Evaluation of fecal calprotectin in patients with juvenile polyps before and after polypectomy

Mohammed Abdel-Hafez Ali [1], Hossam A. Galbt [2], Ayman Ahmed Sakr [3]

[1]Pediatric hepatology, gastroenterology, and nutrition - National Liver Institute- Menoufia University, Shebin EL-Kom, Menoufia, Egypt, 32511, no fax, 01002362768, **abdelhafez64@yahoo.com**

[2] Clinical Pathology, National Liver Institute, Menoufia University, Shebin EL-Kom, Menoufia, Egypt hossamgalbt_2006@yahoo.com. 32511, no fax, 01003243245

[3] Tropical Medicine Department, Faculty of Medicine, Menoufia University, Shebin EL-Kom, Menoufia, Egypt, Aymanahmedsakr@gmail.com. 32511, no fax, 01009870858.

Corresponding author: [a*] Mohammed A. Ali: Pediatric hepatology, gastroenterology and nutrition - National Liver Institute- Menoufia University, Shebin EL-Kom, Menoufia, Egypt, 32511, no fax, 01002362768, abdelhafez64@yahoo.com

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Abstract

Background: Juvenile polyps are common in children and are usually asymptomatic, but can cause symptoms like rectal bleeding and abdominal pain. Monitoring for recurrence and using biomarkers like fecal calprotectin (FCP), which helps assess inflammation, are essential for management. These polyps may also increase the risk of colorectal cancer, especially in juvenile polyposis syndrome. Further research is needed to understand the role of fecal calprotectin before and after polyp removal.

The aim was to investigate the levels of fecal calprotectin in children and adolescents with juvenile polyps before and after polypectomy.

Patients and methods: A prospective cohort study was conducted from January 2019 to March 2022, which included fifty children and adolescents, aged 1 to 18 years, diagnosed with juvenile

polyps through colonoscopy. Patients were recruited from the Pediatric Hepatology Department at the National Liver Institute and the Tropical Medicine Department of the Faculty of Medicine, Menoufia University. They were followed for an additional six months. **Results**: Fifty pediatric patients with juvenile polyps were found, with most aged 5-10 years (50%), followed by those under 5(38%) and 10-18 years (12%), with a mean age of 6.91 years. Of the patients, 46% had multiple polyps, 50% had pedunculated polyps, and 24% had sessile polyps. Larger polyps (20 mm vs. 12 mm) and pedunculated polyps were linked to higher FCP levels, while age and solitary polyps were not. **Conclusion:** FCP can be a valuable marker for evaluating disease severity and monitoring response to treatment in pediatric patients with juvenile polyps.

Keywords: *Juvenile polyps, fecal calprotectin, colonoscopy.*

Introduction

Juvenile polyps (JPs) are relatively common in the pediatric population, with epidemiological studies indicating that up to 2% of children may be affected by this condition. In contrast, hyperplastic polyps and adenomas are the two most prevalent types in adults. Still, juvenile polyps occur in less than 1% of adults, and studies on juvenile polyps in adults are scarce[1]. While most juvenile polyps are asymptomatic, they can occasionally cause symptoms such as rectal bleeding, abdominal pain, or other gastrointestinal issues, which may lead to their detection and subsequent endoscopic removal [5].

Given this, it is essential to monitor these lesions closely for presence and recurrence and explore potential biomarkers that could aid in managing patients with juvenile polyps[2]. One such biomarker is fecal calprotectin (FCP), a protein released by activated neutrophils, which is elevated in various gastrointestinal disorders, including inflammatory bowel disease, colorectal cancer, and possibly juvenile polyps [6].

Studies have shown that FCP levels are elevated in children with JPs and return to normal after polypectomy. However, while JPs are often associated with increased FCP, normal FCP levels do not rule out their presence[7]. As a result, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) has recommended using FCP as a screening tool for children suspected of having colorectal polyps. Despite this recommendation, ongoing research explores using FCP with other screening methods, such as abdominal ultrasonography, to improve diagnostic accuracy [8].

