Diagnostic Value of Fecal Calprotectin in Children with Gastritis, Duodenitis and Helicobacter Pylori

Abstract

Background: Fecal calprotectin (FC) is suggested as a novel biomarker for the diagnosis of gastrointestinal (GI) diseases; however, few studies have investigated its diagnostic value for Helicobacter pylori (H. pylori). Therefore, the current study evaluated the level of FC and its diagnostic value in patients with H. Pylori and its related conditions including gastritis and duodenitis.

Methods: In this case-control study, 120 children with upper GI symptoms, who were indicated to undergo upper GI endoscopic examination, were consecutively selected. The children were categorized into different groups based on their endoscopic findings including H. pylori, gastritis, duodenitis or normal.

Results: Patients with gastritis (P = 0.014) and those with duodenitis (P < 0.001) had significantly higher FC. The level of FC was higher in patients with H. pylori but this difference was marginally significant (P = 0.054). The level of FC had poor ability to diagnose the presence of H. pylori (P = 0.054) and gastritis (area under the curve, AUC = 0.639, P = 0.014). However, it had acceptable power to diagnose patients with or duodenitis (AUC = 0.718, P < 0.001). The sensitivity and specificity of FC for diagnosis of gastritis were 64 and 65 percent (cut-off = 45.2 µg/g), and for duodenitis were 77 and 61 percent (cut-off = 46.2 µg/g), respectively.

Conclusions: FC can be considered as an objective and diagnostic tool for duodenitis. However, due to the low sensitivity and specificity, it is suggested to consider it as an objective supplementary test beside other established diagnostic modalities.

Keywords: Calprotectin, diagnosis, duodenitis, gastritis, Helicobacter pylori

Introduction

H. pylori infection, is one of the most prevalent infections worldwide.[1] A recent meta-analysis estimated that there were about four and a half billion individuals with H. pylori infection globally. Its prevalence varies from 18.9 percent in Switzerland up to 87.7 percent in Nigeria.[2] Moreover, it is reported that 42 percent of children are affected by this infection in Iran.[3] H. pylori is associated with some of the most important GI diseases including peptic ulcer, gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma.[4,5] Gastritis and duodenitis are two other prevalent GI diseases that are caused mainly due to H. pylori.[6,7] There are several diagnostic methods for H. pylori, including urea breath test (UBT), serology and fecal antigens.[8] Over the past 50 years, endoscopy has been used as the diagnostic modality of choice to diagnose H. pylori for patients with upper GI symptoms.[9] However, it is an invasive and operator-based procedure. Furthermore, it is cumbersome to perform an optimal endoscopy in children. Therefore, a lot of investigations are being conducted to find an alternative non-invasive and objective diagnostic test.[10,11] Non-invasive tests such as UBT, serology and stool antigen are usually preferred by the clinicians. However, serology has some limitations particularly in endemic areas and UBT is technically very demanding and did not widely available. Moreover, detection of stool antigen of H. pylori has poor sensitivity. Although, UBT is considered to be specific, there are always some concerns about the presence of many other urease-producing bacteria that may colonize in the stomach and make it questionable to use this test in children.[12,13]

Biomarkers are gaining increasing popularity and interest due to their higher accuracy and applicability to diagnose different diseases.[14-18] FC is one of the most reliable fecal markers for GI-related disease.[19,20] It is known as a biomarker for inflammatory bowel disease.[21] However, its diagnostic value for H. pylori has not been studied in children. Therefore, the current study evaluated the level of FC and its diagnostic value in patients with H. pylori and its related conditions including gastritis and duodenitis.

How to cite this article: Rafeey M, Nikmanesh P, Javadzadeh F. Diagnostic value of fecal calprotectin in children with gastritis, duodenitis and Helicobacter pylori. Int J Prev Med 2022:13:107.
Moreover, the augmented levels of FC are demonstrated in patients with GI infections, non-steroidal anti-inflammatory (NSAID)-induced enteropathy, microscopic colitis or gastric cancer.\textsuperscript{[21,24]} Calprotectin is a heterodimer protein that can bind with calcium and zinc. It is produced by neutrophils, monocytes and macrophages and can exert a strong antimicrobial role against bacteria and fungus.\textsuperscript{[25,26]} Increased levels of calprotectin in plasma, synovial fluid, urine and feces are seen in neutrophil-mediated immune response.\textsuperscript{[11]} Laboratory studies advocate the association of H. pylori and gastritis with neutrophil proliferation.\textsuperscript{[27,28]} Therefore, the objective of this study was to evaluate the level of FC and its diagnostic efficacy in children with H. pylori infection, gastritis and duodenitis.

