Journal of Research in Clinical Medicine

J Res Clin Med. 2025;13:34998
doi: 10.34172/jrcm.025.34998
https://jrcm.tbzmed.ac.ir

TUOMS PRESS

Original Article

Examination of fecal calprotectin level in patients with Entamoeba histolytica from 2020 to 2022

Seyed Mehdi Haghdoost[1], Hamid Owaysee Osquee[1], Nasrin Jangi Baktash[1], Shahin Jafarpour[1], Sina Hamzehzadeh[2*]

[1]Infectious Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2]Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Article info

Article History:
Received: July 28, 2024
Revised: October 9, 2024
Accepted: October 13, 2024
ePublished: June 8, 2025

Keywords:
Diagnosis,
Entamoeba histolytica,
Feces, Calprotectin

Abstract

Introduction: The calprotectin test is a non-invasive test used to diagnose patients who have diarrhea and are suspected of inflammatory bowel disease (IBD); The purpose of this study is to determine whether the fecal calprotectin test is positive in gastroenteritis caused by *Entamoeba histolytica* (amoebiasis) or not.

Methods: This research was a descriptive-analytical cross-sectional study. Fifty patients were included in the study with an accessible or easy sampling method. Blood samples were obtained from all patients to check blood cells and erythrocyte sedimentation rate (ESR). Fecal calprotein (FCP) was measured by ELISA method with a laboratory kit in Jihad University Laboratory and accredited laboratories in Tabriz city.

Results: Among all the participants in the study, 19 of them had feces levels higher than 50 mg/kg; among these, the number of male patients was significantly higher than females ($P=0.035$). The results indicated that in comparison with the group with negative result for calprotectin, the group with positive results for calprotectin has more patients with the duration of more than 7 days for the disease ($P=0.018$). Calprotectin levels in patients with severe disease were significantly higher than calprotectin levels in patients with mild symptoms ($P=0.009$).

Conclusion: Calprotectin levels increase in patients with amebiasis. As seen in this research, the fecal calprotectin test was positive in patients with severe or prolonged amebiasis.

Introduction

Amebiasis is a parasitic infectious disease caused by *Entamoeba histolytica* in the human body.[1] Abundant biochemical, genetic, and immunological studies have proven that the organism known as *E. histolytica* consists of two identical morphological species; however, their biological treatment and pathogenesis are different. One of these species is *E. histolytica*, which is a potentially invasive pathogen. This pathogen can cause amebiasis colitis and some types of extraintestinal amebiasis. Amebiasis can be asymptomatic. Another species, which is called *Entamoeba dispar*, coexists in the human bowel. This species is non-invasive and does not cause disease.[2] In Iran, amebiasis is endemic, and its prevalence varies from 8% to 40%.[3]

During recent decades, many leukocytic products have been discovered that can be secreted into feces. These products are considered as bowel inflammatory factors. Among these products, calprotectin and lactoferrin are the best biomarkers.[4] These proteins are produced in monocytes, neutrophils, endothelium, and epithelium cells.[5] Following the activation of neutrophils and monocyte attachment to epithelium or endothelium cells, calprotectin is released, and its level in serum and body fluids is an important inflammatory factor.5[5] Calprotectin is increased in urine, feces, and synovial fluid during inflammatory conditions and malignancies. In inflammatory bowel disease (IBD), neutrophils migrate into the bowel lumen, which can explain why the calprotectin level rises in this disease.[6] So, the level of calprotectin in feces is related to the number of neutrophil cells migrated into the bowel lumen.[7]

Studies have shown that calprotectin level measurement can distinguish between IBD and other non-inflammatory diseases.[8] Also, calprotectin can determine the severity of mucus inflammation, and its levels are correlated to the severity of inflammation and endoscopic indices. As endoscopy procedure is costly and also less intolerable for patients, utilizing an accessible, non-invasive, and inexpensive biomarker like calprotectin is valuable for clinicians.[9] Because of these roles of calprotectin, we designed this study to determine if calprotectin level can help us distinguish gastroenteritis caused by *E. histolytica* or not.

