

Analysis of type I hypersensitivity-induced inflammatory response in children of different age groups with acute appendicitis

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ABSTRACT

Objective: To explore the differences in type I hypersensitivity-induced inflammatory response among children of different age groups with acute appendicitis.

Methods: We selected children diagnosed with "acute appendicitis" who underwent surgery in the Department of General Surgery of Anhui Provincial Children's Hospital from January 2022 to June 2022 and collected their basic data. We divided them into two groups according to age: the infant group (less than 3 years old) and the pediatric group (3–14 years old). The gender, age, onset time, hospital stay, preoperative white blood cells, percentage of neutrophils, C-reactive protein (CRP), and enzyme-linked immunosorbent assay (ELISA) were collected to determine the levels of immunoglobulin E (IgE), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), and interleukin-9 (IL-9) in appendicular lavage fluid, and the differences between the two groups were compared.

Results: There were 15 children in the infant group and 15 in the pediatric group. There was no significant difference between the two groups with respect to onset time and gender. The hospitalization time in the pediatric group was (5.7 ± 2.1) d, the preoperative white blood cells were $(14.3 \pm 3.7) \times 10^9/\text{mL}$, neutrophil percentage was $(84.5 \pm 6.3)\%$, and CRP was (20.0 ± 17.9) mg/mL. The hospitalization time of the infant group was (8.0 ± 3.1) d, the preoperative white blood cells were $(19.0 \pm 3.8) \times 10^9/\text{mL}$, neutrophil percentage was $(77.8 \pm 10.4)\%$, and CRP was (42.5 ± 25.0) mg/mL. The differences between the two groups were significant. There was no significant difference in IL-5 concentration between the two groups in the appendicular lavage fluid. IgE (610.74 ± 72.56) ng/mL, IL-4 (30.80 ± 12.04) ng/mL, IL-6 (118.09 ± 14.29) ng/mL, IL-9 (133.94 ± 16.00) ng/mL were found in the infant group, and IgE (495.61 ± 95.09) ng/mL, IL-4 (22.68 ± 7.05) ng/mL, IL-6 (98.22 ± 22.18) ng/mL and IL-9 (107.86 ± 27.34) ng/mL were found in the pediatric group, and the differences between the two groups were statistically significant.

Conclusions: The inflammatory response in children with acute appendicitis was associated with type I hypersensitivity-induced inflammatory responses, and the type I hypersensitivity was more intense in children in the lower age group.

1. Introduction

Appendectomy is the current gold standard treatment for acute appendicitis, which is a common acute abdominal emergency in clinical practice. However, if appendicitis is diagnosed early, it can be effectively treated with antibiotics alone. Clinical decision-making can be

complicated when faced with a choice between conservative and surgical treatment, and this choice depends on knowing how inflamed the patient's appendix is and the pathogenesis of the appendicitis. Although the exact causes of acute appendicitis are unknown, it is thought to be caused by pathogenic bacteria invading a lumen that is blocked by appendiceal obstruction (such as fecal stones or fecal impaction),

Abbreviations: ELISA, Enzyme linked immunosorbent assay; CRP, C-reaction protein; IgE, Immunoglobulin E; IL-4, Interleukin-4; IL-5, Interleukin-5; IL-6, Interleukin-6; IL-9, Interleukin-9; CT, Computed tomography; WBC, white blood cell; HE, Hematoxylin-eosin; OD, Optical density.

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causing an increase in lymphoid follicles and intraluminal pressure. However, some patients with appendicitis do not have fecal stones or appendiceal lumen obstruction, implying that the pathogenesis of appendicitis requires further investigation.

Aravindan et al. (1997) suggested that type I hypersensitivity could be a possible cause of acute appendicitis. When bacteria infiltrate the appendix wall, the immunoglobulin E (IgE) antigen binds to high-affinity receptors. This results in the degranulation of mast cells and the release of inflammatory mediators, producing type I hypersensitivity, which is then regulated by cytokines to induce inflammatory reactions. The strength of the immune function of the appendix in children of various ages also differs, and the immune cells in the appendix lumen gradually decrease with age (de Costa, 2020; Dong et al., 2014). Thus, we hypothesize that the younger the child, the stronger the type I hypersensitivity reaction-induced inflammatory response. We hope that by exploring the differences in type I hypersensitivity reaction-induced inflammatory response in children of different age groups with acute appendicitis, we can provide more insights about the pathogenesis of acute appendicitis in children, which can guide the selection of treatment methods.