\textbf{Methods}

In this case-control study, 120 children (3-16 years old) with upper GI symptoms (such as dyspepsia, dysphagia, abdominal discomfort, nausea, vomiting or poor growth) who were indicated to undergo upper GI endoscopic examination at the gastroenterology clinics or gastroenterology ward of the children hospital of Tabriz, Iran, were consecutively included for 2 years (January 2016-January 2018). Patients with polyps, GI bleeding, IBD, colorectal cancer, severe renal or liver diseases, receiving NSAID or treatment for H. pylori were excluded.

\textbf{IRB/IACUC approval}

The study was conducted following the declaration of Helsinki. Written informed consent was obtained from parents and implicit permission from children after sufficient explanations. The study protocol was approved by the medical ethics committee of the Tabriz University of Medical Sciences (IR.TBZMED.REC.1396.276).

\textbf{Patients' evaluation}

Endoscopic examinations were performed by an attending pediatric gastroenterologist with the same procedure for all included patients after adequate sedation. The gastroenterologist was blinded to the result of FC. Full description and captured photos during endoscopy were documented and reviewed by another gastroenterologist. H. pylori was confirmed by histopathological and laboratory evaluations of the obtained specimens. Gastritis and duodenitis were detected by specific signs through the endoscopic examinations (i.e., nodularity, erosions, intramucosal hemorrhage, redness). Biopsies were collected from different sites to assess the presence of H. pylori. Patients were divided into different groups based on their endoscopic findings including H. pylori, gastritis, duodenitis or normal.

FC was measured by obtaining a stool sample once for each patient. The samples were collected by parents at home one day before bowel preparation for endoscopic examination. The parents were instructed to keep the samples in the refrigerator. Moreover, they were stored in a refrigerator (4°C) after they were delivered at the endoscopy session until they were transferred to the study laboratory within 48 hours. Calprotectin remains unchanged at room temperature up to 7 days.\textsuperscript{[29]} FC was measured by means of enzyme-linked immunoabsorbent assay (the ELISA, Calprest; Eurostatus) according to the method of Ton et al.\textsuperscript{[30]}; however, a short description is provided further. The mean of two measurements on each sample was reported as the concentration of FC. The FC cut-off level was considered as 50 µg/g according to the manufacturer’s instructions. The laboratory personnel were blinded to the clinical diagnosis of the patients.

\textbf{FC laboratory measurement}

A 100 mg feces of obtained samples were homogenized in 5 mL extraction buffer. Then, the homogenate was centrifuged in a microcentrifuge for 5 min. Approximately 100 µL of the diluted supernatant (1:50 with incubation buffer) were incubated at room temperature onto a microtiter plate coated with a monoclonal capture antibody highly specific to the calprotectin heterodimeric and polymeric complexes. Next, a second incubation with a specific detection antibody was conducted. After that tetramethylbenzidine (blue color formation) followed by a stop solution (change to yellow color) was added. Spectrophotometry was performed with an optical density of 450 nm.

\textbf{Statistical analysis}

Mean ± standard deviation (SD) or median (interquartile range [IQR]) of quantitative variables with normal or non-normal distribution, respectively, and frequency and percentage for qualitative variables were reported. Mann-Whitney U test was conducted to compare two groups. Multivariate regression analysis was conducted to control the confounding effects and find the independent associations between variables. A receiver operating characteristic (ROC) curve analysis was performed to calculate the AUC as well as the sensitivity and specificity of FC cut-off points. The best cut-off point was calculated using Youden’s J statistic. SPSS version 24 was used to perform statistical analysis. A $P$ value of less than 0.05 was considered significant.