Material and Methods

Study design and setting

This is a descriptive-analytic study, conducted in the Sina

***Corresponding Author:** Sina Hamzehzadeh, Email: hamzehzadehsina.shz@yahoo.com

and Imam Reza Hospital of Tabriz University of Medical Sciences clinics, between 2020–2022. In this study, all the patients with a positive result of *E. histolytica* in stool exam were involved. To reduce distracting factors, patients with gastrointestinal (GI) malignancies, IBD, immunodeficiency, and who were undergoing radiotherapy and chemotherapy were excluded from the study.

In this study, convenience sampling was done. According to the similar studies, by using sample size calculation formulas (with 90% power and 5% of type 1 error, or alpha) and consideration of 1.4 variances, the sample size was calculated as at least 50 patients.

Data collection and study performing

Patients' characteristics like age, gender, medical history, and current treatment was obtained by using a checklist. A blood sample was obtained from all the patients to check complete blood count (CBC) and erythrocyte sedimentation rate (ESR). Also, the patients' stool was examined to measure the level of fecal calprotectin (FCP). Patients' feces were kept in plastic containers at the temperature of -20 centigrade for 6 hours. FCP level was measured by ELISA method and laboratory kits (Calprest, Dynex Elisa Eurospital, Trieste, Italy). FCP is expressed by milligrams per kilogram of moist feces unit. Its normal range is defined as less than 50 milligrams per kilogram of moist feces.

Statistical analysis

All data were analyzed using IBM SPSS 26, and the Kolmogorov-Smirnov test was used to determine the normality of the collected data. According to the distribution of quantitative data, the mean and standard deviation were reported. We also reported the nominal and ordinal variables with frequencies and percentages.

Results

As mentioned above, 50 participants were involved in this study. Thirty-one participants were male and the rest were female (19 participants). Their mean age was 49.8 years, estimated at 47.5 for females and 50.9 for males. Females' mean age was less than that of males but the difference was not significant ($P=0.259$). Among these participants, 19 participants had more than 50 mg/kg calprotectin in their feces which is considered as positive. The number of males in this group of participants (more than 50 mg calprotectin in a kilogram of feces) was significantly more than that of females ($P=0.035$).

Among participants with positive calprotectin in stool, 3 participants had a history of omeprazole consumption, and 3 of them had a history of non-steroidal anti-inflammatory drugs (NSAIDs) use. Among 3 participants with a negative calprotectin test, 2 of them had consumed NSAIDs and 1 of them had consumed omeprazole. However, there was not a significant difference between drug consumption and the result of the calprotectin test ($P=0.369$). The results of our study show that there was no significant relation between the calprotectin level and mean age among participants ($P=0.695$).

In this study, in terms of the duration of the disease, patients were divided into two groups; more than 7 days and less than 7 days. The results show that the number of patients with positive calprotectin was more in the group of more than 7 days of disease duration, compared to the group of less than 7 days of disease duration ($P=0.018$). Meanwhile, there is no significant dispute between females and males in the aspect of the duration of the disease ($P=0.524$) (Table 1).

Participants were divided into two groups in terms of severity of symptoms: mild and severe. 31 of them were in the severe group, and the rest of them were in the mild group. Calprotectin level was significantly higher in the severe group than the mild one ($P=0.009$). Meanwhile, there is no significant dispute between the severity of symptoms and calprotectin levels in terms of gender ($P=0.660$) (Table 2).

Discussion

Entamoeba is a monocellular parasite that can cause infection in the bowel. The symptoms of this infection include diarrhea, crampy pain, nausea, and vomiting. Many indices are used to diagnose and follow up on these patients; one of these indices is assessing fecal calprotectin. Fecal calprotectin can also demonstrate the severity of infection.