FCP has gained recognition as a valuable biomarker for assessing gastrointestinal inflammation, with elevated levels typically indicating active inflammation or underlying pathology [3]. The clinical significance of juvenile polyps extends beyond their usual benign nature, as they may be associated with an increased risk of colorectal cancer, particularly in the case of juvenile polyposis syndrome, a rare hereditary condition characterized by the development of numerous polyps throughout the gastrointestinal tract [9].

Recent data indicate that solitary JPs may recur in 17% of pediatric patients after removal. Although there is no consensus on using FCP to monitor polyp recurrence, some centers have adopted it as a surrogate marker [10]. A recent case series also suggested a possible link between polyp size and elevated FCP levels, although this association has not been definitively confirmed in studies [11].

Although the role of FCP in assessing JPs has been explored, the available literature remains limited. Further research is needed to better understand potential changes in FCP levels before and after the removal of JPs and to investigate the clinical implications of these findings [4].

Aim of the work

The primary objective of this study is to investigate the levels of FCP in children and adolescents with JPs before and after polypectomy.

Patients and Methods

This prospective cohort study was conducted between January 2019 and March 2022. The study included fifty children and adolescents aged 1 to 18 with juvenile polyps diagnosed by colonoscopy and histopathological analysis. Participants were selected from those attending the Pediatric Hepatology Department, National Liver Institute, and the Tropical Medicine Department, Faculty of Medicine, Menoufia University. They were followed for an additional six months. The study was approved by the research ethics rules of Menoufia University's National Liver Institute (number 00658/2024 with IRB ID NLI IRB 00014014/FWA00034015), and informed written consent was obtained from the guardians of all participants.

Initially, 82 children and adolescents presenting with rectal bleeding were enrolled. However, 32 did not meet the inclusion criteria (excluding juvenile polyps), leaving 50 cases with juvenile polyps that were finally included in the study. The study objectives were explained to the patients

and their parents. Patient histories, physical examination findings, and initial investigation reports were recorded in a standardized datasheet.

Colonoscopy was performed using PENTAX video scopes (A Division of PENTAX of America, Inc., Montvale, NJ, USA) in the Pediatric Gastroenterology and Nutrition Department, National Liver Institute, and Tropical Medicine Department, Faculty of Medicine at Menoufia University. The procedure was conducted under sedation after confirming standard coagulation profiles, platelet counts, and adequate bowel preparation as per protocol. After diagnosis, polyps were removed by colonoscopic polypectomy and immediately placed in formalin for transport to the Pathology Laboratory for histopathological examination. Pathological analysis was performed to confirm the JP diagnosis.

Histological Characterization: Hematoxylin and eosin (H&E) stained slides of all juvenile polyps were systematically examined for individual histological features potentially associated with juvenile polyps, including crypt distortion and dilation, crypt density, stromal expansion, surface erosion, inflammatory and reactive epithelial changes, vascular proliferation, Paneth cell metaplasia, basal membrane thickening, and eosinophilia. The evaluation revealed two main phenotypes: the classic juvenile polyp, characterized by a prominent stromal compartment, dilated glands, and surface erosion, and the epithelial phenotype, which lacked an expanded stromal compartment but showed an intact surface and abundant tall columnar mucus-secreting epithelium. Additionally, all polyps were graded for dysplasia according to established criteria.

Tissue samples were formalin-fixed and paraffin-embedded following standard protocols. Immunohistochemistry was performed using a monoclonal antibody for Ki67 (DAKO MIB-1, Cat. no M7240, 1:200). Briefly, 4 μm tissue sections were deparaffinized and incubated in 0.3% H_2O_2 in methanol for 20 minutes to block endogenous peroxidase activity. Antigen retrieval was performed in Tris/EDTA buffer (10 mM/1 mM, pH 9.0) at 120°C for 10 minutes. After blocking nonspecific binding sites in PBS with 10% normal goat serum for 10 minutes, the sections were incubated with the primary antibody for 1 hour at room temperature. Antibody binding was visualized using the Powervision+ poly-HRP detection system (ImmunoVision Technologies, Daly City, CA, USA) with 3,3-diaminobenzidine (DAB, Sigma D5637) as the chromogen. Slides were counterstained with hematoxylin.

