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Is There any Association between Fecal Calprotectin Levels and Cystic Fibrosis?

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Abstract

Background: Early diagnosis of cystic fibrosis (CF) and hence the initiation of symptomatic and prophylactic treatment can help improve the outcomes of CF patients. Fecal calprotectin (FC) is a marker of inflammation in the intestinal tract that is elevated in several gastrointestinal inflammatory diseases, but little is known about its value in CF.

Objectives: This study, therefore, aimed to survey the associations of FC among CF patients and its correlation with the clinical manifestations of CF.

Methods: This descriptive cross-sectional study was performed on 52 children with CF visiting the Children's Hospital of Tabriz, Iran, during 2018-2019, selected through the census sampling method. The calprotectin values of <50 µg/g were considered normal.

Results: There was no clinically relevant association among FC levels concerning pancreatic insufficiency, diabetes, and airway colonization with Pseudomonas. In a comparison of the number of exacerbations and Forced Expiratory Volume in the first second (FEV1), the levels of electrolytes showed no significant difference between patients with normal calprotectin and elevated FC. The results of the performed analysis indicated no significant difference between the low-fecal elastase and the FC levels in patients with CF (P=0.53).

Conclusion: Demographic and clinical parameters, such as age, gender, FEV1, or Body Mass Index, had no clinically significant relationship with FC. However, serial and longitudinal calprotectin levels should also be checked, which may have clinical relevance during symptomatic episodes.

Keywords: Calprotectin, Children, Cystic fibrosis, Mucosal inflammation, Pancreatic insufficiency

1. Background

Cystic fibrosis (CF) is a disease that affects the exocrine glands and is mainly recognized with the involvement of the respiratory and digestive systems. It results in frequent pulmonary infections, progressive pulmonary damage, pancreatic enzyme disorder, and early death (1, 2). In addition, the risk of digestive and hepatobiliary malignancies is higher in CF patients than in healthy people (3).

Crucial advancements in the treatment of these patients over time have increased their survival after diagnosis from several months to several decades. Early diagnosis and hence early treatment initiation can lead to better clinical outcomes in these patients because the lower the organ damage is at the beginning of the treatment, the higher the quality of the patient's life will be (4, 5).

Calprotectin is a protein with a regulatory role in inflammatory processes, as well as antibacterial and pro-apoptotic effects, which may be due to its adhesion to calcium and zinc ions. Secretion of calprotectin in neutrophils, monocytes, and macrophages increases in inflammatory bowel disease (IBD) (6). The increased Fecal Calprotectin (FC) level is associated with the activity of different diseases, including IBD, food allergies, celiac disease, and intestinal bacterial infections. The relationship between FC levels and CF activity has been suggested recently (7).

Given that CF is a progressive multiorgan disease, its early diagnosis and hence the initiation of symptomatic and prophylactic treatment can help improve the outcomes of these patients. In addition, if there is a relationship between the calprotectin level and prognostic factors, as well as the disease activity in each organ, activity and response to treatment can be predicted by measuring the level of this protein.

2. Objectives

Therefore, this study aimed to investigate FC levels in patients with CF and their association with clinical manifestations.

3. Methods

3.1. Study design

This descriptive/cross-sectional study was performed from 22/12/2018 to 23/09/2019 in the

Children's Hospital affiliated with Tabriz University of Medical Sciences, Iranian Cystic Fibrosis Patient Registry (IRCFPR) Northwest Branch, on 52 children visiting the CF registry. The subjects were selected using the census sampling method, considering the inclusion and exclusion criteria.

3.2. Inclusion and exclusion criteria

The inclusion criteria were diagnosis of CF (confirmed by the pediatrician and according to the sweat chloride level of >60 mEq/L), the age range of 3-15 years, and consent of the parents for the participation of their children in the research project. The exclusion criteria were IBD, history of chemotherapy and radiotherapy, infectious enterocolitis (positive fecal sample in terms of *Salmonella*, *Shigella*, *Campylobacter*, and other bacteria), colorectal cancer, urinary incontinence (due to the possibility of stool contamination), inability to collect stool, history of extensive intestinal resection (ileo-sigmoidostomy and ileo-rectostomy), and use of any corticoid or non-steroidal anti-inflammatory drugs for more than two weeks.