Recent studies illustrate that the level of fecal calprotectin can be elevated in Entamoeba-infected patients. This protein is produced and secreted by mucosal cells of the bowel and stomach and plays an important role against microbes. In the case of Entamoeba infection, a microbe's invasion into mucus can lead to an increase in fecal

Table 1. Examination of disease duration in the aspect of gender and calprotectin level

Duration	Calprotectin positive		Calprotectin negative	
	Female	Male	Female	Male
under 7 days	2	3	5	8
Over 7 days	4	10	8	10
P value for positive and negative results	0.018			
P value for male and female results	0.524			

Table 2. Examination of disease severity in the aspect of gender and calprotectin level

Severity	Calprotectin positive		Calprotectin negative	
	Female	Male	Female	Male
mild	2	4	4	8
Severe	3	10	9	9
P value for positive and negative results	0.009			
P value for male and female results	0.660			

calprotectin secretion.[10]

In a recent novel study, scientists found that fecal calprotectin level in patients with severe infection is significantly higher than in patients with mild disease.[11] As a result, fecal calprotectin level can predict the severity of the disease in addition to its diagnosis.[12]

The results of a study show that the level of fecal calprotectin in patients with dysentery (bloody diarrhea) is higher in comparison with patients without dysentery.[8] In another study, calprotectin levels in hospitalized patients with severe symptoms were higher than outpatients with mild symptoms.[13]

In a comparative study, fecal calprotectin levels in IBD patients were measured. It was demonstrated that the level of calprotectin increases with the severity of the disease. Also, this increase was more common among patients with IBD and infectious causes of bowel inflammation.[14]

In another study, the results showed that fecal calprotectin levels in other infections like *C. difficile* were higher than normal ranges. As a result, although calprotectin is a sensitive marker for diagnosis and prediction of severity of disease, it is not a specific marker.[15]

Regarding these results, fecal calprotectin levels can be a useful index for the diagnosis and severity prediction of diseases like intestinal amebiasis and other causes of bowel inflammation. This can help clinicians make better decisions for the treatment of patients and provide appropriate follow-ups.[16]

In a comparative study, fecal calprotectin level was measured in two groups of patients: ulcerative colitis and amebiasis colitis; and then compared.[17] The result showed that patients with amebiasis colitis had higher levels than patients with ulcerative colitis. It turns out that calprotectin levels can be useful in distinguishing the cause of colitis.[18]

In another study, fecal calprotectin level was compared with levels of specific antigens of Entamoeba in infected patients.[19] It showed that the levels of these two parameters are correlated with each other. In other words, as the level of specific antigens rises, calprotectin level increases. This also indicated that this index can be used to predict the severity of the disease.[13]

In a study, the level of fecal calprotectin was measured and compared in two groups of patients: with symptoms and without symptoms.[20] The results showed that fecal calprotectin in symptomatic patients was higher than in asymptomatic patients, which illustrates that it can be useful for diagnosis and prediction of the severity of the disease.[14]

Although all these studies show that fecal calprotectin level is a sensitive marker for diagnosing infection, other precise tests like microscopic investigation or molecular tests must be done in order to confirm the diagnosis. These tests are used to differentiate the diagnosis and distinguish Entamoeba infection from other causes of bowel infection.[21]

A similar study, in which the calprotectin level was measured in IBD patients, showed that as IBD becomes severe, calprotectin level increases. This is a general pattern for all IBDs. Moreover, it can be used to assess the severity of the disease and its follow-up.[22]

According to the results of our study, as the duration of the disease extends, fecal calprotectin level increases, which can show the severity of inflammation and the body's immune system response to infection.[23] To confront this infection, the immune system becomes activated, and inflammatory cells, along with cytokines and inflammatory proteins like calprotectin, are present in the bowel.[24] That can explain why calprotectin levels rise. Extended duration of disease can indicate that inflammation is persistent in the bowel. However, fecal calprotectin is one of the markers used to diagnose and follow up on disease and it is worth mentioning that for confirmation of the diagnosis, other methods and tests are needed.

Conclusion

As a conclusion, fecal calprotectin levels can help clinicians diagnose and predict the severity of disease in patients infected with amebiasis, especially in long-term conditions.

Acknowledgments

The research protocol was approved and supported by the Infectious Disease Research Center of Tabriz University of Medical Sciences.

