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Original Research

Evaluation of some cellular biomarker proteins, oxidative stress and clinical indices as results of laparoscopic appendectomy for perforated appendicitis in children

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Abstract: Laparoscopic appendectomy for perforated appendicitis in children has the advantages of quick recovery, little influence of inflammatory and oxidative stress and low infection rate. Altogether 115 children with perforated appendicitis treated in our hospital from June 2018 to August 2019 were selected and divided into two groups according to different treatment methods. Laparoscopic appendectomy was used as the research group (RG) (67 cases) and open appendectomy (48 cases) as the control group (CG). The clinical indexes (operation time, intraoperative blood loss, ambulation time, incision length, postoperative exhaust time and length of stay) of the two groups were observed. The levels of C- reactive protein (CRP), procalcitonin (PCT), interleukin -6 (IL-6) and tumor necrosis factor-α (TNF-α) before and after treatment were detected by enzyme-linked immunosorbent assay (ELISA). The levels of oxidative stress factors (superoxide dismutase (SOD), malondialdehyde (MDA)) and the incidence of postoperative incision infection were observed in the two groups. A visual analogue scale (VAS) score was used to evaluate the postoperative pain in children. Logistic regression analysis was used to analyze the risk factors of postoperative incision infection in children. The improvement degree of clinical indicators in RG was significantly better than that in CG. The expression levels of CRP, PCT, IL-6 and TNF-α in RG were significantly lower than those in CG. The expression levels of SOD and MDA in RG were significantly better than those in CG. The postoperative incision infection rate in RG was significantly lower than that in CG. The postoperative VAS score in RG was significantly lower than that in CG. Logistic regression analysis results showed that the risk of incision infection was increased in children with operation time over 1h, incision length >3cm, high CRP, high PCT, high IL-6, high TNF-α, high SOD and low MDA. Laparoscopic appendectomy for perforated appendicitis in children can effectively reduce intraoperative hemorrhage, postoperative pain, and the damage to the body of children, and can also reduce oxidative stress and inflammatory reaction in children.

Key words: Laparoscopic appendectomy; Perforated appendicitis in children; Inflammatory factors; Oxidative stress; Infection rate.

Introduction

Appendicitis is the most common disease in children undergoing emergency abdominal surgery (1). In fact, it is a progressive disease, which is caused by acute inflammation secondary to appendiceal orifice obstruction, thus leading to necrosis and perforation of the appendix wall. Inadequate treatment will also lead to a higher incidence of perforated appendicitis (2,3). Appendectomy has always been the first choice for clinical treatment of appendicitis, but there are more postoperative complications (4). Therefore, safe and effective treatment for children with appendicitis is very effective.

Appendectomy is the golden standard for clinical treatment of acute appendicitis (5), such as open appendectomy, but due to slow intestinal recovery, incision infection, intestinal adhesion and intestinal obstruction, the family members of the child gradually refused to accept open appendectomy (6). At present, laparoscopic appendectomy is mostly used to treat acute appendicitis clinically. Compared with open access, it has faster recovery speed and shorter length of stay (7). Laparoscopic surgery is a minimally invasive surgery, which is mainly operated by visualization and two-dimensional video obtained by transferring endoscope to body cavity on the screen. It has the advantages of less postoperative complications, high patient satisfaction and fast recovery speed (8). For example, in studies by Liu Y et al. (9), laparoscopic appendectomy for children with acute appendicitis could shorten the average length of stay, reduce the pain level of children one month after the operation, and improve the QOL compared with children receiving open appendectomy. Research has shown that emergencies during surgery will aggravate the balance of patients' cardiovascular and respiratory systems, body fluids and electrolytes, and surgical intervention will also lead to inflammatory reactions and blood loss, thus increasing the physiological pressure of patients (10).

In this study, laparoscopic surgery and laparotomy were applied to observe the effects of the two groups on recovery, inflammation and oxidative stress of children, and analyze the causes of postoperative incision infection.

Materials and Methods

General information

Altogether 115 children with perforated appendicitis treated in our hospital from June 2018 to August 2019 were selected, with laparoscopic appendectomy (67 cases) as the research group (RG) and open appendectomy (48 cases) as the control group (CG). Inclusion criteria: Both groups of children were diagnosed as perforated appendicitis (11); both groups of children were ≤14 years old and had complete clinical data; the clinical symptoms were tenderness, rebounding pain and other peritonitis symptoms; patients had not taken any drugs that affected the indicators of this study for half a year. Exclusion criteria: patients with preoperative infection history, congenital blood and immune system diseases, previous abdominal surgery history; patients dropped out midway, and patients lost to follow. The study was approved by the Ethics Committee of our hospital. The guardians of the patients were all informed and signed a fully informed consent form. Inclusion criteria applied to all participants.