2. Materials and methods

2.1. Basic information

Children admitted to the Department of General Surgery of Anhui Provincial Children's Hospital with acute appendicitis between January 2022 and June 2022 were included in this retrospective study. Thirty pediatric patient cases were collected and split evenly between two age groups: 15 cases were in the infant group (age less than 3 years old), including 6 males and 9 females, with an age of (2.6 ± 0.5) years old and the time from onset to admission was (33.6 ± 21.8) hours; and 15 cases were in the pediatric group (ages 3–14), including 8 males and 7 females, with an age of (2.6 ± 0.5) years old, and the time from onset to admission was (30.4 ± 13.5) hours. Informed consent was obtained from the family members of the patients, and the study was approved by the Ethics Committee of Anhui Provincial Children's Hospital.

2.2. Inclusion and exclusion criteria

The inclusion criteria for this study were as follows: ① age range of 1–14 years old; ② diagnosis of acute appendicitis based on the patient's medical history, physical examination, laboratory tests, and ancillary examinations. The exclusion criteria were: ① age < 1 year or > 14 years; ② other diseases such as intussusception and allergic purpura; ③ contraindications to anesthesia and/or surgery.

The clinical diagnostic criteria for acute appendicitis were: ① typical signs and symptoms of right lower abdominal pain, migratory abdominal pain, with or without other symptoms (fever, vomiting, etc.); ② increased white blood cell count and neutrophil count on routine blood analysis; ③ B-mode ultrasound and/or CT examination showing appendiceal diameter > 6 mm, thickening of the appendiceal wall, lymphoid follicle hyperplasia, and increased periappendiceal fat echogenicity.

2.3. Research methods

The onset time, gender, age, hospital stay, preoperative white blood cells, neutrophils, and C-reactive protein (CRP) levels were collected, and the levels of IgE, interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), and interleukin-9 (IL-9) in the intraluminal lavage fluid were measured post-surgery via enzyme-linked immunosorbent assay (ELISA). The differences between the two groups were compared.

ELISA analysis of intraluminal lavage fluid for appendix: 3 mL of appendiceal lavage fluid was collected and placed in a silicon plastic bottle (to reduce cell adhesion) and sent for analysis within 1 h. The total

number of cells (mainly neutrophils and eosinophils) was counted on a modified Neubauer cell count plate. Under a light microscope, 200 cells were counted for each specimen and cell sorting was performed. Cell smears were stained with Wright-Giemsa staining, and the manually counted 200 cells were observed under a microscope for classification counting and statistical analysis. ELISA was used to test IgE, IL-4, IL-5, IL-6, and IL-9: The lavage fluid was washed thoroughly with a carbonate-bicarbonate buffer (pH 9.6). The supernatants were collected after centrifugation and diluted with standard dilutions according to the same gradient. The sample to be tested was added to the ELISA plate at 40 μ L/well according to the optimal dilution multiple. Ten μ L/well IgE antibody (or IL-4/L-5/IL-9/IL-6) was added to the ELISA kit. The plate was sealed with adhesive film, and incubated at room temperature for 2 h in a constant temperature oscillator at 100 rpm/min. The liquid was discarded, PBST (phosphate-buffered saline with Tween 20) (pH 7.2–7.4) was added at the rate of 350 μ L/well, and it was let to stand at room temperature for 15 s. The washing liquid was discarded, and fresh washing liquid was added at the rate of 300 μ L/well and oscillated in a constant temperature oscillator at 100 rpm at room temperature for 3 min. The washing liquid was discarded, the board was washed 3 times and patted dry with absorbent paper. Streptavidin labeled with horseradish peroxidase was added at the rate of 50 μ L/well, the plate was sealed with adhesive film, and incubated at room temperature for 45 min in a constant temperature oscillator at 100 rpm/min. The wash-board was reused and cycled three times and patted dry with absorbent paper. Color-developing agent A and B (50 μ L/well) were added for incubation consecutively in an incubator for 30 min in the dark at room temperature. Finally, stopping solution (50 μ L/well) was added to stop the reaction and the optical density (OD) value was measured with a microplate reader.