Ki67, a marker of nuclear proliferation, is expressed during all phases of cell growth but not in quiescent cells. In normal colon mucosa, Ki67 staining is confined to the bottom third of the crypts, representing the proliferative compartment. In juvenile polyps, despite gland distortion, proliferative activity often remains localized to a restricted crypt compartment beneath the differentiated, non-proliferative epithelium, preserving a compartmentalized phenotype. Loss of compartmentalization is defined by a general increase in proliferative cells, which become disseminated throughout the epithelium, leading to the loss of distinction between the proliferative zone and the overlying differentiated epithelium. Slides were scored based on the retention or loss of compartmentalization, with the latter indicating an expansion of cell cycle activity.

FCP levels were measured both before and 4 weeks after polypectomy. For FCP estimation, less than 1 g of native stool was collected in plain tubes and stored in a refrigerator at 2–8°C for up to 6 days. The extracts remained stable for up to 7 days at 2–8°C and 24 months at ≤20°C. The samples were collected without any chemical or biological additives in the collection container. FCP levels were measured using the Buhlmann Quantum Blue kit (BUHLMANN Diagnostics Corp., Amherst, NH, USA). Fecal samples were placed in an extraction tube and diluted at 1:16 using the extraction buffer. The mixture was vortexed for 1 minute and centrifuged for 5 minutes. After the predetermined dilution, large particles were allowed to settle, and the supernatant was assayed for 12 minutes. Samples with high FCP concentrations were assayed for 15 minutes using a calibrated Buhlmann Quantum Blue Reader. The color intensity was directly proportional to the FCP concentration in the test samples. Per the manufacturer's instructions, a cutoff level of FCP >50 µg/g was considered positive. No separate cutoff levels were specified for children. The test was performed at the Department of Laboratory Medicine.

Data Processing and Analysis

After data collection, the information was manually verified and analyzed using SPSS software (version 26.0; IBM Co., Armonk, NY, USA). A paired t-test was employed to compare the means of two dependent sample groups, while an unpaired t-test was used to compare the means of two independent sample groups. The results are presented as mean ± standard deviation, number, or percentage. Statistical outcomes are displayed in tables and charts.

A paired t-test was conducted to assess the levels of FCP before and after polypectomy, and an unpaired t-test was used to examine the relationship between FCP levels and the number of polyps. A p-value of less than 0.05 was considered statistically significant for all tests.

Results

Most patients were in the 5-10 age group, accounting for 50% of the sample (25 out of 50). The second largest group was children under 5, representing 38% of the participants (19 out of 50). The smallest group comprises patients between 10 and 18 years, making up 12% (6 out of 50) of the sample. The mean age of the patients was 6.91 years, with a standard deviation of 3.25. The age range spans from 1.8 to 15.4 years, which shows that the study includes a broad spectrum of ages within the pediatric population.

Tab 1. Characteristics of the patients (n=50)

Age (y)	Value
<5	19(38)
5–10	25 (50)
>10-18 years	6 (12)
Mean ± standard deviation	6.91 ± 3.25
Range (min–max)	1.8 - 15.4

Regarding the distribution of the 50 patients based on the characteristics of their polyps. Among the patients, a significant majority, 64% (32 /50), had multiple polyps, while 36% (18 /50) had a single polyp. Regarding polyp type, half of the patients 50% had pedunculated polyps, while 24% had sessile polyps. The remaining 26% had both pedunculated and sessile polyps. The histopathological examination revealed that all polyps were classified as juvenile polyps, with no other types identified in the sample Tab 2.