Operative method

The children in CG were treated with open appendectomy: after epidural anesthesia, the children were kept in the supine position, and then a 5-7cm oblique incision was taken from the right lower abdomen of the children. After the peritoneum was cut, pus or exudate should be immediately removed by suction. Pull hook was used to pull the incision to both sides, find the appendix along with the cecum, and clamp the mesentery at the tip of the appendix with appendix forceps. After a routine appendectomy, the appendix stump was embedded into the cecum wall, washed with 0.9% sodium chloride solution, placed into drainage management, and then the incision was sutured.

RG adopted laparoscopic appendectomy: after the children were anesthetized by intravenous inhalation, the children were kept at the leg-dividing position, a 1cm arc incision was cut at the umbilical cord of the children, and CO_2 gas film was established by pneumoperitoneum needle. Then, a 10mm trocar was inserted and the intra-abdominal pressure should be maintained at 10-14 mm Hg. The laparoscope was inserted again and a 5mm trocar was used in the left lower abdominal wall and the right abdominal wall above the pubic bone of the child under the view of the laparoscope. Suction devise and grasping forceps were used to suck the pus from the abdominal cavity of the child, and blunt separation of adhesions was applied to find the appendix along the colon, and grasping forceps were used to lift the appendix. After separating the appendix, glomerular mesangium and root were ligated, and then glomerular mesangium was separated by an electric hook. A 0.5 cm appendix was removed from the root and removed from the umbilical cord using a trocar bag. A 0.9% sodium chloride solution was used to wash. After the drainage tube was inserted, the air membrane was removed and the umbilical cord was sutured.

Observation index

A. The clinical indexes (operation time, intraoperative blood loss, ambulation time, incision length, postoperative exhaust time and hospitalization time) of the two groups of patients were recorded.

B. Pain score: visual analog scale (VAS) was used to evaluate the postoperative pain degree of the two groups of children. The total score was 10 points. The high score after statistics was closely related to the high pain level of the children.

C. A 5mL venous blood was collected from the elbow of the child before and 24 hours after the operation, centrifuged at 1500Xg, 4°C, for 10min, and placed in a refrigerator at -70°C for later use. Enzyme-linked immunosorbent assay (ELISA) (12) was applied to detect the concentrations of serum C- reactive protein (CRP), procalcitonin (PCT), interleukin -6 (IL-6), tumor necrosis factor -α (TNF-α), superoxide dismutase (SOD) and malondialdehyde (MDA). The above steps were carried out with reference to the instructions of human CRP (Shanghai Yiyan Biotechnology Co. Ltd., Shanghai, China, EY-D9154), PCT (Yanke Biotechnology Co., Ltd., Xiamen, China, KYMP00030HU), IL-6 (MultiSciences (Lianke) Biotechnology Corporate Limited, Hangzhou, China, 70-EK106/2), TNF-α (Shanghai YuanMu Biological Technology Co. Ltd., Shanghai, China, YM-S0122H), SOD (Chreagen Biotechnology Co., Ltd., Beijing, China, 13800-1), MDA (Yiji Industry Co., Ltd., Shanghai, China, GX6572333).

D. The infection in the incision postoperative in the two groups of children was observed. Diagnostic criteria for incision infection: incision suppuration and drainage were required.

Statistical method

SPSS 21.0 statistical software (EASYBIO, China) was applied for analysis. The intra-group counting data were expressed as the number of cases/percentage [n(%)], the comparison of the inter-group counting data used the chi-square test. When the theoretical frequency in the chi-square test was less than 5, the continuity correction chi-square test was used. The measurement data were expressed as mean±SD, the comparison of the inter-group measurement data used the t-test of independent samples, the paired t test was used in the intra-group comparison before the operation and after the operation, the multivariate Logistic regression analysis was used to analyze the risk factors affecting the incision infection of children, and the GraphPad Prism 6 software was used to visualize the experimental picture. $P<0.05$ was considered to be statistically different.

