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Research Article

Diagnostic Value of Faecal Calprotectin in Children with Chronic Gastrointestinal Disorders at Yaounde General Hospital

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Abstract

Introduction: Chronic gastrointestinal disorders are common in children. Numerous faecal biomarkers, such as faecal calprotectin, are used in the aetiological diagnosis of these digestive disorders. The study aimed to investigate the diagnostic value of faecal calprotectin in paediatric chronic gastrointestinal disease compared with that obtained in healthy children. *Methodology*: This was a comparative, analytical cross-sectional study from October 2022 through June 2023 at Yaound é General Hospital. Participants were children aged between four and eighteen with chronic digestive disorders. Using a pre-established questionnaire, we collected the socio-demographic and clinical characteristics of each participant. The participants' faecal calprotectin was tested at the laboratory of the Yaound é University Hospital Center by Enzyme-Linked Immunosorbent Assay (ELISA). Associations between variables were investigated by linear regression and calculation of the odds ratio (OR). The significance threshold was 5%. *Results*: Sixty stool samples were analysed for faecal calprotectin from 30 patients and 30 healthy participants. The mean age of the population was 9.47 (±3.35) years for patients and 10.67 (±3.70) years for healthy participants, with a sex ratio of 1.14 for patients and 0.87 for healthy participants. The threshold value for faecal calprotectin was 2.75 µg/g, with a sensitivity of 60%, a specificity of 63%, a positive predictive value of 61.20% and a negative predictive value of 62.06%. There were no significant differences in faecal calprotectin concentrations between children with chronic gastrointestinal disorders (peptic ulcer disease: p=0.10; functional gastrointestinal disorder associated with peptic ulcer disease: p= 0.710; functional gastrointestinal disorder: p= 0.143) and healthy children. *Conclusion*: The diagnostic value of faecal calprotectin as a biomarker in the diagnosis of chronic gastrointestinal disease was not observed in this study. However, the biological parameters assessed were measured only once, and given that their concentrations may vary over time, we recommend a subsequent longitudinal study.

Keywords

Chronic Gastrointestinal Disorders, Faecal Calprotectin, Children

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1. Introduction

Symptoms of the gastrointestinal tract such as abdominal pain, constipation, diarrhoea and fever are frequent reasons for parents to take their children to the paediatrician [1]. These symptoms can be found in both functional and organic diseases of the gastrointestinal tract, making diagnosis difficult for doctors. Endoscopy is the gold standard examination for differentiating between them, and we can also use biomarkers of faecal inflammation. However, endoscopy is particularly restrictive for paediatric patients, who often have a normal endoscopy [2]. The use of a biomarker to guide the physician's diagnosis and the prescription of endoscopies would enable rapid and accurate diagnosis of gastrointestinal pathologies. Substitute markers of colorectal inflammation, such as faecal calprotectin, are increasingly recognised as important for diagnostic guidance in children with chronic digestive disorders. Calprotectin is a 36 kDa heterocomplex calcium- and zinc-binding protein with two heavy chains and one light chain [3]. It was first described in 1980 by Fagerhol and is variously named MRP8, MRP14, cystic fibrosis-associated antigen, calgranulin and S100 [4]. When measured in faeces, calprotectin correlates well with neutrophil infiltration of the intestinal mucosal surface and lumen and is a hallmark of inflammatory bowel disease [3]. Calprotectin is also structurally very stable at room temperature for up to 7 days and resists bacterial degradation [4]. This makes calprotectin an ideal marker in the management of gastrointestinal pathologies. In recent years, faecal calprotectin has received a lot of attention and in the last year alone, more than 50 papers have been published on calprotectin measurements in faeces or other body fluids and tissues [2, 5]. In Cameroon, to our knowledge, few studies have been carried out on the measurement of faecal calprotectin in children. This study aimed to investigate the diagnostic value of faecal calprotectin in paediatric gastrointestinal disease by comparing it with that obtained in healthy children to contribute to better management.

