CRP, serological findings, and fecal lactoferrin (22, 23).

### 5.1. Conclusions

FC can be considered as a screening complementary tool for detecting CD with high sensitivity and specificity. One of the main benefits of measuring the FC level could be obviating the need for performing invasive screening

methods. However, due to the broad range of this parameter, there is a need to develop diagnostic criteria incorporating FC with other clinical and serological diagnostic features.

## Footnotes

**Authors' Contribution:** Iraj Shahramian: Study concept, design, and supervision; Ali Bazi: Drafting the manuscript and statistical analysis; Negar Shafie-Sabet: Data collection; Alireza Sargazi: Data collection and analysis; Omolbanin Sargazi Aval: Data analysis; Mojtaba Delaramnasab: Data collection; Mohadeseh Behzadi: Data collection; Zahra Zaer-Sabet: Data collection.

**Conflict of Interests:** The authors report no conflicts of interest.

**Ethical Approval:** This study was approved by the Ethical Committee of Zabol University of Medical Sciences. We also followed principals of Declaration of Helsinki.

**Funding/Support:** Zabol University of Medical Sciences funded the research.

**Patient Consent:** Informed consent was obtained from the children's parents.

## References

- Moreno ML, Rodriguez-Herrera A, Sousa C, Comino I. Biomarkers to monitor gluten-free diet compliance in celiac patients. *Nutrients*. 2017;9(1). doi: [10.3390/nu9010046](#). [PubMed: [28067823](#)]. [PubMed Central: [PMC5295090](#)].
- Hindryckx P, Levesque BG, Holvoet T, Durand S, Tang CM, Parker C, et al. Disease activity indices in coeliac disease: systematic review and recommendations for clinical trials. *Gut*. 2018;67(1):61-9. doi: [10.1136/gutjnl-2016-312762](#). [PubMed: [27799282](#)].
- Tack GJ, Verbeek WH, Schreurs MW, Mulder CJ. The spectrum of celiac disease: Epidemiology, clinical aspects and treatment. *Nat Rev Gastroenterol Hepatol*. 2010;7(4):204-13. doi: [10.1038/nrgastro.2010.23](#). [PubMed: [20212505](#)].
- Rajani S, Huynh HQ, Shirton L, Kluthe C, Spady D, Prosser C, et al. A Canadian study toward changing local practice in the diagnosis of pediatric celiac disease. *Can J Gastroenterol Hepatol*. 2016;2016:6234160. doi: [10.1155/2016/6234160](#). [PubMed: [27446854](#)]. [PubMed Central: [PMC4904635](#)].
- Hejl J, Theede K, Mollgren B, Madsen KV, Heidari A, A. Steig A, et al. Point of care testing of fecal calprotectin as a substitute for routine laboratory analysis. *Pract Lab Med*. 2018;10:10-4. doi: [10.1016/j.plabm.2017.11.002](#). [PubMed: [29234707](#)]. [PubMed Central: [PMC5721267](#)].
- Bunn SK, Bisset WM, Main MJ, Golden BE. Fecal calprotectin as a measure of disease activity in childhood inflammatory bowel disease. *J Pediatr Gastroenterol Nutr*. 2001;32(2):171-7. [PubMed: [11321388](#)].
- Vaos G, Kostakis ID, Zavras N, Chatzemiael A. The role of calprotectin in pediatric disease. *Biomed Res Int*. 2013;2013:542363. doi: [10.1155/2013/542363](#). [PubMed: [24175291](#)]. [PubMed Central: [PMC3794633](#)].
- Caviglia GP, Ribaldone DG, Rosso C, Saracco GM, Astegiano M, Pellitano R. Fecal calprotectin: Beyond intestinal organic diseases. *Panminerva Med*. 2018;60(1):29-34. doi: [10.23736/S0031-0808.18.03405-5](#). [PubMed: [29370679](#)].
- Burri E, Beglinger C. The use of fecal calprotectin as a biomarker in gastrointestinal disease. *Expert Rev Gastroenterol Hepatol*. 2014;8(2):197-210. doi: [10.1586/17474124.2014.869476](#). [PubMed: [24345070](#)].