Results

General information

There was no significant difference in gender, age, residence, nationality, preoperative white blood cell count, course of the disease, systolic blood pressure and diastolic blood pressure between the two groups ($P>0.05$) (Table 1).

Comparison of clinical indexes between two groups of children after the operation

After observing the clinical indexes of the two groups of children, it was found that the operation time, intraoperative blood loss, ambulation time, incision length, postoperative exhaust time and hospitalization

Table 1. General data of two groups of children [N (%)]/(mean±SD).

Classification	Research group (n=67)	Control group (n=48)	t/χ2 value	P value
Gender			0.071	0.789
Male	36 (53.73)	27 (56.25)		
Female	31 (46.27)	21 (43.75)		
Age (years)			0.024	0.624
≤6	31 (46.27)	20 (41.67)		
>6	36 (53.73)	28 (58.33)		
Residence			1.192	0.274
Urban	28 (41.79)	25 (52.08)		
Rural	39 (58.21)	23 (47.92)		
Nationality			0.987	0.321
Han	37 (55.22)	22 (45.83)		
Minorities	30 (44.78)	26 (54.17)		
Preoperative white blood cell count ($\times10^9$/L)			0.115	0.908
	15.02±4.12	14.93±4.09		
Course of disease (d)			1.309	0.193
	1.83±0.21	1.78±0.19		
Systolic pressure (mmHg)			1.407	0.162
	113.01±12.31	109.76±12.09		
Diastolic pressure (mmHg)			0.091	0.927
	75.03±6.98	74.91±6.90		

Table 2. Comparison of clinical indexes between two groups of children after the operation (mean±SD).

Group	n	Operation time (min)	Intraoperative hemorrhage (ml)	Normal activity time (h)	Incision length (cm)	Postoperative exhaust time (h)	Length of stay (d)
Research group	67	48.52±6.78	22.08±3.34	9.34±2.45	2.35±0.98	20.05±5.98	7.12±2.67
Control group	48	62.94±7.22	37.43±3.23	14.07±3.09	4.91±1.09	31.81±6.09	8.42±2.72
t	-	10.950	24.640	9.147	13.180	10.320	2.555
P	-	<0.001	<0.001	<0.001	<0.001	<0.001	0.012

time of RG were significantly better than those of CG (P< 0.05). See Table 2.

Comparison of postoperative VAS scores between the two groups

The postoperative VAS score of the children in RG was (2.19± 0.34) significantly lower than that of the children in CG (3.56± 0.37) (P<0.05) (Figure 1).

Comparison of inflammatory factors between two groups of children before and after the operation

Observing the levels of inflammatory factors before and after the operation in the two groups of children, it was found that there was no significant difference in the levels of CRP, PCT, IL-6 and TNF-α before the operation in the two groups of children (P> 0.05). The levels of inflammatory factors in the two groups of children after operation were significantly increased and higher than before operation (P< 0.05). The levels of CRP, PCT, IL-6 and TNF-α in RG of children after operation were significantly lower than those in CG (P< 0.05) (Figure 2).

Comparison of stress factors between two groups of children before and after the operation

Observing the stress factors of the two groups of children before and after the operation, it was found that there was no significant difference in the levels of SOD and MDA between the two groups before operation (P> 0.05). After the operation, the SOD level of the two groups of children decreased significantly, while

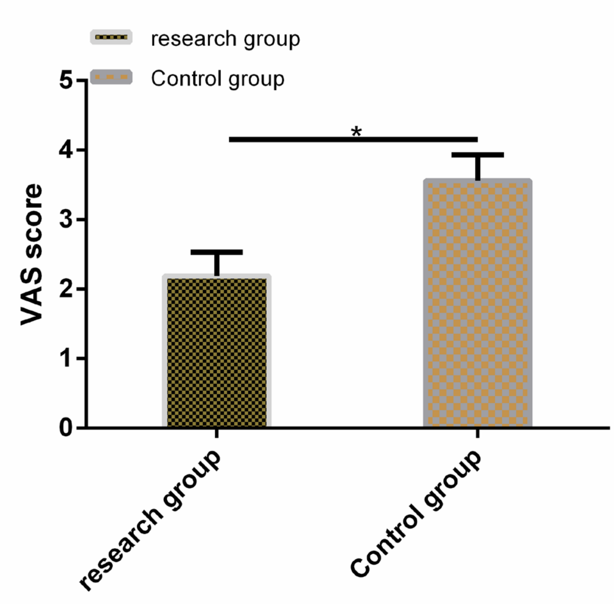

Figure 1. Comparison of postoperative VAS scores between the two groups. The postoperative VAS score of the children in RG was significantly lower than that in CG (P< 0.05). Note: * indicates that compared with the control group, P < 0.05.