2. Method

It was a comparative analytical cross-sectional study carried out at the Yaoundé General Hospital (HGY) from 1er October 2022 to 1er June 2023, i.e. 09 months. Biological analyses were carried out at the biochemistry laboratory of the Yaoundé University Hospital Centre (CHUY). The target population consisted of children aged between 4 and 18 years, divided into 2 groups: sick children and healthy children. The group of sick children was made up of children brought in for consultation for gastrointestinal symptoms or routine follow-up. The group of children who were not ill was recruited from their families. Children aged four to eighteen years with gastrointestinal symptoms during the previous 4 weeks were included. Personal consent and that of parents and/or guardians were obtained beforehand. Children with clinical symptoms of other pathologies associated with their chronic gastrointestinal disorders, such as respiratory infections, were excluded from the study. The formula for sample size calculation adapted to our type of study is contained in the manual by Whitley and Ball [6]. The estimated minimum sample size was 16 participants with chronic gastrointestinal disorders and 16 participants without. Two pre-tested questionnaires with closed- and open-ended questions for children and parents were designed for the study. The equipment used was: a stool jar for collecting the stool sample, care gloves, a timer to respect the time for micropipette analysis, tips for taking precise quantities of the sample, a centrifuge, an ELISA chain: apparatus for analysis, reagents Calprotectin ELISA Kit: to determine the concentration of calprotectin in the stool Saline phosphate for conditioning the stool. The search for faecal calprotectin was carried out in stool samples. A specimen of approximately 3 grams of first-morning stool was collected aseptically in a sterile stool container in a clean toilet at the collection site. Faecal calprotectin was separated from stool samples using PBS buffer (0.01 M, pH = 7.4) (i.e. 1 gram of stool per 9 mL of buffer) by centrifugation at 3000 rpm for 20 minutes. 2 mL of supernatant was then collected in 2 cryotubes and stored at -80 ℃. At the end of the data collection period, all cryopreserved samples were thawed and analysed at the Laboratoire de Biochimie Centre Hospitalier et Universitaire de Yaoundé The direct human sandwich ELISA method of the CALP (Calprotectin) ELISA test kit (Elabscience Biotechnologie Co., Ltd) was used. The results of the test were given to the different participants in the biochemistry laboratory with explanations of its interpretation. The data collected was entered and analysed in SPSS Version 23. Continuous quantitative variables were presented by the mean and standard deviation ($\mu \pm SD$), while discrete or non-normal variables were presented by their median and interquartile range [quartile 25; quartile 75]. The Kolmogorov-Smirnov test was used to confirm the normality of the sample. The categorical variables are presented using their numbers and proportions (n; %). Microsoft Office Excel 2016 ® was used to produce the graphs. The ROC curve was used to determine the intrinsic and extrinsic values of calprotectin. The relationship between gastrointestinal disease and faecal calprotectin concentration was investigated using the Mann-Whitney test. The statistical significance threshold was 0.05. Research authorisation was obtained from the Centre Region Human Health Research Ethics Committee (References: CE N ℃00269/CRERSHC/2023).

3. Results

Of a total of 60 children (30 sick and 30 healthy), the ages of the participants ranged from 4 to 18 years, with an average age of 9.47 ± 3.35 years in the sick subjects and 10.67 ± 3.70

years in the healthy subjects. The sex ratio was 1.14 for patients and 0.875 for healthy subjects. The most common age group was [7-10] years for patients (n=13, 43.3%) and [7-10] years for healthy subjects (n=12, 40%). The chronic gastrointestinal pathologies found were functional gastrointestinal disorders n= 25(90%) and an organic gastrointestinal disorder, peptic ulcer n= 5 (10%). The most frequent functional gastrointestinal disorder was functional abdominal pain: n=15 (60%). Figure 1 shows the distribution of children according to gastrointestinal disorders.

