Gastrointestinal manifestations and their relation to faecal calprotectin in children with autism

Hanan Galal Azouz¹, Nermine Hossam El-din Zakaria², Ahmed Fouad Khalil¹, Sara Mohammad Naguib¹, Mona Khalil³

¹Department of Paediatrics, Faculty of Medicine, Alexandria University, Alexandria, Egypt
²Department of Clinical and chemical Pathology, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Gastroenterology Rev 2021; 16 (4): 352–357
DOI: https://doi.org/10.5114/pg.2021.111420

Key words: autism spectrum disorder, faecal calprotectin, gastrointestinal symptoms.

Address for correspondence: Mona Khalil, Department of Paediatrics, Faculty of Medicine, Alexandria University, Alexandria, Egypt, mobile: 00201001116562, e-mail: drmonakhalil@yahoo.com

Abstract

Introduction: A common comorbidity in autism spectrum disorder (ASD) children is gastrointestinal problems, and a possible link between active gastrointestinal inflammation and autism has been suggested. Faecal calprotectin (FC) is a non-invasive marker for gastrointestinal inflammation.

Aim: To study the level of FC as a marker of bowel inflammation in children with ASD and its possible relation to gastrointestinal manifestations.

Material and methods: Calprotectin levels were assessed in stool samples of 40 ASD children. Autism severity was assessed by the Childhood Autism Rating Scale (CARS). Severity of gastrointestinal symptoms was assessed using a modified version of the 6-Item Gastrointestinal Severity Index (6-GSI) questionnaire. A control group of 40 healthy children matched for age and sex with the cases was also included to compare their levels of FC.

Results: Gastrointestinal symptoms were present in 82.5% of children with autism; the most reported offensive stool odour (70%) and the least diarrhoea (17.5%), and a high 6-GSI score was observed in 35% of ASD children. FC levels were elevated in 35% of the cases and in 25% of the control group. The mean levels of FC of cases were significantly elevated compared to levels of controls. FC levels positively correlated with severity of gastrointestinal symptoms (6-GSI) in autistic patients. There was positive correlation between CARS and 6-GSI.

Conclusions: Gastrointestinal manifestations are a common comorbidity in autistic patients. ASD patients have significantly higher FC levels than healthy controls. FC levels are strongly correlated with the severity of gastrointestinal manifestations in ASD children. So, gastrointestinal manifestations among autistic patients could be caused by gastrointestinal inflammation.
Calprotectin in stool signifies intestinal tract infiltration with neutrophils. The level of faecal calprotectin (FC) correlates with intestinal tract inflammation histologically and macroscopically [5]. FC has been considered as a non-invasive marker for some gastrointestinal disorders that can be used before more invasive procedures [7].

Some studies demonstrated that intestinal inflammation is more prevalent in children with autism, while other research failed to discover intestinal inflammation among autistic children.

**Aim**

The aim of this work was to study the level of faecal calprotectin as a marker of bowel inflammation in children with autism and its possible relation to gastrointestinal manifestations.

**Material and methods**

The study included 40 autistic children aged 3 to 12 years and fulfilling the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) diagnostic criteria [8]. A control group of 40 healthy children matched for sex and age was also included to compare their level of faecal calprotectin with the cases group.

The cases were selected from those attending the outpatient neurobehavioral clinic at Alexandria University Children’s Hospital. Written informed consent was obtained from parents/caregivers of children after explanation of the steps and nature of the study. The study was started after the agreement of the Medical Ethics Committee of Faculty of Medicine, Alexandria University. Patients with dysmorphic features suggestive of syndromic developmental delay and children with any chronic gastrointestinal disease such as chronic gastritis or celiac disease were excluded.

All the studied children were subjected to a thorough history taking and complete physical examination with special emphasis on neurological examination. The severity of autism was assessed using the Childhood Autism Rating Scale (CARS) [9]. It was classified as mild to moderate if from 30 to 36.5 or severe if more than or equal to 37.