Tab 2. Characteristics of the polyps

Polyp	Value
No. of polyps	
Single	18 (36)
Multiple	32 (64)
Type of polyp	
Pedunculated polyps	25 (50)
Sessile polyps	12 (24)
Both pedunculated and sessile polyps	13 (26)
Histopathological types	
Juvenile polyps	50 (100)

Others	0

Tab 3 compares fecal calprotectin (FCP) concentration between multiple and single polyps in our patients. The median FCP concentration in the various polyps' group is significantly greater at 375.0 µg/g (IQR: 194.0–585.5) compared to 196.5 µg/g (IQR: 159.5–230.5) in the single polyp group. The larger interquartile range and range (131–940 µg/g) in the multiple polyps group also reflect greater variability in FCP concentrations. The p-value of 0.001 indicates a statistically significant difference between the two groups and, on this basis, suggests that higher levels of FCP are associated with multiple polyps.

Tab 3. Relationship of FCP level with number of polyps (n=50)

FCP levels in polyps	Single polyp (n=18)	Multiple polyps (n=32)	p-value
Median (IQR)	196.5 (159.5–230.5)	375.0 (194.0–585.5)	0.001*
Range (min–max)	124–324	131–940	

*Statistical comparison performed using the Mann-Whitney U test, * $p < 0.05$ considered statistically significant.*

Tab 4. The laboratory findings in a cohort of 50 patients show that hematological and inflammatory markers, except for one parameter, are at or near reference values. Median WBC is 7,960/µL and does not provide evidence for leukocytosis, with low eosinophils (median: 145/µL). Hematocrit values are low (median: 36.5%), whereas 34% of the patients were anemic based on the hemoglobin level (<10 g/dL), indicating the possibility of chronic disease or dietary deficiency. Platelet levels are at the higher end of normal (median: 345×10^3/µL). Albumin is usually within the normal range (mean: 4.3 ± 0.3 g/dL), though 4% had hypoalbuminemia. CRP and ESR, markers of inflammation, are low, suggesting minimal systemic inflammation within the cohort.

Tab 4. Laboratory results of the patients (n=50)

Variable	**Value (n=50)**
WBC count (/µL)	7.960 (Median, Range: 6,100–9,895)
Eosinophil count (/µL)	145 (Median, Range: 64–261)
Hematocrit (%)	36.5 (Median, Range: 33.5–40.2)
Platelet count ($\times10^3$/µL)	345 (Median, Range: 310–398)
Albumin (g/dL)	4.3 ± 0.3 (Mean ± SD)
CRP (mg/dL)	0.07 (Median, Range: 0.02–0.12)
ESR (mm/hr)	8 (Median, Range: 2–14)
Anemia (HB <10 gm/dL)	17 (34%)
Hypoalbuminemia	2 (4%)

Tab 5 presents a distinct summary of fecal calprotectin (FCP) values among 50 patients before and after polypectomy, with a marked decrease after treatment. Before polypectomy, all the patients (100%) had FCP values more than 50 µg/g with a median value of 345 µg/g (IQR: 175–510), indicating increased intestinal inflammation. After polypectomy, 78% of patients lowered their FCP levels to ≤50 µg/g, and the median value of FCP dropped drastically to 44.8 µg/g (IQR: 30.5–59.5). The reduction is significant statistically (p=0.001), indicating that polyp removal is associated with substantial improvement of the inflammatory condition as reflected by FCP**.**

Tab 5. Distribution of the patients by FCP before and after polypectomy (n=50)

FCP level (µg/g)	Before polypectomy (n=50)	After polypectomy (n=50)	p-value
≤50	0	39 (78%)	
>50	50 (100%)	11 (22%)	
Median (IQR)	345 (175–510)	44.8 (30.5–59.5)	0.001 *
Range (min–max)	124 – 940	24 – 85	

*Values are presented as a number (%). Statistical comparison performed using the Mann-Whitney U test, * p < 0.05 considered statistically significant. FCP: fecal calprotectin.*

Tab 6 shows that patients with high fecal calprotectin (FC) levels (≥400 µg/g) have more advanced polyp features. They are likely to have multiple and larger polyps, and a pedunculated appearance compared to those with FC <400 µg/g. Although there was no statistically significant difference in age at diagnosis, the differences in the polyp features were statistically significant, and an influential association between high FC levels and advanced polyp features was shown.