3.3. Methodology

Patient information collected and recorded in a questionnaire included age, gender, weight, age at diagnosis, history of meconium ileus, electrolyte disorders, frequent diarrhea, serum albumin level, pancreatic insufficiency (by measuring fecal pancreatic elastase-1 using ELISA), respiratory symptoms, pulmonary infections, pulmonary insufficiency [by measuring Forced Expiratory Volume in the first second (FEV1)], patients with FEV1<50 have acute pulmonary insufficiency, patients with 50<FEV1<80 moderate pulmonary insufficiency, and patients with FEV1>80 normal pulmonary function), and liver insufficiency (using serum tests, ultrasound if required and final confirmation by the pediatrician). In addition, the FC level was measured using an ELISA kit (Thermo Fisher Scientific, USA). According to the ELISA kit used in this study, the calprotectin level of <50 μg/g was normal.

3.4. ELISA

The stool samples were kept in plastic containers at -20°C for 6 h before measuring FC levels. Afterward, 5 g of the stool was weighed, mixed in a roller shaker for 20 min, and centrifuged at 3,000 rpm for 5 min. In addition, the supernatant was micro-centrifuged and stored at -20°C. The obtained sample was then analyzed using fecal calprotectin ELISA test kits (Buhlmann Co., Switzerland). Moreover, the FC level was expressed in mg per kg of wet stool, and its normal level was considered <50 μg/g of feces.

3.5. Ethical considerations

The research was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1398.116 and IR.TBZMED.REC. 1395.1301). Informed consent was obtained from the parents of all the participants, no costs were incurred by the patients, and all the expenses were paid by Tabriz University of Medical Sciences. The study objectives were explained to the parents in simple words, and they were informed that participation in the study was voluntary and the children could withdraw from the study at any time during the research without penalty. The researchers did their best not to succumb to the temptation of falsification or plagiarism.

3.6. Statistical analysis

Data were analyzed in the SPSS software (version 18) using descriptive statistics (frequency, percentage, mean, and standard deviation). The qualitative data were analyzed employing Chi-squared (χ^2) test. The quantitative variables between the groups were determined using the independent t-test and Mann-Whitney U test, and the qualitative data employing the Chi-squared test. The relationship between the two quantitative variables was investigated using the bivariate Pearson and Spearman tests. P-values of <0.05 were considered significant.

4. Results

The mean±SD of patients' age was 8.8±5.0 years with a min of 1 month and a max of 144 months. The mean±SD of height was 121.2±12.5 cm with min and max of 60 and 167 cm, respectively. The mean±SD of weight was 24.6±12.5 kg with min and max of 6 and 53 kg, respectively. The mean±SD of Body Mass Index (BMI) was 15.7±2.2 with min and max of 10.88 and 21.67, respectively.

The mean±SD of the number of exacerbations was 4.11±0.98, with the min and max of 0 and 30, respectively. Fifty-one patients (98%) had pancreatic insufficiency, and 19 (36.5%) had Pseudomonas infections. None suffered from diabetes, and the low-fecal elastase was positive in most patients (98%). The mean±SD of the FC levels was 60.3±34.53 μg/g, with the lowest level of 11 μg/g and the highest level of 158 μg/dL. According to the measurement kit, calprotectin levels of <50 μg/g were considered normal. Therefore, 22 (42.3%) and 30 (57.7%) patients had normal and high calprotectin levels, respectively. Spirometry was not performed, and FEV1 was not obtained in six patients as they were very young. In the remaining 46 patients, the mean±SD of FEV1 was 79.8±12.28%, with the min and max of 40% and 95%, respectively.