Gastrointestinal (GI) symptoms and the symptom severity were assessed using a modified version of the GI Severity Index, i.e. a shortened version called the 6-GI Severity Index (6-GSI) [10]. It included 6 items, which were constipation, abdominal pain, diarrhoea, stool smell, stool consistency, and flatulence. Each variant was scored 0, 1, or 2 according its frequency per week; a zero score of any variant was interpreted as the symptom is not present, and a 1 or 2 score of any variant denoted the presence of the symptom with different severity. Total score equal to or less than 3 was classified as low score, and more than 3 was a high score.

Faecal samples were collected and stored at below −20°C. After thawing, the extracts were diluted and run on enzyme linked immunosorbent assay (ELISA) plates. Calprotectin levels were measured in stool samples using an EDITM Quantitative faecal calprotectin ELISA [11]. FC levels were classified as follows: < 50 µg/g = normal, ≥ 50 µg/g = elevated. A comparison between cases and control as regards faecal calprotectin levels was done, and the following correlations were investigated among cases: between autism severity (CARS) and GI symptoms severity (6-GSI), FC and GI symptoms severity (6-GSI), and between FC and autism severity (CARS).

**Statistical analysis**

Data were entered into the computer and analysed using IBM SPSS software package version 20.0 (Armonk, NY: IBM Corp) [12]. Qualitative data were presented as percentages and numbers. The Kolmogorov-Smirnov test was utilized to demonstrate the normality of distribution. Quantitative data were demonstrated using mean, range (minimum and maximum), median, and standard deviation. The significance of the results was judged at the 5% level. The χ2 test was used to test the association between qualitative variables. Fisher’s Exact or Monte Carlo correction was used when in the χ2 more than 20% of the cells had an expected count of less than 5 and required correction. The Mann-Whitney test was used to make comparisons between 2 studied independent subgroups that were not normally distributed.

**Results**

Out of the 40 ASD children, 28 (70.0%) were males and 12 (30.0%) were females. Their age ranged from 3 to 12 years with a mean of 6.53 ±2.10 years. Twenty-five (62.5%) children were from urban areas, and consanguinity was positive only in 6 (15%) ASD children.

Cases were diagnosed at ages ranging from 18.0 to 36.0 months with a mean of 23.40 ±5.07 months. Twenty-six (65%) children had regressive type of autism and 14 (35%) had non-regressive autism. According to CARS, 23 (57.5%) of the ASD children were mild to moderate and 17 (42.5%) were severe. CARS ranged from 30 to 48.5 with a mean of 36.18 ±5.22.

Gastrointestinal symptoms were present in 33 (82.5%) ASD children. The most frequent symptom was offensive stool odour (70%), and the least was diarrhoea (17.5%). The total 6-GSI score was low in 26 (65%) cases and high in 14 (35%) cases. The total score ranged from 0 to 9 with a mean of 2.85 ±2.05 (Table I).
A control group of 40 healthy children matched for sex and age was included to compare their level of faecal calprotectin with the cases group. The faecal calprotectin level was elevated ($\geq 50 \, \mu g/g$) in 14 (35%) children with ASD and in 10 (25%) of the control group. Comparing the mean levels of faecal calprotectin, it showed that the mean level of FC in cases was 47.03 ±26.68 while in the control group it was 37.08 ±21.55, and this showed statistical significant difference ($p = 0.049$) (Table II).

Correlations between CARS, 6-GSI, and the levels of faecal calprotectin among cases were investigated and revealed a significant positive correlation between CARS and 6-GSI at $p = 0.003$ and a significant positive correlation between faecal calprotectin and 6-GSI at $p = 0.002$. However, no significant correlation was found between CARS and faecal calprotectin ($p = 0.280$) (Table III).