- Mosli MH, Zou G, Garg SK, Feagan SG, MacDonald JK, Chande N, et al. C-reactive protein, fecal calprotectin, and stool lactoferrin for detection of endoscopic activity in symptomatic inflammatory bowel disease patients: A systematic review and meta-analysis. *Am J Gastroenterol*. 2015;110(6):802-19. quiz 820. doi: [10.1038/ajg.2015.120](#). [PubMed: [25964225](#)].
- Biskou O, Gardner-Medwin J, Mackinder M, Bertz M, Clark C, Svolos V, et al. Faecal calprotectin in treated and untreated children with coeliac disease and juvenile idiopathic arthritis. *J Pediatr Gastroenterol Nutr*. 2016;63(5):e112-5. doi: [10.1097/MPG.0000000000001384](#). [PubMed: [27540707](#)].
- Shahramian I, Dehghani SM, Haghighat M, Noori NM, Teimouri AR, Sharafi E, et al. Serologic evaluation of celiac disease in patients with beta thalassemia major and control. *Gastroenterol Hepatol Bed Bench*. 2015;8(2):153-9. [PubMed: [25926941](#)]. [PubMed Central: [PMC4403028](#)].
- Shahramian I, Dehghani SM, Haghighat M, Noori NM, Teimouri A, Sharafi E, et al. Serological evaluation of celiac disease in children with congenital heart defect; a case control study. *Middle East J Dig Dis*. 2015;7(2):98-103. [PubMed: [26106470](#)]. [PubMed Central: [PMC4430799](#)].
- Theede K, Kiszka-Kanowitz M, Nordgaard-Lassen I, Nielsen AM. [Faecal calprotectin is a useful biomarker for intestinal inflammation]. *Ugeskr Laeger*. 2014;176(37). Danish. [PubMed: [25294035](#)].
- Ertekin V, Selimoglu MA, Turgut A, Bakan N. Fecal calprotectin concentration in celiac disease. *J Clin Gastroenterol*. 2010;44(8):544-6. doi: [10.1097/MCG.0b013e3181cadbc0](#). [PubMed: [20054281](#)].
- Tola MD, Marino M, Casale R, Borghini R, Donato G, Vitolo D, et al. Sa1336 anti-actin antibodies, fatty acid-binding proteins, and calprotectin as new serological markers in the diagnosis and monitoring of celiac disease. *Gastroenterology*. 2012;142(5):S-276. doi: [10.1016/S0016-5085\(12\)61043-9](#).
- Montalto M, Santoro L, Curigliano V, D'Onofrio F, Cammarota G, Panunzi S, et al. Faecal calprotectin concentrations in untreated coeliac patients. *Scand J Gastroenterol*. 2009;42(8):957-61. doi: [10.1080/00365520601173632](#). [PubMed: [17613925](#)].
- Capone P, Rispo A, Imperatore N, Caporaso N, Tortora R. Fecal calprotectin in coeliac disease. *World J Gastroenterol*. 2014;20(2):611-2. doi: [10.3748/wjg.v20.i2.611](#). [PubMed: [24574734](#)]. [PubMed Central: [PMC3923040](#)].
- Balamtekin N, Baysog Y, Uslu N, Orhan D, Akcoren Z, Ozen H, et al. Fecal calprotectin concentration is increased in children with celiac disease: relation with histopathological findings. *Turk J Gastroenterol*. 2012;23(5):503-8. doi: [10.4318/tjg.2012.0366](#). [PubMed: [23161294](#)].
- Kolho KL, Turner D, Veereman-Wauters G, Sladek M, de Ridder L, Shaoul R, et al. Rapid test for fecal calprotectin levels in children with Crohn disease. *J Pediatr Gastroenterol Nutr*. 2012;55(4):436-9. doi: [10.1097/MPG.0b013e318253cffi](#). [PubMed: [22412699](#)].
- Kostakis ID, Cholidou KG, Vaiopoulos AG, Vlachos IS, Perrea D, Vaos G. Fecal calprotectin in pediatric inflammatory bowel disease: A systematic review. *Dig Dis Sci*. 2013;58(2):309-19. doi: [10.1007/s10620-012-2347-5](#). [PubMed: [22899243](#)].
- Joishy M, Davies I, Ahmed M, Wassel J, Davies K, Sayers A, et al. Fecal calprotectin and lactoferrin as noninvasive markers of pediatric inflammatory bowel disease. *J Pediatr Gastroenterol Nutr*. 2009;48(1):48-54. doi: [10.1097/MPG.0b013e31816533d3](#). [PubMed: [19172123](#)].
- Bremner A, Roked S, Robinson R, Phillips I, Beattie M. Faecal calprotectin in children with chronic gastrointestinal symptoms. *Acta Paediatr*. 2005;94(12):1855-8. doi: [10.1111/j.1651-2227.2005.tb01870.x](#). [PubMed: [16421055](#)].