The mean±SD of patients with normal FC levels was 28.86±12.09 μg/g, and for patients with abnormal FC levels, it was 83.40±26.26 μg/g, which

Table 1. Comparison of demographic information of patients with Calprotectin Fibrosis with Fecal Calprotectin

Variable		Calprotectin level of :50 -50 µg/g	calprotectin level of <50 µg/g	P-Value
Age		4.84±8.86	5.55±8.82	0.971
Sex	**Male**	18 (45%)	5 (41.6%)	0.555
	Female	22 (55%)	7 (63.5%)	
Body Mass Index		2.3±15.23	2.01±16.45	0.0511
Age at the time of diagnosis (month)		14.48±11.23	7.21±16.56	0.446

Calprotectin of< 50 µg/g is normal, P<0.0005= significant

Table 2. Differences between patients with Calprotectin Fibrosis in terms of pancreatic insufficiency, and airway colonization with Pseudomonas

Variable		Calprotectin level of >50µg/dL	Calprotectin level of<50 µg/dL	P-value
Pancreatic insufficiency	Yes	30 (100%)	21 (95.5%)	0.531
	No	-	1 (4.5%)	
Pseudomonas colonization	Yes	13 (43%)	6 (27%)	0.183
	No	17(56%)	16 (72%)	

Calprotectin of <50 µg/g is normal, P<0.0005=significant

indicated a significant difference between the groups (calprotectin <50 µg/g and calprotectin >50 µg/g, P=0.001). The results indicated no significant difference between patients with normal and abnormal FC levels in terms of gender, age at visiting the clinic and the emergency department, height, weight, BMI, and age at diagnosis (Table 1).

In addition, no significant difference was found between the patients with normal and abnormal FC levels in terms of pancreatic insufficiency, diabetes, and airway colonization with Pseudomonas (Table 2).

Table 3. Difference between exacerbation and FEV1 in Calprotectin Fibrosis patients with Calprotectin levels

Variable	Calprotectin level >50 µg/dL	Calprotectin level of <50 µg/dL	P-value
Exacerbation	8.67±5.56	0.74±2.13	0.084
FEV1 (%)	13.67±78.14	9.53±82.38	0.251

Calprotectin of <50 µg/g is normal, P<0.0005=significant
FEV1: Forced Expiratory Volume in the first second

Table 4. Electrolytes in patients with calprotectin levels

	Calprotectin level <50 µg/dL	Calprotectin level>50 µg/dL	P-Value
Na (meq/l)	2.34±138.18	3.66±139.03	0.372
K (meq/l)	0.18±3.95	0.16±3.97	0.650
Ca (mg/dl)	0.52±9.52	0.54±9.68	0.311
Mg (mg/dl)	0.18±2.15	0.22±2.16	0.856
P (mg/dl)	0.52±5.48	0.46±5.48	0.917

Calprotectin of <50 µg/g is normal, P<0.0005=significant

There was also no significant difference in low-fecal elastase between patients with calprotectin levels of <50 and >50 (P=0.53). A comparison of the number of exacerbations and FEV1 also showed no significant difference between patients with normal and abnormal calprotectin levels. (Table 3).

Table 4 presents the levels of electrolytes in patients with CF, according to which no statistically significant difference existed between patients with different calprotectin levels.

5. Discussion

The present study aimed to investigate FC levels in children with CF and showed an evaluation of FC values in patients with CF. In total, 58% of patients had increased FC values (>50 µg/g). There were no differences in FC values between patients with pancreatic insufficiency and those with normal pancreatic function. Demographic and clinical parameters, such as age, gender, FEV1, and BMI, had no clinically significant relationship with FC.

Calprotectin is a complex of two light (S100A8) and heavy (S100A9) heterodimers and exists in the cytoplasm of neutrophils and the membrane of monocytes. It is released in the serum and body fluids following the activation of neutrophils and monocytes and can be considered an inflammation-related biomarker in inflammatory diseases, such as arthritis, periodontitis, CF, sclerosing cholangitis, and IBD, including Crohn's disease and ulcerative colitis (8,9, 10). Calprotectin stimulates the inflammatory response by increasing the production of special inflammatory chemokines and the expression of adhesion molecules. Increased FC levels can be used as a useful biomarker to determine the status of patients with IBD, including Crohn's disease and ulcerative colitis (11, 12). According to recent literature, healthy newborns and young infants have much higher FC concentrations than adults (13). The

