Original paper

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

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## 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 of 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.

## Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by difficult communication, impaired reciprocal social interaction, and repetitive, restricted, and stereotyped patterns of interests or behaviours. These deficits start early in life, vary widely in severity, and often change with the gain of other developmental skills [1].

Many children diagnosed as ASD also have gastrointestinal symptoms such as chronic diarrhoea, abdominal pain, constipation, vomiting, and gastroesophageal reflux. An association between ASD and inflammatory intestinal mucosal pathology has been proposed, and a link between gastrointestinal problems and behavioural signs and symptoms in autistic patients has been suggested [2]. Endoscopy results in children with ASD have demonstrated that the inflammation could occur anywhere along the gastrointestinal tract [3]. Some procedures can be used for detecting bowel inflammation including endoscopy, biopsy, colonoscopy, and analysis for biochemical markers in stool [4].

Calprotectin is a cytoplasmic protein that is present mainly in neutrophils; it is released by cell death and disruption [5]. During some inflammatory processes, calprotectin is released with the intracellular exudates in high amounts and can be found in body fluids and serum; therefore, it can be considered as a useful marker for inflammation [6].

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 EDI™ 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 $\chi^2$ test was used to test the association between qualitative variables. Fisher's Exact or Monte Carlo correction was used when in the $\chi^2$ 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).

**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 (≤ 3) | 26 | 65.0 |
| High (> 3) | 14 | 36.0 |
| Min.–max. | 0.0–9.0 | |
| Mean ± SD | 2.85 ±2.05 | |
| Median | 3.0 | |

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 (≥ 50 µg/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 Karkelis *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. Karkelis *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 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 |
|---|---|---|---|---|---|---|
| | N | % | N | % | | |
| < 50 | 26 | 65.0 | 30 | 75.0 | $\chi^2$ = 0.952 | 0.329 |
| ≥ 50 | 14 | 35.0 | 10 | 25.0 | | |
| Min.–max. | 13.0–100.0 | | 11.35–90.0 | | $U$ = 595.5* | 0.049* |
| 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 ≤ 0.05.*

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 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].

**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 $p \leq 0.05$.*

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 diarrhoea 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,

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.

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