2.4. Experimental equipment

Microplate reader (RT-6000 Rayto), centrifuge (JW3021HR, Anhui Jiawen Instrument and Equipment Co., Ltd), vortex-type mixer (GL-88B, Kylin-Bell Instruments Manufacturing Co., Ltd.), pipettes/volley of various models (all eppendorf), electric incubator (DNP-9052BS-III, Shanghai Sanfa Scientific Instruments), human immunoglobulin IgE, IL-4, IL-5, IL-6, and IL-9 (Wuhan Gene Beauty Biotechnology Co., Ltd.).

2.5. Statistical analysis

Statistical analysis was conducted using SPSS22.0. Normally distributed data with homogeneous variances, such as age and white blood cell count, are expressed as mean $\pm$ standard deviation, and the independent samples t-test was used to compare the two groups. The comparison of count data, such as gender and complications, was performed using the chi-squared test, while Fisher's exact probability test was used when the sample size (n) was less than 40 or the frequency of at least one cell in the contingency table was less than 1. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Baseline level display

There was no significant difference in the onset time and gender between the infant and pediatric groups ($P < 0.05$). The hospitalization time was (5.7 ± 2.1) days for the pediatric group, with preoperative white blood cell count of (14.3 ± 3.7) $\times 10^9$ /mL, neutrophil percentage of (84.5 ± 6.3) %, and CRP level of (20.0 ± 17.9) mg/mL. The hospitalization time was (8.0 ± 3.1) days for the infant group, with preoperative white blood cell count of (19.0 ± 3.8) $\times 10^9$ /mL, neutrophil percentage of (77.8 ± 10.4)%, and CRP level of (42.5 ± 25.0) mg/mL. The differences between the two groups were statistically significant ($P < 0.05$) (Table 1).

Table 1

Comparison of clinical data between the two groups of children hospitalized with acute appendicitis.

	Infant group	Pediatric group	Statistics	P value
Gender (male/female)	6(40 %) / 9 (60)	8(53.3 %) / 7 (46.7 %)	0.536	0.464*
Age (y)	2.6 ± 0.5	7.6 ± 2.1	-8.975	< 0.010 [#]
Onset time (h)	33.6 ± 21.8	30.4 ± 13.5	0.483	0.633 [#]
Operation time (mins)	54.9 ± 19.3	44.0 ± 17.4	1.631	0.114 [#]
Length of stay (d)	8.0 ± 3.1	5.7 ± 2.1	2.445	0.021 [#]
White blood cell count (10 ⁹ /mL)	19.0 ± 3.8	14.3 ± 3.7	3.385	0.002 [#]
Neutrophil proportion (%)	77.8 ± 10.4	84.5 ± 6.3	-2.134	0.042 [#]
C-reactive protein (mg/mL)	42.5 ± 25.0	20.0 ± 17.9	2.759	0.010 [#]

*, compared by Chi-square test; [#], compared by Student's t-test.

3.2. Pathological results

All the children completed surgical treatment successfully, and the postoperative pathology results indicated acute appendicitis. Fig. 1 depicts the appendix with a large amount of surrounding neutrophil infiltration. Fig. 2 depicts the appendix with local infiltration of eosinophils through Giemsa staining.

3.3. Determination of results of post-surgery intraluminal lavage fluid of appendix via ELISA

The level of IL-5 in the lavage fluid from the appendiceal cavity did not differ significantly between the two groups ($P > 0.05$). The concentration of IgE, IL-4, IL-6, and IL-9 were 610.74 ± 72.56 ng/mL, 30.80 ± 12.04 ng/mL, 118.09 ± 14.29 ng/mL, and 133.94 ± 16.00 ng/mL, respectively, in the infant group, while they were 495.61 ± 95.09 ng/mL, 22.68 ± 7.05 ng/mL, 98.22 ± 22.18 ng/mL, and 107.86 ± 27.34 ng/mL, respectively, in the pediatric group. The differences in the concentrations between the two groups were significant ($P < 0.05$). These data suggest that the infant group had a stronger inflammatory response induced by type I hypersensitivity than the pediatric group, consistent with our hypothesis (Fig. 3).

