The effect of probiotics on fecal calprotectin in patients with cystic fibrosis

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SUMMARY: Fallahi G, Motamed F, Yousefi A, Shafieyoun A, Najafi M, Khodadad A, Farhmand F, Ahmadvand A, Rezaei N. The effect of probiotics on fecal calprotectin in patients with cystic fibrosis. Turk J Pediatr 2013; 55: 475-478.

Cystic fibrosis (CF) is a common autosomal recessive disorder with different clinical manifestations, mainly in the gastrointestinal and respiratory tracts. This study was performed to access the effect of probiotics in the status of intestinal inflammation in a group of children with CF by measuring the calprotectin level in the fecal samples.

Forty-seven patients with CF were enrolled in this study. The fecal calprotectin levels were measured by enzyme linked immunosorbent assay. In a randomized systematic method, the children were divided into two groups - one group received probiotic powder and another received placebo for four weeks. After this period, fecal calprotectin was re-measured.

Thirty-one of 47 enrolled patients (65.9%) had abnormal fecal calprotectin levels (>50 μg/g). After the intervention, the fecal calprotectin levels decreased in 29 patients (21 patients in the drug group, and only 8 patients in the placebo group; p<0.001).

This study showed that about two-thirds of patients with CF had intestinal inflammation based on fecal calprotectin levels. Probiotic administration was shown to decrease calprotectin concentrations and subsequently intestinal inflammation in CF patients.

Key words: cystic fibrosis, calprotectin, probiotics.

Cystic fibrosis (CF) is a common genetic disorder occurring in about 1 of 2,500 live births in the Caucasian population. CF is a chronic debilitating disorder, in which pancreatic enzyme replacement therapy cannot resolve patients' gastrointestinal complaints completely. High levels of inflammatory markers in the blood of CF patients have been reported, while their concentrations seem to be correlated with the disease severity[1-4].

A recent study showed several inflammatory findings in patients with CF, such as edema, erythema, mucosal breaks, and frank ulcerations using capsule endoscopy[1]. The patients with pancreatic insufficiency had higher levels of fecal calprotectin, which could also correlate with CF findings[1]. Calprotectin is in a family of Ca^{2+} binding proteins that is basically produced by neutrophils, but smaller amounts can be found in monocytes and reactive macrophages. This molecule is known to be bacteriostatic and fungistatic with minimum inhibitory concentrations comparable to those of antibiotics[5,6].

There are some evidences showing increased levels of calprotectin in stool specimens of patients with some gastrointestinal disorders, including inflammatory bowel diseases (IBDs). Recently, calprotectin concentrations have also been used to identify the severity of intestinal inflammation and the activity of the disease in children with IBD[5,6]. Gray et al.[7] found serum and sputum calprotectin levels to change informatively following treatment of CF exacerbations.

This study was performed to assess the

effect of probiotics in the status of intestinal inflammation in a group of children with CF by measuring the calprotectin level in the fecal samples.

Material and Methods

Subjects

This study was performed in a group of patients with CF, whose diagnosis was made based on two positive sweat tests, and who were aged over four years. All the patients had steatorrhea as a sign of pancreatic insufficiency. They had not used any non-steroidal anti-inflammatory drug (NSAID) during the last two weeks. The patients were not on antibiotic therapy at the time of the study. All these patients were pediatric cases referred to the gastrointestinal clinic of the Children's Medical Center, the Pediatrics Center of Excellence in Iran.

This study was approved by the local Ethics Committee of the hospital. Written informed consents were obtained from the subjects. This study was also registered in the Iranian Registry of Clinical Trials (IRCT201201258778N3). Although 50 patients were initially selected for this study, three patients later decided not to participate in this study and did not bring the stool samples. Therefore, 47 patients were enrolled in the study.

Methods

In a double-blind randomized controlled trial, patients were simply randomly divided into two groups to receive either probiotic or placebo. The probiotic group received one probiotic sachet each day for four weeks and the placebo group received maltodextrin instead. As a double-blind study, neither the patients nor the doctors/researchers were aware of the placebo or probiotic packs. The probiotic powder used in this study was 1 g Protexin Restor sachet, which contains of FOS (fructooligosaccharides) and a mixture of 1×10^9 CFU/sachet bacteria (*Lactobacillus casei, Lactobacillus rhamnosus, Streptococcus thermophilus, Bifidobacterium breve, Lactobacillus acidophilus, Bifidobacterium infantis* (child-specific), *Lactobacillus bulgaricus*). All the families were contacted by telephone each week to ensure that the patients were using the packs regularly.