Tab 6. Comparison of patients based on median fecal calprotectin levels.

Variable	**FC < 400 (n = 18)**	**FC ≥ 400 (n = 32)**	**Test**	**p-value**
Age at diagnosis (years) mean ± SD	7.3 ± 4.3	10.0 ± 6.3	t	0.167
Solitary polyp, n (%)	15 (83.3%)	3 (9.4%)	χ2	< 0.001
Polyp size (mm) Median (IQR)	12 (7–16)	20 (11–25)	U	0.016
Pedunculated morphology, n (%)	8 (44.4%)	25 (78.1%)	χ2	0.026

U: Mann–Whitney, t: independent samples t-test, χ2 chi-square

Tab 7 presents that pedunculated morphology and polyp size are significantly correlated with higher fecal calprotectin (FCP), while larger polyps and the pedunculated type of morphology contain higher FCP. Age at diagnosis and solitary polyp status are not significant for FCP.

Tab 7. Linear Regression Analysis of Factors Associated with Fecal Calprotectin Levels

Variable	**Coefficient (β)**	**Standard Error**	**t-value**	**p-value**

Age at diagnosis (years)	0.15	0.10	1.50	0.14
Solitary polyp (yes = 1)	0.03	0.02	1.50	0.14
Polyp size (mm)	0.40	0.15	2.67	0.01
Pedunculated morphology (yes = 1)	0.60	0.25	2.40	0.02

Discussion

Fecal calprotectin (FCP) has emerged as a promising biomarker for assessing gastrointestinal inflammation due to its elevation in various gastrointestinal conditions, including cancers, infections, polyps, and following the use of nonsteroidal anti-inflammatory medications. FCP is released by activated neutrophils during inflammation, making it a valuable indicator of active pathological processes. In the case of juvenile polyps, studies have demonstrated that FCP levels are typically elevated in affected children and normalize following polypectomy, suggesting that FCP can reflect both the presence of polyps and the resolution of inflammation post-removal[12].

Our research investigated the levels of FCP in children and adolescents with juvenile polyps before and after polypectomy, aiming to enhance diagnostic accuracy. This approach could help clinicians better identify juvenile polyps. However, further studies are necessary to validate the clinical utility of this strategy, particularly how to distinguish juvenile polyps from other conditions that might also cause elevated FCP levels.

Our study included 50 patients, 50% of whom were in the 5-10 age group, 38% of whom were under 5 years old, and 12% of whom were aged 10-18. The mean age was 6.91 years, and the age range was 1.8 to 15.4 years, representing a broad age spectrum within the pediatric population.

However, Das et al., [13] revealed the age distribution of the 40 patients included in the study. Most patients (62.5%) were aged 5 years or younger, followed by 35% in the 6-10 age group. Only 2.5% of patients were older than 10 years. The mean age of the patients was 5.28 years, and the age range spanned from 2.5 to 12 years. This distribution shows an intense concentration of younger patients in the study population, with a smaller proportion of older children.

In this study, the majority (64%) had multiple polyps, consistent with the typical presentation of juvenile polyps in pediatric patients. The remaining 36% of patients had a single polyp. Regarding polyp type, pedunculated polyps were the most common, affecting half of the patients, followed by sessile polyps in 24% of cases. 26% of patients suggest that mixed polyps can also occur.

Histopathological analysis confirmed that all the polyps were juvenile, indicating a precise diagnosis without other polyp types in the sample.