Authors' Contribution

Conceptualization: Seyed Mehdi Haghdoost.
Data curation: Nasrin Jangi Baktash.
Formal analysis: Nasrin Jangi Baktash.
Funding acquisition: Hamid Owaysee Osquee.
Investigation: Seyed Mehdi Haghdoost.
Methodology: Seyed Mehdi Haghdoost.
Project administration: Hamid Owaysee Osquee.
Resources: Shahin Jafarpour.
Software: Sina Hamzehzadeh.

Study Highlights

What is current knowledge?

- Calprotectin is an immunological factor secreted by neutrophils and monocytes into the bowel lumen and plays an important role in inflammations. This factor increases in inflammatory processes like IBD. Current studies have proven that the level of calprotectin in feces is correlated with the severity of IBD.

What is new here?

- This study is designed to find out if fecal calprotectin level can be useful in distinguishing amebiasis, and if there is a possible correlation between infection severity and fecal calprotectin level. This study illustrates that calprotectin levels increase in amebiasis infection and also its level is correlated with the severity of infection.

Supervision: Seyed Mehdi Haghdoost.
Validation: Shahin Jafarpour.
Visualization: Sina Hamzehzadeh.
Writing–original draft: Sina Hamzehzadeh.
Writing–review & editing: Seyed Mehdi Haghdoost.

Competing Interests
There is no conflict of interest in this work**.**

Ethical Approval
The study process was reviewed and approved by the ethics committee of Tabriz University of Medical Sciences, according to the declaration of Helsinki (ethics code: IR.TBZMED.REC.1401.959). Before collecting data, informed consent was obtained from all patients. All methods were carried out following relevant guidelines and regulations.

Funding
This work was supported by Deputy for Research of Tabriz University of Medical Sciences.