results of this study indicated no significant relationship between patients with normal and abnormal calprotectin levels in terms of age at diagnosis, the age at which stool samples were collected, BMI, and gender. Similar results have been reported in various studies. For example, Więcek *et al.* showed that FC levels had no significant relationship with age and disease symptoms (14). In addition, Ellemunter *et al.* (15) and Neumann *et al.* (16) found no significant clinical relationships between FC levels and age at which stool samples were collected, age at diagnosis, gender, and BMI. According to the results of this study, the mean±SD of FC levels in patients with normal FC levels was 28.86±12. µg/g, and in patients with abnormal FC levels, it was 83.40±26.2 µg/g, which was significantly higher (P=0.001). Dhaliwal *et al.* reported similar results in that they also found that the mean FC level in patients with CF was 93.4 (17). Similarly, the mean FC level in the study by Neumann *et al.* was 94.29 µg/g (16).

The results of the above-mentioned study showed no significant difference between patients with normal and abnormal calprotectin levels in terms of pancreatic insufficiency. In contrast to the results of the present study, Ellemunter *et al.* reported that pancreatic insufficiency was associated with high FC levels (15). The results of the study by Dhaliwal *et al.* were also opposed to the present findings since they reported that abnormal FC levels could be observed only in patients with pancreatic insufficiency (17). In a similar study, Więcek *et al.* found that FC levels had no significant relationship with advanced digestive tract damage and pancreatic insufficiency in patients with CF (14).

In addition, no significant relationship was observed between the number of exacerbations and FC levels. Similarly, Neumann *et al.* also found no significant relationship between patients with normal and abnormal FC levels in terms of exacerbation (16).

Given the inconsistent results between different studies and the limited number of studies in this field, it is suggested that further multi-center studies be performed with larger sample sizes and several variables be included to provide more reliable results. In future studies, serial and longitudinal calprotectin levels may have clinical relevance during symptomatic episodes and whether certain interventions impact these levels.

5.1. Limitations

The present study suffered from some limitations. It was a small study conducted in only one CF center, and there was no control group. Furthermore, the high cost of the calprotectin test and the availability of the elastase test were other limitations of this study. Therefore, more accurate results can be obtained by performing multi-center studies with large sample sizes.

6. Conclusion

This study showed no relationships between FC levels and pancreatic insufficiency, pulmonary insufficiency, and pulmonary infections in patients with CF. FC is used as a marker of inflammation, although it may not be a perfect indicator of intestinal inflammation in CF as in other inflammatory conditions.

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Footnotes

Conflicts of Interest: The authors declare no conflict of interest regarding the publication of the present study.

Authors' contributions: M.R. participated in the design of the study, recruiting patients and collecting data, and wrote the manuscript. A.H.J.R participated in recruiting patients and collecting data. M.H.S and E.F. performed the statistical analysis and helped to draft the manuscript. S.H. participated in the design of the study, coordinated and supervised the ELISA tests, and helped to draft the manuscript. S.Y. participated in the design of the study and helped to draft and edit the manuscript. All authors read and approved the final manuscript.

References

1. Bell SC, Mall MA, Gutierrez H, Macek M, Madge S, Davies JC, et al. The future of cystic fibrosis care: a global perspective. *Lancet Respir Med*. 2020;**8**(1):65-124. doi: 10.1016/S2213-2600(19)30337-6. [PubMed: 31570318].
2. De Boeck K. Cystic fibrosis in the year 2020: A disease with a new face . *Acta Paediatr*. 2020;**109**(5):893-9. doi: 10.1111/apa.15155. [PubMed: 31899933].
3. Ortiz-Muñoz G, Michelle AY, Lefrançais E, Mallavia B, Valet C, Tian JJ, et al. Cystic fibrosis transmembrane conductance regulator dysfunction in platelets drives lung hyperinflammation. *J Clin Invest*. 2020;**130**(4):2041-53. doi: 10.1172/JCI129635. [PubMed: 31961827].
4. Bryon M. Breaking the diagnosis of cystic fibrosis to parents: a process not a one-off event. *Paediatr Respir Rev*. 2020;**35**:103-5. doi: 10.1016/j.prrv.2020.04.006. [PubMed: 32409170].
5. Fatima T, Ajjarapu S, Shankar VK, Rangappa S, Shivakumar HN, Biswas SK, et al. Topical pilocarpine formulation for diagnosis of cystic fibrosis. *J Pharm Sci*. 2020;**109**(5):1747-51. doi: 10.1016/j.xphs.2020.01.030. [PubMed: 32035925].
6. Kopi TA, Shahrokh S, Mirzaei S, Aghdaei HA, Kadijani AA. The role of serum calprotectin as a novel biomarker in inflammatory bowel diseases: a review study. Gastroenterol Hepatol Bed Bench. 2019;**12**(3):183-9. [PubMed: 31528300].
7. Talebi S, Day AS, Rezaiyan MK, Ranjbar G, Zarei M, Safarian M, et al. Fecal calprotectin and phenotype severity in patients with cystic fibrosis: a systematic review and meta-analysis. *Pediatr Gastroenterol Hepatol Nutr*. 2022;**25**(1):1-12.