Calprotectin

A stool specimen was collected from the patients at the beginning of the study. After four weeks, another fecal sample was taken from the patients. The samples were stored at -80°C in the laboratory of the hospital until the time of assay. Once fecal calprotectin concentrations were measured, 0.1 g frozen feces were suspended in extraction buffer, homogenized and centrifuged. The supernatant was collected and assayed using enzyme linked immunosorbent assay (ELISA kit, Hycult Biotech, Uden, Netherlands) with a specific polyclonal antibody. The upper normal limit for fecal calprotectin is 50 μg/g.

Statistics

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software, version 16.0. For the qualitative variables, descriptive methods were used and the frequencies of variables were presented, while for quantitative variables, the results were expressed as mean±SD (standard deviation). In order to analyze the changes in fecal calprotectin levels before and after probiotic administration, t test was used.

Results

Forty-seven children with CF were enrolled in this study and divided into two groups, as probiotic (n=24), with mean age of 8.56±4.19 years, or placebo (n=23), with mean age of 8.65±3.29 years.

Table I. Fecal Calprotectin Concentrations in Patients with CF Before and After Intervention

Groups	Before intervention		After intervention	
	Fecal calprotectin >50 μg/g	Fecal calprotectin <50 μg/g	Fecal calprotectin >50 μg/g	Fecal calprotectin <50 μg/g
Placebo	13	10	8	15
Probiotic	18	6	21	3
Total	31	16	29	18

Mean fecal calprotectin concentrations in the probiotic and placebo groups before intervention were 101.38 μg/g and 70.22 μg/g, respectively, and there was no significant difference between the two groups (p=0.1). However, the mean fecal calprotectin concentration after using probiotic powders was 56.2 μg/g, which was significantly lower than 182.1 μg/g in the placebo group (p=0.031).

Among the 47 CF patients, 31 patients (65.9%) had fecal calprotectin levels of more than 50 μg/g. Although 13 patients (41.9%) belonged to the placebo and 18 patients (58.1%) belonged to the probiotic group in random allocation (Table I), this difference was not significant (p=0.230). However, after intervention, 29 patients had fecal calprotectin concentrations less than 50 μg/g; among them, 8 patients (27.6%) were in the placebo and 21 patients (72.4%) were in the probiotic group. There was a significantly lower number of cases with high calprotectin level in the patients who used probiotic (p<0.001).

Discussion

Fecal calprotectin concentrations have been used as a non-invasive valid marker for intestinal inflammation[3,5]. In this study, we used this marker to evaluate the effect of a probiotic in a group of patients with CF.

Fagerberg et al.[3] showed that the mean fecal calprotectin levels in children with colorectal inflammation were greater than the ones without colorectal inflammation. The level of 50 μg/g is considered as the upper normal limit for fecal calprotectin concentration, with 95% sensitivity and 93% specificity. At the beginning of our study, we found that about two-thirds of the patients with CF had fecal calprotectin concentrations of more than 50 μg/g. Bruzzese et al.[8] also reported that fecal calprotectin levels were significantly higher in children with CF compared to healthy controls. It seems that there is a compromised intestinal barrier function in CF patients, while there are increased concentrations of inflammatory substances in the whole gut lavage of CF patients[1].

There are some controversies on the initial event causing inflammatory states in CF patients; some groups believe that bacterial infections initiate the inflammatory responses, while others believe that dysregulation in inflammatory processes and cellular defects can predispose them to infections[2,9]. In support of this link between intestinal bacterial overgrowth and inflammation, the expression of inflammatory-related genes decreased when treating CF mice with proven intestinal dysbiosis with antibiotics[10]. One theory about the persistent abdominal complaints in CF patients is the presence of abdominal inflammation, which could be due in part to microflora changes. Therefore, if probiotic administrations in CF patients palliate the intestinal symptoms and reduce the fecal calprotectin concentrations, it could support this hypothesis.

Probiotic bacteria seem to be beneficial in treating some intestinal disorders, including acute gastroenteritis and IBD[8]. In our study, probiotic administration significantly changed the fecal calprotectin concentrations compared to the placebo group, so the microflora modifications can improve the intestinal inflammation. The result of this study was in agreement with a previous study by Bruzzese et al.[8], which showed that the calprotectin level is lessened after probiotic administration. In a study by Lisowska et al.[9], they found significantly higher fecal calprotectin levels in CF patients in comparison with controls, but calprotectin levels did not differ between small bowel bacterial overgrowth-positive and -negative patients. Thus, fecal calprotectin level may not be a sufficient marker of intestinal inflammatory status. It should be noted that since the number of cases included in this study was limited, further studies are needed to check different inflammatory markers in patients with CF, while the efficacy of probiotics should be checked over longer time periods.

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