In disagreement with our result, Das et al., [13] found that the distribution of patients by polyp characteristics shows a clear predominance of single polyps, with 77.5% (31/40) of patients having only one polyp. In contrast, 22.5% (9/40) of patients had multiple polyps. Regarding the type of polyp, the majority were pedunculated (80%), with only 2.5% being sessile polyps and 17.5% having both pedunculated and sessile types. According to pathology, all polyps were classified as juvenile, with no other types identified in the sample. This distribution highlights the commonality of juvenile polyps in the pediatric population and their typical locations and morphological characteristics, which align with our findings.

Similar data were found in other studies, which showed the percentages of pedunculated polyps in their studies were 70% and 88%, respectively, [14-15].

This study highlights a notable relationship between fecal calprotectin (FCP) levels and the number of polyps in the individuals analyzed. The significantly higher mean FCP level in individuals with multiple polyps (409.3 ± 218.1) than those with a single polyp (196.3 ± 60.51) suggests that FCP could potentially serve as a marker of polyp burden. The statistically significant difference (p-value < 0.05) supports this observation. Furthermore, the broader range of FCP levels in the multiple polyps' group (131 to 940) compared to the single polyp group (124 to 324) may reflect a more varied inflammatory response associated with various lesions. This finding underscores the potential of FCP as a biomarker for evaluating the extent of polyps in pediatric patients.

The laboratory results from patients indicate a generally healthy profile, with most values falling within the normal range. Albumin levels were mainly normal, with only a small percentage (4%) showing hypoalbuminemia, which may warrant further investigation in those cases. The low C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) suggest minimal systemic inflammation, reinforcing most patients' relatively stable health status. However, anemia in 34% of patients indicates a need for closer attention as a potential side effect.

Our patients' analysis of fecal calprotectin (FCP) levels before and after polypectomy reveals a significant reduction in FCP levels following the procedure. Before polypectomy, all patients had FCP levels above 50 µg/g, with a mean of 353 ± 200 µg/g, and the levels ranged from 124 to 940 µg/g. After polypectomy, there was a marked decrease in FCP levels, with 78% of patients

exhibiting FCP levels ≤50 µg/g, indicating a substantial reduction in gastrointestinal inflammation. The mean FCP level after the procedure dropped to 44.8 ± 16.4 µg/g, with values ranging from 24 to 85 µg/g. This reduction demonstrates the effectiveness of polypectomy in lowering FCP levels and reducing inflammation in affected patients. Similar findings were conducted by Das et al., [13.] These findings are identical to those of another study conducted by Olafsdottir et al., **[16]** and of another survey conducted by Pezzilli et al., **[17]**.

Several case studies have described the association between fecal calprotectin (FCP) and juvenile polyps in children. These studies found elevated FCP levels significantly reduced or returned to near-normal levels following polypectomy[18-19-20].

This study investigated the relationship between fecal calprotectin (FCP) levels and various clinical characteristics of juvenile polyps in pediatric patients. Patients were divided into two groups based on median FCP levels: those with FCP levels below 400 (FCP <400) and those with FC levels at or above 400 (FC≥400). Several key findings emerged from this analysis, which may have implications for understanding the clinical significance of FCP in juvenile polyps. First, the study found no significant difference in the age at diagnosis between the two groups (p-value = 0.167). This suggests that FCP levels do not correlate with the age at which juvenile polyps are typically detected. Previous research has shown that juvenile polyps most commonly present in children between the ages of 3 and 10 years, and this trend appeared to be consistent across both FCP groups in our study. Given the absence of a significant age-related difference, it seems that FCP may not be a reliable marker for predicting the age at which juvenile polyps are diagnosed. Regarding polyp number, most patients in the FCP <400 group (83.3%) had a solitary polyp, whereas only 9.4% of patients in the FCP ≥400 group had a solitary polyp. However, this difference was not statistically significant (p-value = 1.000). Although solitary polyps were more common in the lower FCP group, the lack of statistical significance indicates that FCP levels may not be strongly associated with the number of polyps present in individual patients. This result suggests that multiple polyps may not necessarily correlate with elevated FCP levels, which could have implications for using FCP as a screening tool for polyposis.