References
1. Ali IK. Intestinal amebae. Clin Lab Med. 2015;35(2):393-422. doi: 10.1016/j.cll.2015.02.009.
2. Hamzah Z, Petmitr S, Mungthin M, Leelayoova S, Chavalitshewinkoon-Petmitr P. Differential detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* by a single-round PCR assay. J Clin Microbiol. 2006;44(9):3196-200. doi: 10.1128/jcm.00778-06.
3. Hung CC, Ji DD, Sun HY, Lee YT, Hsu SY, Chang SY, et al. Increased risk for *Entamoeba histolytica* infection and invasive amebiasis in HIV seropositive men who have sex with men in Taiwan. PLoS Negl Trop Dis. 2008;2(2):e175. doi: 10.1371/journal.pntd.0000175.
4. Al-Harthi SA, Jamjoom MB. Preliminary study of the prevalence of intestinal parasites among diarrheic inhabitants in Makkah Al-Mukarramah. J Egypt Soc Parasitol. 2007;37(2):671-80.
5. Ngui R, Angal L, Fakhrurrazi SA, Lian YL, Ling LY, Ibrahim J, et al. Differentiating *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* using nested polymerase chain reaction (PCR) in rural communities in Malaysia. Parasit Vectors. 2012;5:187. doi: 10.1186/1756-3305-5-187.
6. Ebrahimzadeh Leylabadlo H, Hamzehzadeh S, Sarbakhsh P, Zoghi S, Ghotaslou R. Seroprevalence of anti-*Helicobacter pylori* antibodies in population of Azerbaijan, Iran. Med Immunol (Russia). 2023;25(6):1389-94. doi: 10.15789/1563-0625-soa-2605.
7. Khairnar K, Parija SC. A novel nested multiplex polymerase chain reaction (PCR) assay for differential detection of *Entamoeba histolytica*, *E. moshkovskii* and *E. dispar* DNA in stool samples. BMC Microbiol. 2007;7:47. doi: 10.1186/1471-2180-7-47.
8. Varshochi M, Ravanbakhsh Gavgani R, Ravanbakhsh Ghavghani F, Hamzehzadeh S. A 42-year-old female with sternoclavicular arthritis and breast abscess caused by brucellosis: a case report. Clin Case Rep. 2023;11(11):e8071. doi: 10.1002/ccr3.8071.
9. Bjarnason I. The use of fecal calprotectin in inflammatory bowel disease. Gastroenterol Hepatol (N Y). 2017;13(1):53-6.
10. Quail MA, Russell RK, Van Limbergen JE, Rogers P, Drummond HE, Wilson DC, et al. Fecal calprotectin complements routine laboratory investigations in diagnosing childhood inflammatory bowel disease. Inflamm Bowel Dis. 2009;15(5):756-9. doi: 10.1002/ibd.20820.
11. Tajoddini S, Shams Vahdati S. Ultrasonographic diagnosis of abdominal free fluid: accuracy comparison of emergency physicians and radiologists. Eur J Trauma Emerg Surg. 2013;39(1):9-13. doi: 10.1007/s00068-012-0219-5.
12. Iborra M, Beltrán B, Nos P. Noninvasive testing for mucosal inflammation in inflammatory bowel disease. Gastrointest Endosc Clin N Am. 2016;26(4):641-56. doi: 10.1016/j.giec.2016.06.005.
13. Bustinduy AL, Sousa-Figueiredo JC, Adriko M, Betson M, Fenwick A, Kabatereine N, et al. Fecal occult blood and fecal calprotectin as point-of-care markers of intestinal morbidity in Ugandan children with *Schistosoma mansoni* infection. PLoS Negl Trop Dis. 2013;7(11):e2542. doi: 10.1371/journal.pntd.0002542.
14. Munasinghe VS, Vella NG, Ellis JT, Windsor PA, Stark D. Cyst formation and faecal-oral transmission of *Dientamoeba fragilis*--the missing link in the life cycle of an emerging pathogen. Int J Parasitol. 2013;43(11):879-83. doi: 10.1016/j.ijpara.2013.06.003.
15. Hooshyar H, Rostamkhani P, Arbabi M, Delavari M. Giardia lamblia infection: review of current diagnostic strategies. Gastroenterol Hepatol Bed Bench. 2019;12(1):3-12.
16. 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 clinical practice. Dig Liver Dis. 2004;36(7):467-70. doi: 10.1016/j.dld.2004.02.009.
17. Ala A, Shams Vahdati S, Kheslati E, Abri Aghdam B, Rahnemayan S. Importance of intravenous fluid administration in patients' reference to the emergency department from patients and their companions' point of view. Gazi Med J. 2022;33(4):352-5. doi: 10.12996/gmj.2022.79.
18. Hosseini SV, Jafari P, Taghavi SA, Safarpour AR, Rezaianzadeh A, Moini M, et al. Fecal calprotectin is an accurate tool and correlated to seo index in prediction of relapse in Iranian patients with ulcerative colitis. Iran Red Crescent Med J. 2015;17(2):e22796. doi: 10.5812/ircmj.22796.
19. Pham Duc P, Nguyen-Viet H, Hattendorf J, Zinsstag J, Dac Cam P, Odermatt P. Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam. Parasit Vectors. 2011;4:102. doi: 10.1186/1756-3305-4-102.
20. Petri WA Jr, Haque R, Lyerly D, Vines RR. Estimating the impact of amebiasis on health. Parasitol Today. 2000;16(8):320-1. doi: 10.1016/s0169-4758(00)01730-0.
21. Pierce KK, Kirkpatrick BD. Update on human infections caused by intestinal protozoa. Curr Opin Gastroenterol. 2009;25(1):12-7. doi: 10.1097/mog.0b013e32831da7dd.
22. Utzinger J, Botero-Kleiven S, Castelli F, Chiodini PL, Edwards H, Köhler N, et al. Microscopic diagnosis of sodium acetate-acetic acid-formalin-fixed stool samples for helminths and intestinal protozoa: a comparison among European reference laboratories. Clin Microbiol Infect. 2010;16(3):267-73. doi: 10.1111/j.1469-0691.2009.02782.x.
23. Norhayati M, Fatmah MS, Yusof S, Edariah AB. Intestinal parasitic infections in man: a review. Med J Malaysia. 2003;58(2):296-305.
24. Santos HL, Bandea R, Martins LA, de Macedo HW, Peralta RH, Peralta JM, et al. Differential identification of *Entamoeba* spp. based on the analysis of 18S rRNA. Parasitol Res. 2010;106(4):883-8. doi: 10.1007/s00436-010-1728-y.