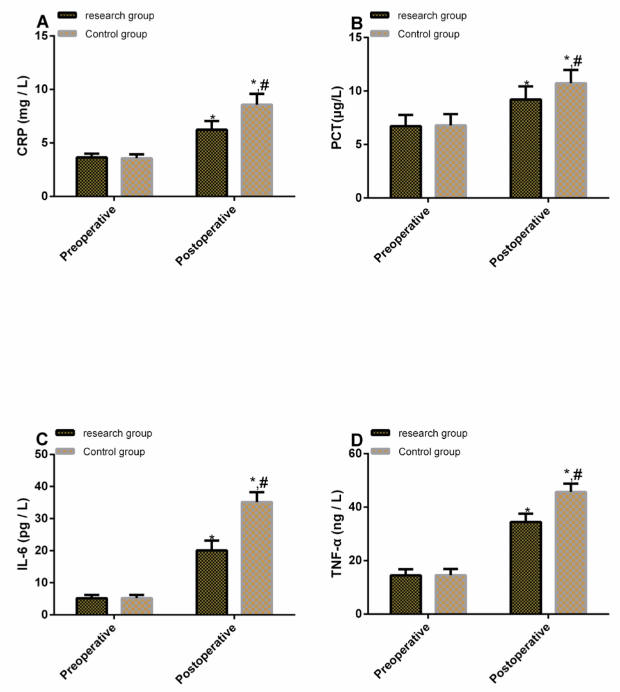

Figure 2. Comparison of inflammatory factors before and after operation between the two groups of children. (A) There was no difference in CRP between the two groups before the operation. CRP in RG was significantly lower than that in CG after the operation. (B) There was no difference in PCT between the two groups before the operation. PCT in the postoperative RG was significantly lower than that in CG. (C) There was no difference in IL-6 between the two groups before the operation. IL-6 in RG was significantly lower than that in CG after the operation. (D) There was no difference in TNF-α between the two groups before the operation. TNF-α in RG was significantly lower than that in CG after the operation.

the SOD level of CG was significantly lower than that of RG (P<0.05). The MDA level of the two groups of children increased significantly, the MDA level of CG was significantly higher than that of RG (P<0.05). See Figure 3.

Comparison of incision infection rate between two groups of patients after operation

The postoperative incision infection rate in RG was (5.97)%, significantly lower than that in CG (27.08)% (P<0.05). See Table 3.

Multivariate Logistic regression analysis of postoperative incision infection in children

There were 17 children with postoperative incision infection (infection group) and 98 children without infection (non-infection group). The clinical parameters and related indexes of postoperative incision infection and non-infection were compared. There was no significant difference in the clinical parameters between incision infection and non-infection children (P>0.05), but there was a statistical difference between them in operation time, incision length, CRP, PCT, IL-6, TNF-α, SOD, MDA (P<0.05). Multivariate Logistic regression analysis was carried out on the factors with differences. The results showed that operation time (P=0.023), incision length (P=0.029), CRP (P=0.009), PCT (P=0.011),

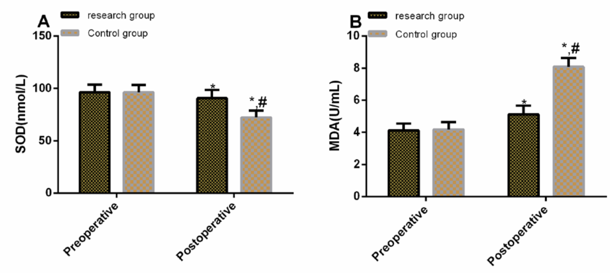

Figure 3. Comparison of stress factors between the two groups before and after the operation. (A) There was no difference in SOD between the two groups before the operation. SOD in RG was significantly lower than that in CG after the operation. (B) There was no difference in MDA between the two groups before the operation. MDA in RG was significantly lower than that in CG after the operation. Note: * indicates that compared with before treatment, P< 0.05; # indicates that compared with the control group after treatment, P< 0.05.