**Discussion**

GI problems are common morbidities in ASD children; numerous studies have suggested a probable gut-brain axis that could be explained by inflammatory, immunological, or genetic factors [2]. Afferent gut-brain pathway includes inflammatory mediators, entero-endocrine system, intestinal microbiota, and sensory epithelial cells, while efferent pathway involves neuroendocrine and autonomic nervous systems [13].

In the current study we investigated faecal calprotectin as a marker of inflammation in the gastrointestinal tract in children with ASD. It was found that faecal calprotectin levels were elevated in 35% of patients in comparison to 25% of the controls, and the mean levels of faecal calprotectin in cases were significantly more elevated than in the control group.

This finding is consistent with the findings of Karkeles et al. [14], de Magistris et al. [15], Babinská et al. [16], and Eduardo et al. [17], who observed that higher levels of calprotectin were detected in the stools of autistic children than in normal children. Karkeles et al. conducted their study on 45 autistic children aged 2.5 to 8 years and detected increased levels of faecal calprotectin in ASD children in comparison with healthy

### Table I. Distribution of children with ASD according to 6-GSI score ($n = 40$)

| GI symptoms                | N  | %  |
|----------------------------|----|----|
| Constipation:              |    |    |
| 0                         | 27 | 67.5 |
| 1                         | 10 | 25.0 |
| 2                         |  3 |  7.5 |
| Diarrhoea:                |    |    |
| 0                         | 33 | 82.5 |
| 1                         |  7 | 17.5 |
| 2                         |  0 |  0.0 |
| Loose stool consistency:  |    |    |
| 0                         | 20 | 50.0 |
| 1                         | 20 | 50.0 |
| 2                         |  0 |  0.0 |
| Offensive stool smell:    |    |    |
| 0                         | 12 | 30.0 |
| 1                         | 26 | 65.0 |
| 2                         |  2 |  5.0 |
| Flatulence:               |    |    |
| 0                         | 25 | 62.5 |
| 1                         | 15 | 37.5 |
| 2                         |  0 |  0.0 |
| Abdominal pain:           |    |    |
| 0                         | 21 | 52.5 |
| 1                         | 18 | 45.0 |
| 2                         |  1 |  2.5 |
| Total score:              |    |    |
| Low ($\leq 3$)            | 26 | 65.0 |
| High ($> 3$)              | 14 | 36.0 |
| Min.–max.                 | 0.0–9.0 |
| Mean ± SD                 | 2.85 ±2.05 |
| Median                    | 3.0 |

### Table II. Comparison between the two studied groups according to levels of faecal calprotectin

| Faecal calprotectin | Cases ($n = 40$) | Control ($n = 40$) | Test of sig. | P-value |
|--------------------|-----------------|------------------|--------------|---------|
| < 50               | 26              | 30               | $\chi^2 = 0.952$ | 0.329 |
| ≥ 50               | 14              | 10               | $U = 595.5^*$ | 0.049* |
| Min.–max.          | 13.0–100.0      | 11.35–90.0       |              |         |
| Mean ± SD          | 47.03 ±26.68    | 37.08 ±21.55     |              |         |
| Median (IQR)       | 36.0            | 27.20            |              |         |

$\chi^2$ – Chi-square test, $U$ – Mann-Whitney test, $p$-value for comparison between the studied groups, $^*$statistically significant at $p \leq 0.05$. 

Gastroenterology Review 2021; 16 (4)
children [14]. De Magistris et al. investigated 90 children with ASD and 146 of their first-degree relatives; they found that FC was elevated in 24.4% of patients with autism and in 11.6% of their relatives, and the mean pathological value of FC found in these patients indicated a mild degree of inflammation of the bowel [15]. Babinska et al. studied the level of faecal calprotectin in 3 groups (autistic patients, their siblings, and non-related controls); ASD children and their siblings had significantly higher levels of FC than non-related controls [16]. Eduardo et al. detected increased levels of FC in 75% of their autistic patients indicating nonspecific GI inflammation in ASD children [17].

