Intestinal barrier as a silent driver of gut-brain disorders

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Background
The disorder of the gut-brain interaction, in adolescence, is of high prevalence worldwide. Up till now there is no clear aetiology for this gastrointestinal dysfunction.
Objective
To assess the status of the intestinal barrier in those having gut dysfunction compared with control group.
Materials and methods
A case-control study involved 180 Egyptian adolescents. They were distributed into two groups, the cases with positive ROME criteria and the controls with negative ROME criteria. Serum anti-flagellin antibodies (IgA and IgG) and intestinal fatty acid binding protein were assessed in both groups.
Results and conclusion
Ninety-nine adolescents out of 180 were positive for gastrointestinal dysfunction. The values of anti-flagellin antibodies and intestinal fatty acid binding protein were equivocal in both groups. Therefore, screening for gut-brain interaction disorders by ROME criteria is worthful for all adolescents. The positivity of ROME criteria does not always denote an underlying intestinal barrier defect.

Keywords:
adolescents, FLiC ab, iFABP, intestinal barrier, ROME criteria

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Introduction

The nomenclature "Functional Gastrointestinal Disorders" (FGID) is recently substituted by 'Disorders of Gut-Brain Interaction' (DGBI). It is an enigmatic disorder characterized by deregulated gut motility and abnormal sensory manifestations with a lack of organic aetiology. FGID is aggravated by stressful conditions and has a remarkable drawback on quality of life [1,2]. The dysfunctional gastrointestinal complaints are frequently encountered in children and in adolescence which is the stressful phase of pervasive maturation (physically, mentally and psychologically). Their academic achievement and relationship with their peers are affected and sometimes even ruined by the symptomatology and the stigmatizing diagnosis of FGID in this critical stage of life [3,4]. A multitude of pathophysiological mechanisms were investigated to clarify the cause for this dysfunction. Many researchers studied the role of deranged gut microbiota in the emergence of FGID but non-conclusive results were obtained. This ended up by noting that it is uncertain whether dysbiosis share in the aetiology or not. However, even if it does, definitely its contribution is not exclusive [5,6].

Ethnicity counts enormously in determination of intestinal microbiome. For this reason we established our study in Egypt to assess the magnitude of FGID in adolescents. Moreover, for further evaluation of gut barrier's status, we assessed the levels of serum immunoglobulins anti-flagellin IgG and IgA (FliC ab-IgA and IgG). These antibodies are formed as defensive mechanism against the leakage of gut bacteria to systemic circulation [7]. Their presence will be our marker of a dehiscent intestinal barrier. In addition, we evaluated the integrity of enterocytes through systemic detection of the cytosolic intestinal fatty acid binding protein (iFABP). Its extracellular presence points to damaged intestinal cells [8].

Subjects and methods

The research aim and steps were explained to the legal guardians of all participants. They approved and signed an informed consent prior to enrolment in the study. An ethical allowance was provided by the ethical committee of the National Research Centre (registered by number 19224). This was done according to the ethics of the Helsinki Declaration of 1975, as revised in 2000 [9].

A case-control study involved one hundred eighty Egyptian children and adolescents, from 10 to 18 years old. They were randomly recruited from four schools in Giza governorate.

Inclusion criteria

Adolescents of both sexes from 10 to 18 years of age.

Exclusion criteria

Children with organic gastrointestinal illness or other significant chronic health condition with potential gastrointestinal manifestations (e.g. cystic fibrosis, coeliac disease, etc). Those who were on antibiotics currently or for the preceding 6 months. Lastly, those who refused to participate or gave incomplete information or refused blood samples' withdrawal.

All participants were screened for FGID or DGBI. Then further stratification was done according to ROME criteria into a group of cases involving 99 adolescents diagnosed to have FGID or DGBI and a control group including 81 adolescents with negative ROME criteria. The tool used for FGID's diagnostic criteria was a valid Questionnaire on Pediatric Gastrointestinal Symptoms-Rome III (QPGS-RIII) [10,11]. It was extracted from the Questionnaire on Pediatric Gastrointestinal Symptoms (QPGS) [12]. The final form was constructed and supported by the Rome Foundation. In the current study, we used the self administered questions convenient for adolescents. The scaling of: how frequent, how severe and for how long was graded by five points. The answers were scored to evaluate the fitting criteria for the diagnosis of FGID or DGBI [13].

Blood samples were withdrawn. Three markers of defective intestinal barriers were estimated in both groups. Human FliC ab-IgA and IgG and iFABP. Serum sample preparation and procedure by ELISA technique was followed according to instruction manual.

Clinical characteristics were presented as mean ± SD. Comparison between groups were done using Student's *t* test. A *P* value of less than 0.05 was regarded as significant. Categorical data were evaluated using the χ^2 test. All statistics were performed using the SPSS statistics 17.0 software program.

Results and discussion

The cases and control children were homogenous as regards age with Mean±SD of 13.79±2.11 and 13.96 ±2.10, respectively, with an insignificant *P* value between the two groups (*P*=0.417). There were more females (*n*=59) than males (*n*=40) among cases and the inverse among controls with males (*n*=46) and females (*n*=35) with a significant *P* value=0.023, the four body mass index categories were of similar distribution in both groups with a non-significant *P*=0.207, as shown in (Table 1).

The FGID categories were determined as number and percent within the studied groups as shown in (Table 2).

Three markers of dehiscent gut barrier were equivocal in FGID group (FliC IgA 2.91±1.83 FliC IgG 2.36 ±2.09 iFABP 419.42±278.77) and in control group (FliC IgA 3.03±2.94 FliC IgG 2.43±2.17 iFABP 523.68±542.28) with insignificant *P* values of 0.295 and 0.753 and 0.084, respectively, as shown in Table 3.