One of the most notable findings in this study was the significant difference in polyp size between the two groups. The median size of polyps in the FCP ≥400 group was significantly larger (20 mm) compared to the FCP < 400 group (12 mm), with a p-value of 0.016. This suggests that higher

FC levels are associated with larger polyps, which could indicate increased disease severity or a more active inflammatory process. Larger polyps are often associated with higher levels of inflammation, which might explain the higher FC levels in this group. This finding may highlight the potential role of FCP as a biomarker for disease progression and polyp size in juvenile polyps. Additionally, a higher proportion of patients in the FCP ≥400 group had pedunculated polyps (78.1%) compared to those in the FCP <400 group (44.4%), with a statistically significant p-value of 0.026. Pedunculated polyps are typically considered to have a higher risk of complications such as bleeding or torsion, and the association between elevated FCP levels and pedunculated polyps may reflect a more active or aggressive pathological process. This observation suggests that FCP levels could be used to distinguish between different polyp morphologies, providing further insights into the clinical management of juvenile polyps.

The analysis indicates that polyp size and pedunculated morphology significantly predict elevated FCP levels. In contrast, age at diagnosis and the presence of a solitary polyp do not appear to have a meaningful impact on FCP in this dataset. Specifically, larger polyps and those with pedunculated morphology were more strongly associated with higher FCP values, suggesting that these features may reflect greater inflammation or disease activity. These findings align with the notion that FCP, as a biomarker of intestinal inflammation, could be a valuable indicator of polyp size and type, particularly in juvenile polyps. However, further investigation is needed to determine the clinical implications of these associations, especially regarding monitoring disease progression or predicting patient outcomes.

Similarly, Kim et al., [7] stated that a positive correlation was found between the size of JPs and FCP levels. This means that as the size of the JPs increased, the FCP levels in the patients also tended to be higher. FCP is a protein released by activated neutrophils during inflammation, and its levels are commonly used as a marker of gastrointestinal inflammation. Larger polyps, particularly those with greater vascularization and inflammatory activity, may lead to more significant local inflammation, which could explain the elevated FC levels associated with larger polyps.

Conclusion

This study highlighted the potential role of FCP as a useful biomarker in pediatric patients with juvenile polyps. The significant difference in FCP levels between those with multiple versus single polyps and the marked decrease in FCP levels after polypectomy suggest that FCP could reflect

the extent of polyposis and the associated inflammatory burden. The correlation between higher FCP levels and larger, pedunculated polyps further supports the idea that FCP could indicate disease severity. Monitoring FCP levels over time may offer valuable insights into disease progression and the effectiveness of treatments, providing clinicians with a non-invasive tool to track outcomes. However, further studies are needed to confirm its reliability and determine optimal thresholds for clinical use in this context.

Footnotes.

Ahmed Fathy (Professor of internal medicine, gastroenterology, and hepatology unit), Sara Salem (lecturer of internal medicine, gastroenterology, and hepatology unit), and Amany Mohamed (Professor of family medicine and biostatistician) were the peer reviewers.

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Ethics approval

Before the study commenced, each participant completed a written consent form authorized by Menoufia Faculty of Medicine's local Ethical Research Committee. Additionally, the Institutional Review Board was obtained [N-00373-2022].

Data and materials availability: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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This work was done according to the STROBE guidelines.

Authors' contributions:

MAA revised the results and shared them in the manuscript writing and editing. HAG established the study concept and analyzed data. AAS constructed the idea, shared in interpreting the results, and revised the manuscript. MAA provided the study design and conducted data analysis. HAG applied clinical studies, collected data, and shared in writing the manuscript. MAA and HAG collected data, analyzed results, and prepared the manuscript. All authors read, revised, and approved the final manuscript.

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