In contrast, other previous studies by Fernell et al. [18], Wos et al. [19], and Strati et al. [20] revealed that faecal calprotectin levels of autistic patients were not elevated more than in normal populations.

Regarding GI manifestations, it was found that 82.5% of ASD patients had at least one GI symptom, with offensive stool odour being the most common (in 70% of patients) and diarrhoea the least reported (in 17.5% of patients). High 6-GSI scores were observed in 35% of ASD children.

Horvath and Perman detected GI manifestations in 84.1% of ASD children [21]. Valicenti-McDermott et al. compared the frequency of GI manifestations in 3 groups: normally developed children, autistic children, and a group with other developmental disorders. They detected GI symptoms in 28% of children with normal development, in 70% of autistic children, and in 42% of children with other developmental disorders [22].

In contrast, Ibrahim et al. [23] and Black et al. [24] found that GI symptoms were not detected more in autistic than in normal children. Ibrahim et al. in 2009 found no significant difference in the overall incidence of GI symptoms between autistic children and controls, although constipation and feeding problems/food selectivity were detected more in ASD children [23]. Another study by Black et al. reviewed hospital records and found that GI problems were not detected in autistic children more than in the normal population (9% vs. 9%) [24].

A wide range of variations of prevalence of GI symptoms in autism were observed by Buie et al. [25], McElhanon et al. [26], and Holingue et al. [27]. Buie et al. revealed that in autistic children the prevalence of GI tract symptoms ranges from 9% to 84% versus 9–37% for normal children [25]. In 2014, McElhanon et al. in a meta-analysis that involved 15 studies over 30 years, revealed that general GI symptoms ranged from 0.39 to 48.25, with the observation that GI symptoms in children with ASD are 4 times more prevalent than for children without ASD [26]. In 2018, Holingue et al. reviewed studies dating back to 1980; the ranges were quite wide. Among the 62 studies, for the category of “any” GI symptom the range was 4.2–96.8% of participants [27].

Constipation, diarrhoea, and abdominal pain were reported as the most common GI symptoms in autistic patients in several studies. Holingue et al. in their review on GI symptomatology in ASD, revealed that the median prevalence of constipation was 22.2% and of diarrhea 13% [27]. Gorrindo et al. found that functional constipation was the most frequent type of GI manifestation in children with ASD (85%) [28]. In another study by Wang et al., parents reported that the most common GI symptoms in children with ASD were constipation (20%) and chronic diarrhoea (19%), and that increased autism symptom severity was associated with a higher score of GI problems [29]. However, a study done by Parracho et al. found that diarrhoea was the most common GI symptom (75.6%), followed by excess wind (55.2%), abdominal pain (46.6%), constipation (44.8%), and abnormal faeces (43%) [30]. Also, another study by Molloy and Manning-Courtney described diarrhoea as being more common in ASD children (17%) [31].

Wasilewska and Klukowski [2] reported that the most common GI symptoms were overproduction of intestinal gasses/flatulence (60%), bloating (38%), abdominal pain (378%), diarrhoea (28%), burping/belching (25%), gastroesophageal reflux symptoms (16%), and constipation (10%). This was similar to the current study, in which abdominal pain and flatulence were more frequent than diarrhoea and constipation.

The wide variations in presentations of gastrointestinal tract affection in ASD patients may be attributed to high methodological variability including the person who reported the symptoms (parents, caregivers, or physician), different scales used to evaluate GI symptoms, different environment, study design, age group, and sample size.