Table 3. Comparison of postoperative incision infection rate between two groups of children [n(%)].

Group	n	Infection rate (%)	
		Cases	%
Research group	67	4	5.97
Control group	48	13	27.08
$\chi 2$	-	9.896	
P	-	0.002	

Table 5. Logistic multivariate regression analysis assignment.

Factor	Variable	Assignment
Operation time	X1	≤1=0, >1=1
Incision length	X2	≤3=, >3=1
CRP	X3	Continuous variable
PCT	X4	Continuous variable
IL-6	X5	Continuous variable
TNF-α	X6	Continuous variable
SOD	X7	Continuous variable
MDA	X8	Continuous variable
Operative method	X9	Laparoscopy =0, laparotomy =1

Table 6. Multivariate Logistic regression analysis of postoperative incision infection in children.

Factor	Variable	Assignment
Operation time	X1	≤1=0, >1=1
Incision length	X2	≤3=00, >3=1
CRP	X3	Continuous variable
PCT	X4	Continuous variable
IL-6	X5	Continuous variable
TNF-α	X6	Continuous variable
SOD	X7	Continuous variable
MDA	X8	Continuous variable
Operative method	X9	Laparoscopy =0, laparotomy =1

IL-6 (P=0.015), TNF-α (P=0.013), SOD (P=0.004), MDA (P=0.003), and operation method (P=0.001) were

Table 4. Relationship between clinical parameters and indexes of children and incision infection [n(%)].

Classification	n	Non-infection group (n=98)	Infection group (n=17)	t/χ2 value	P-value
Gender				0.131	0.716
Male	63	53(84.13)	10 (15.87)		
Female	52	45(86.54)	7 (13.46)		
Age (years)				1.803	0.179
≤6	51	46 (90.20)	5 (9.80)		
>6	64	52 (81.25)	12 (18.75)		
Residence				0.194	0.659
Urban	53	46 (86.79)	7 (13.21)		
Rural	62	52 (83.87)	10 (16.13)		
Nationality				0.021	0.883
Han	59	50 (84.75)	9 (15.25)		
Minorities	56	48 (85.71)	8 (14.29)		
Operation time (h)				11.970	0.001
≤1	76	71 (93.42)	5 (6.58)		
>1	39	27 (69.23)	12(30.77)		
Incision length (cm)				9.675	0.002
≤3	78	72 (92.31)	6 (7.69)		
>3	37	26 (70.27)	11 (29.73)		
CRP(mg/L)				18.340	<0.001
	115	3.32±0.42	5.68±0.79		
PCT(μg/L)				10.220	<0.001
	115	6.98±1.08	9.87±1.05		
IL-6(pg/L)				46.530	<0.001
	115	6.03±1.07	26.33±3.54		
TNF-α(ng/L)				35.820	<0.001
	115	15.26±2.37	39.25±3.44		
SOD(nmol/L)				10.050	<0.001
	115	93.22±6.44	76.35±6.07		
MDA (U/mL)				16.700	<0.001
	115	4.67±0.52	7.12±0.75		
Operative method				9.896	0.002
Laparoscopic	67	63 (94.03)	4 (5.97)		
Open abdomen	48	35 (72.92)	13 (27.08)		

Table 6. Multivariate Logistic regression analysis of postoperative incision infection in children.

Factor	Variable	Assignment
Operation time	X1	≤1=0, >1=1
Incision length	X2	≤3=0, >3=1
CRP	X3	Continuous variable
PCT	X4	Continuous variable
IL-6	X5	Continuous variable
TNF-α	X6	Continuous variable
SOD	X7	Continuous variable
MDA	X8	Continuous variable
Operative method	X9	Laparoscopy =0, laparotomy =1

independent risk factors for postoperative incision infection in children. The risk of incision infection was increased in children with operation time over 1h, incision length >3cm, high CRP, high PCT, high IL-6, high TNF-α, high SOD and low MDA. See Tables 4-6.

Discussion

The high incidence period of appendicitis is in childhood and is also the most common emergency abdominal surgery. One-third of children with appendicitis will develop complicated diseases with perforation or gangrene, and the incidence rate is extremely high (13). Perforated appendicitis is a progressive inflammatory process caused by obstruction of the appendix cavity, which will also cause appendiceal infection and ischemic necrosis (14). Surgery is the main method for clinical treatment of perforated appendicitis (15).