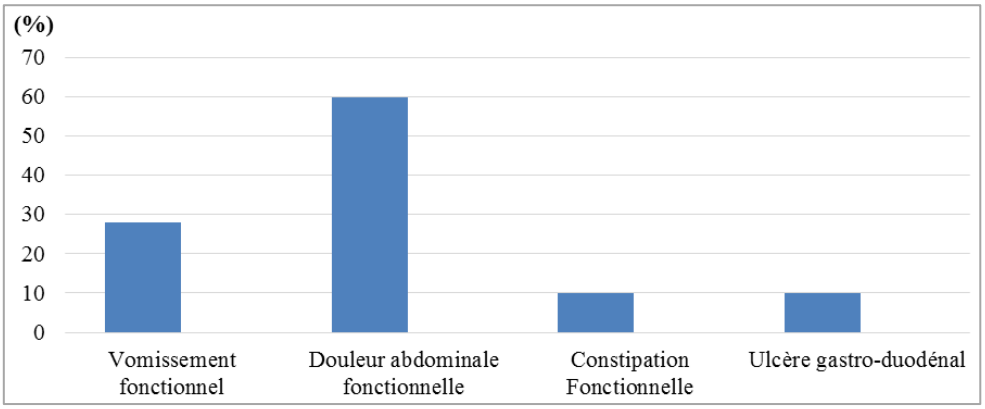

Figure 1. *Distribution of gastrointestinal disorders in patients (N=30).*

There was no difference between the median concentration of faecal calprotectin in sick children 4.1 [1.6; 8] and in healthy children 2.2 [1;7.5]. P= 0.19 (Figure 2).

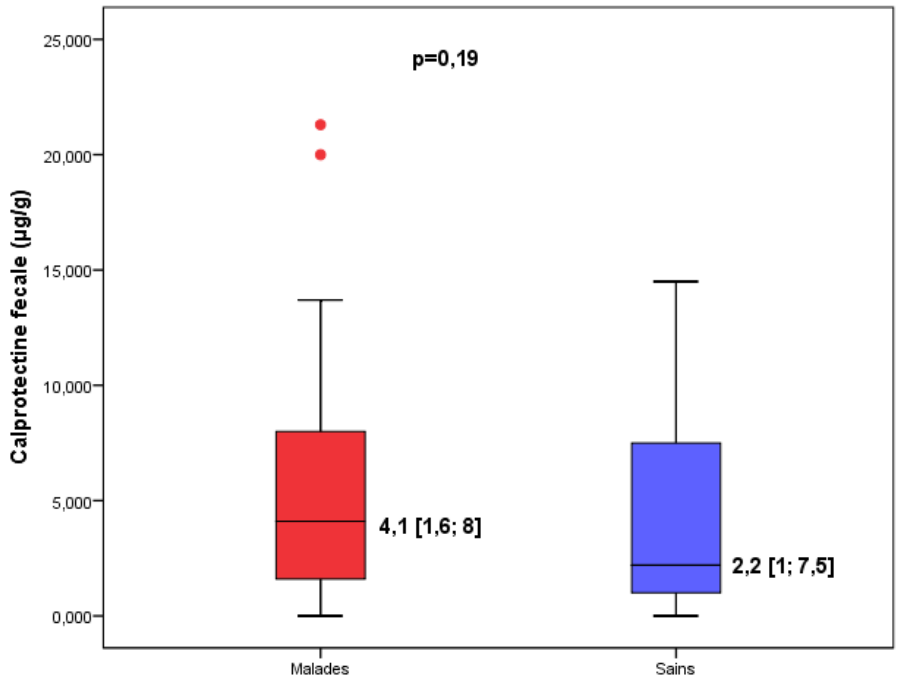

Figure 2. *Comparison of faecal calprotectin concentrations in sick and healthy children.*

At the faecal calprotectin cut-off value of 2.75 µg/g as shown in Table 1, sensitivity and specificity were 60% and 63%. At this threshold value, a positive predictive value of 61.29% and a negative predictive value of 62.06% were obtained. The diagnostic cut-off value for faecal calprotectin was 2.75 µg/g (Table 1).

Table 1. *Threshold value for faecal calprotectin.*

Calprotectin threshold (µg/g)	Sensitivity (%)	Specific (%)	PPV (%)	VPN (%)
0,2	96	20	54.71	85.71
2.75	63	60	61.29	62.06
10,50	13	90	57.14	50.94

The concentration of faecal calprotectin was not significantly higher in the diseased group than in the healthy group. There was no significant difference between the faecal calprotectin values of the different gastrointestinal pathologies as presented in Table 2.