In the current study, we correlated 3 variables among cases: severity of autism (CARS), severity of GI symptoms (6-GSI score), and levels of FC as a marker of intestinal inflammation. Correlations were found to be significantly positive between CARS and GI severity score, and between FC and GI severity score; however,

---

**Table III. Correlation between different parameters in the ASD group**

| Variable                           | \( r_s \) | \( P \)-value |
|-----------------------------------|-----------|--------------|
| CARS vs. faecal calprotectin       | 0.175     | 0.280        |
| CARS vs. 6-GSI score              | 0.462*    | 0.003*       |
| Faecal calprotectin vs. 6-GSI severity | 0.471*    | 0.002*       |

\( r_s \) = Pearson coefficient, *statistically significant at \( p \leq 0.05 \).
no significant correlation was found between CARS and FC level.

In 2011, Adams et al. used 6-GSI to assess GI severity in ASD children and found that the total score was low in 39% of patients and high in 61% of patients. Also, a strong positive correlation between autism severity and GI symptom severity was detected [10]. Similarly, in 2011, Wang et al. demonstrated that increased autism severity was associated with more frequent GI problems [29].

According to our best knowledge, limited studies have correlated GI severity index (as a clinical method of detection of GI problems) and faecal calprotectin (as a laboratory method) in ASD patients. Further studies are required because a positive correlation between the level of FC and the GI severity index was found.

One of the limitations of the current study was the lack of objective confirmation of an absence of a concomitant bowel disease of inflammatory origin, such as endoscopy, in children with autism. This could be justified by the fact that the severity of symptoms was not sufficient to arrange for an invasive procedure. The elevation of calprotectin was mild, and in the absence of specific clinical presentation suggestive of a serious disease like haematemesis or bleeding per rectum, most of the anticipated findings in endoscopy in such a presentation (type of patients and severity) is usually mild and non-specific [4, 32].

Conclusions

Gastrointestinal manifestations are a common comorbidity in autistic patients, and the severity of their GI manifestations is strongly correlated with autism severity. ASD patients have significantly higher FC levels than healthy controls, and their FC levels are strongly correlated with the severity of gastrointestinal manifestations in autistic children. FC as a lab marker and GI severity score could be utilized as an indicator of GI problem severity in autistic patients with GI symptoms.

Conflict of interest

The authors declare no conflict of interest.