Prognosis of perforated appendicitis is closely related to incision infection, postoperative recurrence and complete surgical resection (16). Research shows that the incision infection rate of patients after laparoscopic intervention for appendicitis is significantly lower than that of open surgery (17). For example, in the studies by Zwintscher NP et al. (18), laparoscopic appendectomy for children with perforated appendicitis has a lower incidence of postoperative complications and shorter length of stay. In Wang D et al. (19), laparoscopic surgery for elderly appendicitis patients can better reduce the incidence of complications, wound infection rate and shorten the length of stay than open surgery. This observation of the impact of laparoscopic and open surgery on clinical indicators of children with perforated appendicitis found that the operation time and intraoperative blood loss of the children in RG were lower than those in CG, and the postoperative normal activity time, incision length, postoperative exhaust time and length of stay were also better than those in CG, indicating that laparoscopic surgery can avoid damage to blood vessels, nerves and muscles of children, which is similar to the research results of Zwintscher NP and XX. Research shows that chronic postoperative pain is one of the most common postoperative complications of patients, which not only has a negative impact on the QOL of patients (20), but also has an impact on the recovery of the functions of various systemic organs of patients, and is also an unfavorable factor for the recovery of respiratory function and mental state of patients (21). This study observed the postoperative pain degree of the two groups of children and found that the VAS score of the children in RG was significantly lower than that in CG, which was related to the smaller incision of laparoscopic resection, so it could avoid the injury to the muscles and nerves of the children, relieve the pain of the children, and better promote the postoperative rehabilitation of the children.

Clinical studies show that appendicitis is characterized by perforation and necrosis of the appendix. Inflammatory factors are very important in the inflammatory appendix. For example, IL-6 and CRP are early markers of tissue injury and systemic inflammatory response (22). The results of this study showed that there was no significant difference in the expression levels of CRP, PCT, IL-6 and TNF-α between the two groups of children before the operation. The expression levels of CRP, PCT, IL-6 and TNF-α in the serum of the two groups of children after operation were significantly increased, while the expression levels in RG were significantly lower than those in CG. It showed that laparoscopy could reduce the trauma to the body of children and cause a less inflammatory reaction. SOD is a metal enzyme and a usable antioxidant, which can protect the human body from superoxide radicals generated in the biological system (23). Targeted delivery to endothelial cells can also protect a variety of pathological environments associated with vascular oxidative stress (24). MDA is one of the final products of peroxidation of duo unsaturated fatty acids in cells, and high levels of MDA are often associated with the increase of free radicals and are often applied as antioxidants and markers of oxidative stress in cancer patients (25). Some studies have shown that SOD and MDA are abnormally expressed in acute perforated appendicitis (26). The results of this study showed that the SOD level in the two groups of children after surgery was significantly reduced, while MDA level was significantly increased, and the expression level of children in RG was better than that in CG, indicating that SOD and MDA may participate in the pathological process of children with perforated appendicitis, while laparoscopic surgery can significantly reduce the oxidative stress in children. Clinical research has shown that laparoscopic surgery has small incision, visualization, and less postoperative pain. It can also shorten the length of stay of patients, reduce wound infection, and reduce incision infection after surgery (27). The results of this study showed that the postoperative incision infection rate in RG was significantly lower than that in CG. However, some studies have shown that although laparoscopic appendectomy is the standard strategy for children with acute appendicitis, complications of incision infection, intestinal obstruction and abdominal infection are still difficult to avoid (28). We hereby carried out a multi-factor Logistic regression analysis on incision infection in children with perforated appendicitis. The results showed that operation time, incision length, CRP, PCT, IL-6, TNF-α, SOD and MDA were independent risk factors leading to incision infection in children.

This study confirmed that laparoscopic appendectomy could better promote the recovery of children's perforated appendicitis, but there is still room for improvement. First, we can increase the investigation of the postoperative recurrence rate of children with perforated appendicitis. Second, we can supplement the research on specific infection types and regulation mechanisms of children with perforated appendicitis. In addition, the number of cases included in this study is relatively small, thus it has certain limitations. These deficiencies need to be further supplemented in future research to further support the results of this study.

To sum up, laparoscopic appendectomy for perforated appendicitis in children can effectively reduce intraoperative bleeding, postoperative pain, and damage to the body of the child, and can also reduce oxidative stress and inflammatory response of the child. In addition, new technologies such as genome editing (29) can be used in this regard.

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