4. Discussion

Acute appendicitis is one of the most common surgical emergencies in children, with current causes including appendicular intraluminal obstruction, infection, dietary habits, and mucosal blood circulation disorder at the appendix wall (Hryn et al., 2022). Bacteria in the appendix lumen infiltrate the appendix wall, stimulating immune cells

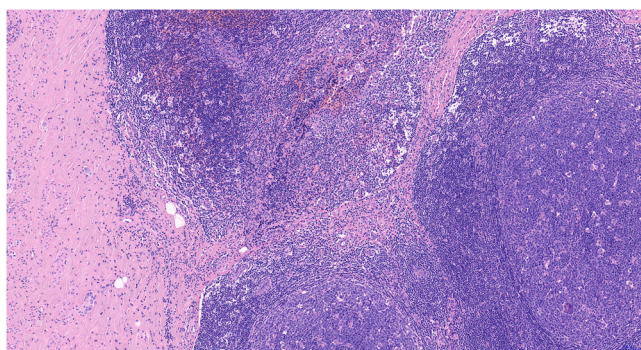


Fig. 1. HE staining: Massive neutrophil infiltration.

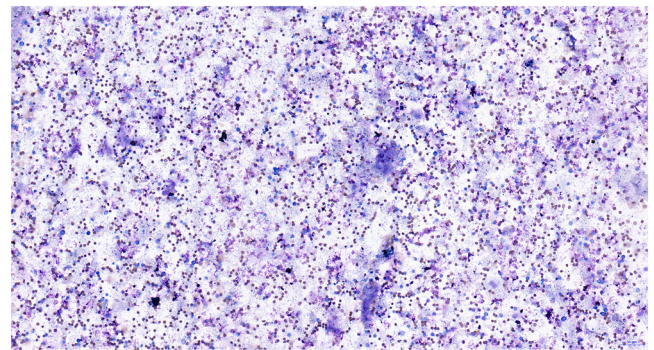


Fig. 2. Giemsa stain: Shows eosinophilic infiltration.

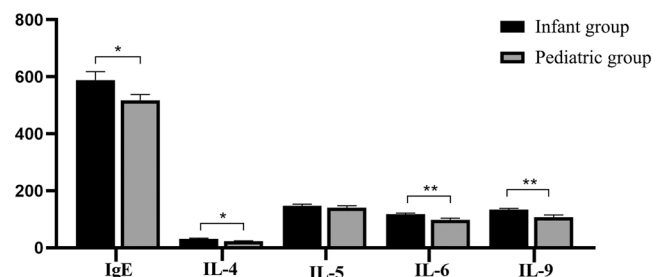


Fig. 3. Analysis of appendicular intraluminal lavage fluid by ELISA in both the groups.

such as macrophages and dendritic cells, to initiate a cascade of immune responses that further regulate the inflammation by mobilizing cytokines. However, it is unknown why some children develop a persistent exacerbation of inflammation that progresses to complex appendicitis, while others develop inflammation gradually. Some researchers believe that eosinophil infiltration, mast cell degranulation and muscle tissue edema can be seen in the histological manifestations of acute appendicitis, which are common histological characteristics of allergic reactions (Aggelidou et al., 2019; Carvalho et al., 2021). Therefore, whether the allergic reaction-mediated inflammatory response influences the progression of appendicitis deserves further investigation.

Among the pathologies of acute appendicitis, fecaliths are found in about 30 % of appendix specimens, but there is no obstruction in most cases. Appendicular fecaliths are less common in children due to their unique dietary structure (Walter, 2021). As a result, the theory of appendicular allergic reaction has been proposed, which states that when bacteria in the appendix lumen infiltrate the appendix wall, the allergic antigen stimulates the contraction of the appendicular muscular layer, resulting in intraluminal stenosis and obstruction, and eventually, acute appendicitis (Costa-Pinto et al., 2007). Allergic reaction occurs when the allergen binds to the antibodies on the surfaces of sensitized mast cells and basophils. It is characterized by rapid occurrence, strong reaction, and rapid regression, which is consistent with the characteristics of acute clinical onset and strong reaction in acute appendicitis. In our study, hematoxylin and eosin (HE) staining of all the 30 children revealed a large number of neutrophil infiltrations, whereas Giemsa staining revealed a large number of eosinophil infiltrations and mast cell degranulation, indicating the possibility of allergic reaction in the early stages of acute appendicitis in children. There were no significant differences in pathological findings between the infant and pediatric groups.