Table 2. *Association between faecal calprotectin and gastrointestinal diseases.*

Gastrointestinal diseases	Median (± CI)	p-value
Functional constipation		
Yes	3.0 [0.4;21.3]	0.89
No	4.2 [1.6;8.0]	
Functional abdominal pain		
Yes	1.6 [1.0;5.3]	0.77
No	5.1 [3.0;8.0]	
Functional vomiting		
Yes	2.0 [1.0;4.0]	0.27
No	5.1 [1.6;8.0]	
Functional gastrointestinal disorders		
Yes	1.6 [1.0; 6.0]	0.14
No	6.0 [3.0; 8.0]	
Peptic ulcer		
Yes	7.0 [5.1;9.0]	0.10
No	3.5 [1.5;7.5]	

4. Discussion

The general objective was to study the diagnostic value of faecal calprotectin in paediatric gastrointestinal disease by comparing it with that obtained in healthy children followed at the Yaoundé General Hospital. Some limitations should be borne in mind when interpreting the results of this study. Firstly, the biological parameters evaluated were measured only once, given that their concentrations may vary over time. However, we used fairly strict selection criteria to limit measurement bias. Secondly, standard faecal calprotectin values have not yet been validated in the child population. However, the size of the sample and the method used enabled conclusions to be drawn. The ages of the participants ranged from 4 to 18 years, with an average of 9.47 ± 3.35 years in the sick population and 10.67 ± 3.70 years in the healthy population. A study carried out in Poland found an average age of 12.5 years for patients and 6.5 years for healthy subjects [7]. This result could be explained by the presence of chronic gastrointestinal symptoms in school-age children [8]. The sex ratio was 1.14, with a male predominance of 53.3% in the sick group. For the healthy group, the sex ratio was 0.87, with a predominance of females. The male predominance noted in this study was found in Poland [9]. The most frequent functional gastrointestinal disorder (FGID) was functional abdominal pain 15 (60%). These results contrast with the study carried out in Poland, where a predominance of gastrointestinal diseases of organic origin was observed [8]. Given the inadequate technical facilities in Cameroon and the patient's therapeutic itinerary, with self-medication as the first recourse, and the lack of security for carrying out certain tests necessary for diagnosis, organic gastrointestinal pathologies are generally diagnosed when the child is already at the stage of complications [10]. The mean concentration of faecal calprotectin in healthy and sick children was 4.1 µg/g (1.6;8) and 2.2 µg/g (1;7.5) respectively, with a p= 0.19 value. The study carried out in Poland showed no significant difference in faecal calprotectin concentration between the group of healthy patients and patients with functional gastrointestinal disorders. However, in children with inflammatory gastrointestinal disorders (gastrointestinal ulcers), CF concentrations were significantly higher than in the healthy group [8]. This predominance of high calprotectin values in inflammatory gastrointestinal disorders can be explained by the fact that faecal calprotectin is an inflammatory marker. Its value increases significantly during inflammation. [11] The sensitivity, specificity, and positive and negative predictive values of faecal calprotectin in functional gastrointestinal disorders were 60% and 63% respectively at a threshold value of 2.75 µg/g. Studies of calprotectin in children in the UK and Norway found a cut-off value of 50 µg/g [12, 13] In Cameroon, a study of the diagnostic performance of faecal calprotectin in adults with chronic inflammatory bowel disease found a cut-off value of 2.515 µg/g close to that found in this study. [14] The kit used and the method for extracting faecal calprotectin from faeces could explain this difference in the threshold values found [15].

5. Conclusion

The most common chronic gastrointestinal disorders were functional gastrointestinal disorders. The threshold value for faecal calprotectin was 2.75 µg/g, with a sensitivity of 60% and a specificity of 63%. The diagnostic value of faecal calprotectin as a biomarker in the diagnosis of chronic gastrointestinal disease was not observed in this study. However, the biological parameters evaluated were measured only once, and given that their concentrations may vary over time, we recommend a subsequent longitudinal study.

Abbreviations

CALP: Cal Protectine
ELISA: Enzyme Linked Immunosorbent Assay
ROC: Receiver Operating Characteristic
OR: Odds Ratio
CHUY: Yaoundé University Hospital Centre
HGY: Yaoundé General Hospital

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Author Contributions

Isabelle Mekone Nkwele: Project administration Conceptualization, Data curation, Writing – original draft, Writing – review & editing
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Jeannette Epee Ngoué: Writing – review & editing
Ngogang Marie Paul: Writing – review & editing
Nguefack Félicité: Methodology, Writing – review & editing
Ama Moor Vicky Jocelyne: Supervision, Validation

Conflicts of Interest

The authors declare no conflicts of interest.

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