\textbf{Results}

An overall 120 patients were included. The mean age of included patients was 8.07 ± 2.71 and 51.7% were female. H. pylori was detected in 60 patients (50%). Gastritis was present in 82 (68.3%) of included patients of whom 43 patients (52.4%) had positive H. pylori. Duodenitis was seen in 40 patients (33.3%) of whom 32 cases (40%) had positive H. pylori and 38 (95%) had concomitant gastritis. Among patients with gastritis, 43 patients (52.4%) had acute gastritis and 39 patients (47.6%) had chronic gastritis.
An overall 58 patients (48.3%) had a FC above 50 μg/g. The proportions of patients with increased FC are described in Figure 1. The level of FC in patients with H. pylori was higher than those without, but this difference was marginally significant (median [IQR], 51 [32.5–71] vs 42.5 [18–63] μg/g, respectively, P = 0.054). Patients with gastritis had significantly higher FC than non-gastritis patients (53.5 [32–70] vs 38.7 [18–57] μg/g, respectively, P = 0.014). Those with duodenitis had significantly higher FC than non-duodenitis patients (60 [47.5–124.5] vs 39 [17.5–60] μg/g, respectively, P < 0.001). The levels of FC in different categories are depicted in Figure 2. Higher FC level was detected in patients with acute gastritis as compared with those having chronic gastritis but the difference was not statistically significant (59 [32–99] vs 52 [18–69] μg/g, respectively, P = 0.294).

The results of multivariate analysis are described in Table 1. FC was independently associated with duodenitis (adjusted OR, 3.147 [95% CI, 1.217–8.142]; P value, 0.018). However, no significant independent association was detected between FC and gastritis (adjusted OR, 1.713 [95% CI, 0.685–4.283]; P value, 0.250) and H. pylori (adjusted OR, 1.161 [95% CI, 0.517–2.606]; P value, 0.717).

ROC curve analysis demonstrated that the level of FC had poor ability to diagnose the presence of H. pylori (AUC = 0.602, 95% CI = 0.501–0.703, P = 0.054) or gastritis (AUC = 0.639, 95% CI = 0.534–0.744, P = 0.014) [Figure 3]. However, it had acceptable power to diagnose patients with or duodenitis (AUC = 0.718, 95% CI = 0.624–0.812, P < 0.001). The sensitivity and specificity of FC higher than 45.2 μg/g for diagnosis of gastritis were 64 and 65 percent, respectively. The sensitivity and specificity of FC higher than 46.2 μg/g for diagnosis of duodenitis were 77 and 61 percent, respectively.

**Discussion**

H. pylori has been identified as the main cause of peptic ulcers and a significant risk factor for gastric cancer.[31] Therefore, due to the high importance of the diagnosis of this bacterium and its related conditions including gastritis and duodenitis, the current study was conducted to evaluate the levels of FC in patients who were referred for endoscopic examinations. The results of the current study showed that patients with H. pylori, gastritis and duodenitis had a higher level of FC. However, further analysis showed that the level of FC could not be a good diagnostic factor for H. pylori and gastritis. But, the FC level was demonstrated to be an appropriate diagnostic test for duodenitis. Nevertheless, the sensitivity and specificity of FC for gastritis and duodenitis were relatively low to be considered as the single diagnostic test but it can be used as a supplementary test besides other established diagnostic modalities. Accordingly, a study by Manz et al. showed that the level of FC in patients with gastritis was significantly higher than in healthy individuals.[32] The study by Ataee et al. further confirmed that the level of FC had a significant relationship with the presence of gastritis as well as the severity of H. pylori infection.[33] Similar to our findings, Montalto et al. failed to find any significant relationship between the levels of calprotectin with gastritis.[34] Also, the study of Wang et al. showed that patients with gastritis, peptic ulcer or duodenitis had the same level of FC as healthy people.[35] However, it is noteworthy that gastritis patients included in both of these studies consisted of only those with chronic gastritis, and no patient with acute gastritis was included. We demonstrated that the level of FC in patients with acute gastritis was higher than those with chronic gastritis possibly due to higher inflammatory status. The concentration of FC in the study of Ataee et al. was significantly higher in chronic active gastritis than chronic non-active gastritis.[33] Therefore, the lack of increase in the level of FC in gastritis patients in the studies of Wang et al. and Montalto et al. may be explained by the exclusion...
of the patients with acute gastritis. Previous studies have also shown that inflammation in the gastrointestinal tract can increase the level of FC.\textsuperscript{[11,35]} Furthermore, it has been postulated that the level of FC is associated with the severity of inflammations and the extent of the lesions within the GI tract.\textsuperscript{[32]}

\textit{In vivo} studies have shown that H. pylori is resistant to the antimicrobial effects of calprotectin by means of specific lipid changes and biofilm formation.\textsuperscript{[36]} Therefore, increased calprotectin during inflammation against H. pylori cannot inhibit the activity of this microorganism. However, it may have a diagnostic value for detecting gastritis and duodenitis caused by H. pylori.