In type I hypersensitivity (allergic reaction), inflammatory factors such as IL-4, IL-5, IL-9, and IL13 are released through IgE-mediated Th2 activation to participate in systemic or local allergic reactions (Carvalho et al., 2019; Harlak et al., 2008). These cytokines, which exist on the mucosal surface of the appendix, are primarily secreted by B cells, mast

cells, T cells, and eosinophils (Soussi-Gounni et al., 2001). In this study, we obtained the cytokines adhered to the appendix mucosa by collecting the appendix specimen lavage fluid from the children in the infant and the pediatric groups. These cytokines include IL-4, IL-5, IL-6, and IL-9, among which IL-4 and IL-9 can induce the activation of mast cells and basophils, resulting in a series of allergic cascade reactions. IL-6 is an effective inducer of acute reaction, whereas IL-5 can induce the differentiation and activation of eosinophils.

Our experimental results revealed that there were significant differences in the levels of IgE, IL-4, IL-6, and IL-9 among different age groups. The cytokine level in the infant group was significantly higher than that in the pediatric group. There was no significant difference in the IL-5 level. This may be because the immune mechanism of children is not yet mature, and the appendix has a strong immune response. The type I hypersensitivity reaction becomes more intense when the allergen is stimulated. Studies have found that an increase in the number of B cells may promote continuous inflammation and the progression to complex appendicitis, which is more common in younger children (Gorter et al., 2017). This is consistent with the increased expressions of IgE, IL-4, IL-6, and IL-9 that we found in the infant group – they may promote the activation of B cells to accelerate the progression of appendicitis in young children.

Not all cases of appendicitis progress to perforation in the clinical setting, implying that simple and complex appendicitis may represent two distinct pathogenic mechanisms. Complex appendicitis is commonly seen in pediatric patients, implying that the concentrations of IgE and Th2-related cytokines IL-4, IL-5, IL-6, and IL-9 in appendiceal lavage fluid may be associated with the risk of severe appendicitis. This warrants further investigation. This study further illustrates that the appendix plays an active role in the human body. The younger the patient, the more obvious the immune effect. Non-surgical treatment of appendicitis has received attention in recent years (Virchow et al., 1996). The results of our study confirm that type I hypersensitivity-induced inflammatory response is a possible cause of acute appendicitis and can provide new insights into the treatment of appendicitis.

This study has certain limitations. First, we lack negative appendectomy data for comparative analysis. Additionally, the sample size of this study was relatively small, and future multi-center studies with larger sample sizes may be required to further support the specific mechanism of allergy in the pathogenesis of appendicitis.

5. Conclusions

In conclusion, there were differences in IgE, IL-4, IL-6, and IL-9 in the appendicular lavage fluid between the infant and pediatric groups. Type I hypersensitivity induced an inflammatory response, which could be a possible cause of acute appendicitis. In addition, in younger children, the stronger immune function of the appendix plays an important role in the overall or local immune resistance of children.

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CRedit authorship contribution statement

Conception and design of the research: **Zhu-Bin Pan, Tao Zhang**. Acquisition of data: **Tao Zhang, Yu-Liang Zhou**. Analysis and interpretation of the data: **Yu-Liang Zhou, Zhen-Qiang Zhang**. Statistical analysis: **Tao Zhang, Yuan Cheng**. Obtaining financing: **Zhu-Bin Pan**. Writing of the manuscript: **Zhu-Bin Pan, Tao Zhang**. Critical revision of the manuscript for intellectual content: **Yuan Cheng, Zhen-Qiang Zhang**. All authors read and approved the final draft.

Competing interests

The authors declare that they have no competing interests.

Data availability

No data was used for the research described in the article.

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