doi: 10.5223/pghn.2022.25.1.1. [PubMed: 35087728].
8. Shabestari MS, Rafeey M, Shoaran M, Shirvani S. Association between fecal calprotectin concentration and mesenteric lymphadenopathy in children. Crescent *J Med Biol Sci.* 2020;**7**(2):238-42.
9. Mosavi A, Mosavi H, Zare S, Gadarjani R. Evaluation of fecal calprotectin in the assessment of patients with inflammatory bowel disease in the Shahid Mohammadi Hospital in Hormozgan in 2013-2014. *J Rafsanjan Univ Med Sci*. 2017;**15**(12):1107-18.
10. Xiang BJ, Jiang M, Sun MJ, Dai C. Optimal range of fecal calprotectin for predicting mucosal healing in patients with inflammatory Bowel Disease: a systematic review and meta-analysis. *Visc Med.* 2021;**37**(5):338-48. doi: 10.1159/000514196. [PubMed: 34722717].
11. D'Amico F, Bonovas S, Danese S, Peyrin-Biroulet L. faecal calprotectin and histologic remission in ulcerative colitis. *Aliment Pharmacol Ther.* 2020;**51**(7):689-98. doi: 10.1111/apt.15662. [PubMed: 32048751].
12. Xiang B, Dong Z, Dai C. The diagnostic and predictive value of Fecal calprotectin and capsule endoscopy for small-bowel Crohn's disease: A systematic review and meta-analysis. *Rev Esp Enferm Dig*. 2021;**113**(3):193-201. doi: 10.17235/reed.2020.6996/2020. [PubMed: 33207888].
13. Kapel N, Campeotto F, Kalach N, Baldassare M, Butel M-J, Dupont C. Faecal calprotectin in term and preterm neonates. *J Pediatr Gastroenterol Nutr.* 2010;**51**(5):542-7. doi: 10.1097/MPG.0b013e3181e2ad72. [PubMed: 20818270].
14. Więcek S, Woś H, Kordys-Darmolińska B, Sankiewicz-Szkółka M, Grzybowska-Chlebowczyk U. The concentration of calprotectin in the stools of children with diagnosed cystic fibrosis. *Gastroenterology Rev.* 2017;**12**(1):38–43. doi: 10.5114/pg.2016.58897.
15. Ellemunter H, Engelhardt A, Schüller K, Steinkamp G. Fecal calprotectin in cystic fibrosis and its relation to disease parameters: a longitudinal analysis for 12 years. *J Pediatr Gastroenterol Nutr*. 2017;**65**(4):438-442. doi: 10.1097/MPG.0000000000001544. [PubMed: 28207476].
16. Rumman N, Sultan M, El-Chammas K, Goh V, Salzman N, Quintero D, et al. Calprotectin in cystic fibrosis. *BMC Pediatr*. 2014;**14**(1):1-7. doi: 10.1186/1471-2431-14-133. [PubMed: 24885444].
17. Dhaliwal J, Leach S, Katz T, Nahidi L, Pang T, Lee J, et al. Intestinal inflammation and impact on growth in children with cystic fibrosis. *J Pediatr Gastroenterol Nutr.* 2015;**60**(4):521-6. doi: 10.1097/MPG.0000000000000683. [PubMed: 25539196].