The distinctive structural and biological characteristics of H. pylori cause considerable resistance sometimes even against heavy therapeutic regimes. Therefore, timely detection of this microorganism can play an important role in the treatment and management of the disease before it leads to more severe complications such as gastric cancer. However, FC was not validated by our results to be an appropriate candidate for this purpose. Therefore, future studies are warranted to investigate other modalities for this goal.

This study was the first study that evaluated the applicability of FC in the diagnosis of H. pylori, gastritis and duodenitis; however, there were some limitations in our study. The study was conducted only on children due to the paucity of related studies in this regard. Therefore, the results may not be valid in adult patients. Thus, it could be better if this study was conducted as a multi-center study in all age groups which can be considered for future studies.

\textbf{Conclusion}

FC is increased in patients with H. pylori, gastritis and duodenitis. It can be considered as an objective and diagnostic tool for duodenitis. However, due to the low sensitivity and specificity of FC, it can be considered as an objective supplementary test besides other established diagnostic modalities.

\textbf{Declaration of patient consent}

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the scientific journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity.
Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

Received: 28 Oct 20 Accepted: 29 Dec 20 Published: 08 Aug 22

References
1. Zamani M, Ebrahimtabar F, Zamani V, Miller WH, Alizadeh-Navaei R, Shokri-Shirvani J, et al. Systematic review with meta-analysis: The worldwide prevalence of Helicobacter pylori infection. Aliment Pharmacol Ther 2018;47:868-76.
2. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of Helicobacter pylori infection: Systematic review and meta-analysis. Gastroenterology 2017;153:420-9.
3. Moosazadeh M, Lankarani KB, Afshari M. Meta-analysis of the prevalence of Helicobacter pylori infection among children and adults of Iran. Int J Prev Med 2016;7:48.
4. McColl KE. Clinical practice. Helicobacter pylori infection. N Engl J Med 2010;362:1597-604.
5. Rafeey M, Nikvash S. Detection of Helicobacter pylori antigen in stool samples for diagnosis of infection in children. East Mediterr Health J 2007;13:1067-72.
6. Owen DR, Owen DA. Celiac disease and other causes of duodenitis. Arch Pathol Lab Med 2018;142:35-43.
7. Azer SA, Akhondi H. Gastritis. StatPearls [Internet]: StatPearls Publishing; 2019.
8. Garza-Gonzalez E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol 2014;20:1438-49.
9. Kalach N, Bontems P, Raymond J. Helicobacter pylori infection in children. Helicobacter 2017;22(Suppl 1):e12414.
10. McColl KE, Murray LS, Gillen D, Walker A, Wirz A, Fletcher J, et al. Randomised trial of endoscopy with testing for Helicobacter pylori compared with non-invasive H pylori testing alone in the management of dyspepsia. BMJ 2002;324:999-1002.
11. Erbayrak M, Turkyay C, Eraslan E, Cetinkaya H, Kasapoglu B, Bektas M. The role of fecal calprotectin in investigating inflammatory bowel diseases. Clinics (Sao Paulo) 2009;64:421-5.
12. Korkmaz H, Kesli R, Karabagli P, Terzi Y. Comparison of the diagnostic accuracy of five different stool antigen tests for the diagnosis of Helicobacter pylori infection. Helicobacter 2013;18:384-91.
13. Khaliilpour A, Kazemzadeh-Narbat M, Tamayol A, Oktlu R, Khademhosseini A. Biomarkers and diagnostic tools for detection of Helicobacter pylori infection. Appl Microbiol Biotechnol 2016;100:4723-34.
14. Edelman CL. Biomarkers in acute kidney injury. Biomarkers of Kidney Disease. Elsevier; 2017. p. 241-315.
15. Nakamura A, Kaneko N, Villemagne VL, Kato T, Doeecke J, Dore V, et al. High performance plasma amyloid-beta biomarkers for Alzheimer’s disease. Nature 2018;554:249-54.