References

1. Faras H, Al Ateeqi N, Tidmarsh L. Autism spectrum disorders. Ann Saudi Med 2010; 30: 295-300.
2. Wasilewska J, Klukowski M. Gastrointestinal symptoms and autism spectrum disorder: links and risks – a possible new overlap syndrome. Pediatric Health Med Ther 2015; 6: 153-66.
3. Bramati-castellarin I, Patel VB, Drysdale IP. Faecal calprotectin and a twenty-four-parameter questionnaire in autistic children with gastrointestinal symptoms. SM J Psychiatry Mental Health 2017; 2: 1009.
4. Kushak RI, Buie TM, Murray KF, et al. Evaluation of intestinal function in children with autism and gastrointestinal symptoms. J Pediatr Gastroenterol Nutr 2016; 62: 687-91.
5. Sidler MA, Leach ST, Day AS. Fecal S100A12 and fecal calprotectin as noninvasive markers for inflammatory bowel disease in children. Inflamm Bowel Dis 2008; 14: 359-66.
6. Sipponen T, Kolho KL. Fecal calprotectin in diagnosis and clinical assessment of inflammatory bowel disease. Scand J Gastroenterol 2015; 50: 74-80.
7. Herrera OR, Christensen ML, Helms RA. Calprotectin: clinical applications in pediatrics. J Pediatr Pharmacol Ther 2016; 21: 308-21.
8. American Psychiatric Association (APA). Diagnostic and statistical manual of mental disorders. 5th edn. APA, Arlington 2013; 501.
9. Chlebowski C, Green JA, Barton ML, Fein D. Using the childhood autism rating scale to diagnose autism spectrum disorders. J Autism Dev Disord 2010; 40: 787-99.
10. Adams JB, Johansen LJ, Powell LD, et al. Gastrointestinal flora and gastrointestinal status in children with autism—comparisons to typical children and correlation with autism severity. BMC Gastroenterol 2011; 11: 22.
11. Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. Clin Exp Gastroenterol 2016; 9: 21-9.
12. Kirkpatrick LA, Feeney BC. A simple guide to IBM SPSS statistics for version 20.0. Student ed. Belmont, Calif.: Wadsworth, Cengage Learning; 2013.
13. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Ann Gastroenterol 2015; 28: 203-9.
14. Karkelis S, Papadaki Q, Lyrogeorgou M, et al. Fecal calprotectin in autistic children. Paediatr Child Health 2010; 15: 66A.
15. de Magistris L, Familiari V, Pascotto A, et al. Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr 2010; 51: 418-24.
16. Babinska K, Tomova A, Celusaková H, et al. Fecal calprotectin levels correlate with main domains of the autism diagnostic interview-revised (ADI-R) in a sample of individuals with autism spectrum disorders from Slovakia. Physiol Res 2017; 66 (Suppl 4): S517-22.
17. Eduardo UK, Andrea CM, Zulbey RD, et al. Nonspecific gastrointestinal inflammation not associated with enteropathogens in children with Autism Spectrum Disorder. Curr Top in Biochem Res 2017; 18: 103-15.
18. Fernell E, Fagerberg UL, Hellsstrom PM. No evidence for a clear link between active intestinal inflammation and autism based on analyses of faecal calprotectin and rectal nitric oxide. Acta Paediatr 2007; 96: 1076-9.
19. Wos H, Komraus M, Kazek B, et al. Faecal calprotectin in children with autistic spectrum disorders. Arch Dis Child 2008; 93: 215.
20. Strati F, Cavaleri D, Albanese G, et al. New evidences on the altered gut microbiota in autism spectrum disorders. Microbime 2017; 5: 24.
21. Horvath K, Perman JA. Autism and gastrointestinal symptoms. Curr Gastroenterol Rep 2002; 4: 251-8.
22. Valicenti-McDermott M, McVicar K, Rapin I, et al. Frequency of gastrointestinal symptoms in children with autistic spectrum disorders and association with family history of autoimmune disease. J Dev Behav Pediatr 2006; 27: S128-36.

23. Ibrahim SH, Voigt RG, Katusic SK, et al. Incidence of gastrointestinal symptoms in children with autism: a population-based study. Pediatrics 2009; 124: 680-6.

24. Black C, Kaye JA, Jick H. Relation of childhood gastrointestinal disorders to autism: nested case-control study using data from the UK General Practice Research Database. BMJ 2002; 325: 419-21.

25. Buie T, Campbell DB, Fuchs GJ, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. Pediatrics 2010; 125 (Suppl 1): S1-18.

26. McElhanon BO, McCracken C, Karpen S, Sharp WG. Gastrointestinal symptoms in autism spectrum disorder: a meta-analysis. Pediatrics 2014; 133: 872-83.

27. Holingue C, Newill C, Lee LC, et al. Gastrointestinal symptoms in autism spectrum disorder: a review of the literature on ascertainment and prevalence. Autism Res 2018; 11: 24-36.

28. Gorrindo P, Williams KC, Lee EB, et al. Gastrointestinal dysfunction in autism: parental report, clinical evaluation, and associated factors. Autism Res 2012; 5: 101-8.

29. Wang LW, Tancredi DJ, Thomas DW. The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected members. J Dev Behav Pediatr 2011; 32: 351-60.

30. Parracho HM, Bingham MO, Gibson GR, McCartney AL. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol 2005; 54: 987-91.

31. Molloy CA, Manning-Courtney P. Prevalence of chronic gastrointestinal symptoms in children with autism and autistic spectrum disorders. Autism 2003; 7: 165-71.

32. Kang V, Wagner GC, Ming X. Gastrointestinal dysfunction in children with autism spectrum disorders. Autism Res 2014; 7: 501-6.

Received: 15.11.2020
Accepted: 12.03.2021