The current study revealed a widespread of FGID in Egyptian adolescents. Almost half 55% of the participants (99 out of 180) showed positive ROME criteria for at least one category. This percent is more than double that one found in Colombia study by Velasco-Benítez *et al.* [14] 20.8% and by Saps *et al.*

Table 1 Distribution of age, sex and body mass index in the study group

Parameters	FGID (*n*=99) Mean±S.D.	Control (*n*=81) Mean±S.D.	*P* value
Age in years	13.79±2.11	13.96±2.10	0.417
Sex Male : Female	40 : 59	46 : 35	0.023
Body mass index value	24.30±6.77	23.07±6.36	0.207

FGID, functional gastrointestinal disorder.

Table 2 Distribution of functional gastrointestinal disorder among the study group

Diagnostic criteria	Study group *n*=180	
	+ve criteria *N*, (%)	–ve criteria *N*, (%)
1—Functional dyspepsia	2 (1.1)	178 (98.9)
2—IBS	37 (20.5)	143 (79.5)
3—Abdominal migraine	15 (8.5)	165 (91.5)
4—Functional abdominal pain syndrome	33 (18.5)	147 (81.5)
5—Functional abdominal pain	45 (25)	135 (75)
6—Functional constipation	44 (24.5)	136 (75.5)
7—Non-retention faecal incontinence	3 (1.67)	177 (98.33)
8—Aerophagia	59 (32.78)	121 (67.22)
9—Cyclic vomiting syndrome	1 (0.56)	179 (99.44)
10—Abdominal rumination syndrome	0	180 (100)

Table 3 Markers of defective intestinal barriers in cases compared with controls

Parameters	FGID (*n*=99) Mean±S.D.	Control (*n*=81) Mean±S.D.	*P* value
FLIC Ab IgA (mg/l)	2.91±1.83	3.03±2.94	0.295
FLIC Ab IgG (mg/l)	2.36±2.09	2.43±2.17	0.753
FABP (pg/ml)	419.42±278.77	523.68±542.28	0.084

FABP, fatty acid binding protein; FGID, functional gastrointestinal disorder; FLIC Ab IgA, flagellin antibodies IgA; FLIC Ab IgG, flagellin antibodies IgG.

[15] 29% among Colombians and that of 26.6% detected by Scarpato E *et al*. [16], in Europe, 23.5% by Bouzios *et al*. [17] in Greece and 23.1% by Lewis *et al*. [18] in United States. While Vernon-Roberts *et al*. [1] stated that FGID was prevalent in children and adolescents 4–18 years old and ranged from 19 to 40%. This discrepancy in prevalence may be attributed to geographical dietary heterogeneity, ethnicity, genetics, socio-economic and environmental factors. FGID was founded in females (*n*=59) more than males (*n*=40). This sex predilection reached a statistical significance compared to sex distribution in control group (46:35) *P*=0.023. This goes in accordance with the Goyal and colleagues and with Peralta-Palmezano and colleagues who noticed higher percent of FGID in females [19,20].

The commonest four results of FGID were as follows in descending order of frequency: Aerophagia 32.78% followed by functional abdominal pain 25% followed by functional constipation (FC) 24.5% followed by irritable bowel syndrome 20.5%. This matches the findings of Peralta-Palmezano *et al*. in their study where the predominant categories proved to be FC (13.2%), irritable bowel syndrome (6.9%), and aerophagia (3.1%) [20]. Similarly, FC (13.7%) was the commonest subtype found among eight Mediterranean countries in a research done by Strisciuglio *et al*. [21]. On the other hand, the least common presentations of FGID were abdominal migraine 8.5% non-retention faecal incontinence 1.67% and cyclic vomiting syndrome 0.56%. No one had abdominal rumination 0%.

In the current study we investigated the back leak of Gram-negative bacteria from gut to blood which is pathognomonic of barrier destruction. We searched whether a systemic immune reaction is present or absent against the byproducts of Gram-negative bacteria as a reflection of gut's barrier status. We considered the presence of the anti-FliC IgA and IgG as the markers of a disintegrated barrier. On the opposite, we considered their absence as a confirmation of an integral gut wall. We found equivocal levels of anti-FliC IgA and IgG in cases and controls. Accordingly, we deduced that the gut barrier was intact. This goes in harmony with the results found by Cook and colleagues in their study. They confirmed that the barrier destruction is more prevalent in inflammatory bowel diseases rather than the simple dysfunction in FGID [22]. Moreover, we investigated the circulating level of the (iFABP The iFABP is an intestinal intracellular protein. It remains cytosolic as long as the enterocytes are intact. But once the enterocytes are injured, a rise in iFABP will be noted [23]. Our results revealed no elevation of iFABP among the cases compared to controls. Thus, we derived that an intestinal mucosal injury does not underlie FGID. Corresponds to our results, neither the bacterial translocation nor the enterocytes' damage was proven in cases with FGID by many researches [24,25].

Up till now there is controversy about the aetiology of FGID. Many pathologies were considered as visceral hypersensitivity, stress, depression, anxiety and neurotransmitters' signals from brain to gut [26]. Unfortunately the symptomatology of FGID is misleading. A lot of functional disorders are misdiagnosed as organic diseases (e.g. inflammatory bowel diseases and coeliac disease). Thus, we would suggest using the markers of organic defects (iFABP, anti-FliC IgA and IgG) as a tool for a definite differential diagnosis.

Conclusion

FGID is a multifactorial cryptic disorder commonly encountered in adolescence. Although an association exists between dysbiosis and FGID, still a causative attribution remains uncertain.

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Conflicts of interest

There are no conflicts of interest.

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