16. Wang J, Chen J, Sen S. MicroRNA as biomarkers and diagnostics. J Cell Physiol 2016;231:25-30.
17. Molaei A, Khomahani A, Sadeghi-Shabestari M, Ghaffari S, Sadat-Ebrahimim S-R. Cardiac biomarkers for early detection of cardiac involvement in children with Kawasaki disease: A cross-sectional study. Int J Pediatr 2019;7:10573-82.
18. Sadat-Ebrahimim S-R. Diagnostic and prognostic value of cardiac biomarkers in children with Kawasaki disease: A state-of-the-art review. Int J Pediatr 2020;8:10911-28.
19. Poullis A, Foster R, Shetty A, Fagerhol MK, Mendall MA. Bowel inflammation as measured by fecal calprotectin: A link between lifestyle factors and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2004;13:279-84.
20. Shabestari MS, Rafeey M, Shooran M, Shirvani S. Association between fecal calprotectin concentration and mesenteric lymphadenopathy in children. Crescent J Medical Sci 2020;7:338-42.
21. Hanek K, Hausken T, Morken MH, Strand EA, Morch K, Coll P, et al. Persisting symptoms and duodenal inflammation related to Giardia duodenalis infection. J Infect 2007;55:524-30.
22. Berni Canani R, Rapacciuolo L, Romano MT, Tanturri de Horatio L, Terrin G, Manguso F, et al. Diagnostic value of faecal calprotectin in paediatric gastroenterology clinic practice. Dig Liver Dis 2004;36:467-70.
23. Poullis A, Foster R, Mendall MA, Fagerhol MK. Emerging role of calprotectin in gastroenterology. J Gastroenterol Hepatol 2003;18:756-62.
24. Gisbert JP, McNicholl AG. Questions and answers on the role of faecal calprotectin as a biological marker in inflammatory bowel disease. Dig Liver Dis 2009;41:56-66.
25. Montalto M, Gallo A, Ianiro G, Santoro L, D’Onofrio F, Ricci R, et al. Can chronic gastritis cause an increase in fecal calprotectin concentrations? World J Gastroenterol 2010;16:3406-10.
26. Costa F, Mummolo MG, Bellini M, Romano MR, Ceccerelli L, Arpe P, et al. Role of faecal calprotectin as non-invasive marker of intestinal inflammation. Dig Liver Dis 2003;35:642-7.
27. Chu TH, Huang ST, Yang SF, Li CJ, Lin HW, Weng BC, et al. Hepatoma-derived growth factor participates in Helicobacter Pylori-induced neutrophils recruitment, gastritis and gastric carcinogenesis. Oncogene 2019;38:6461-77.
28. Whitmore LC, Weems MN, Allen LH. Cutting edge: Helicobacter pylori induces nuclear hypersegmentation and subtype differentiation of human neutrophils in vitro. J Immunol 2017;198:1793-7.
29. Roseth AG, Fagerhol MK, Aadland E, Schjonsby H. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol 1992;27:793-8.
30. Ton H, Brandsnes, Dale S, Holtlund J, Skuibina E, Schjonsby H, et al. Improved assay for fecal calprotectin. Clin Chim Acta 2000;292:41-54.
31. Kyburz A, Muller A. Helicobacter pylori and extragastric diseases. Curr Top Microbiol Immunol 2017;400:325-47.
32. Manz M, Burri E, Rothen C, Tchanguizi N, Niederberger C, Rossi L, et al. Value of fecal calprotectin in the evaluation of patients with abdominal discomfort: An observational study. BMC Gastroenterol 2012;12:5.
33. Atae P, Afsariabi V, Nirkkohoo B, Sani MN, Rahhegh R, Ghaderi E, et al. Relationship between fecal calprotectin and upper endoscopy findings in children with upper gastrointestinal symptoms. Iran J Pediatr 2017;27:e8658.
34. Wang S, Wang Z, Shi H, Heng L, Juan W, Yuan B, et al. Faecal calprotectin concentrations in gastrointestinal diseases. J Int Med Res 2013;41:1357-61.
35. Hestvik E, Tumwine JK, Tylleskar T, Grahnquist L, Ndeezi G, Kaddu-Mulindwa DH, et al. Faecal calprotectin concentrations in apparently healthy children aged 0-12 years in urban Kampala, Uganda: A community-based survey. BMC Pediatr 2011;11:9.
36. Gaddy JA, Radin JN, Cullen TW, Chazin WJ, Skaar EP, Trent MS, et al. Helicobacter pylori resists the antimicrobial activity of calprotectin via lipid a modification and associated biofilm formation. mBio 2015;6:e01349-15.

International Journal of Preventive Medicine 2022, 13: 107