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Expression and analysis of NLRP6 in chronic gastritis in children

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Abstract

Background Chronic gastritis (CG) in children refers to chronic inflammatory lesions of the gastric mucosa caused by physical, chemical, or biological factors. We investigated the expression of NLRP6 and its downstream factors Caspase-1, IL-1β, and IL-18 in gastric tissues of children with chronic gastritis, and analysed the effect of *Helicobacter pylori* (Hp) infection on NLRP6 expression.

Methods In this case-control study, 160 children with CG, who visited the Department of Paediatrics at the First People's Hospital of Lianyungang from May 2023 to February 2024, were divided into six groups by the degree of gastric mucosal damage under endoscopy, pathological diagnosis, and Hp infection status: A, mild CG Hp-negative; B, moderate CG Hp-negative; C, severe CG Hp-negative; D, mild CG Hp-positive; E, moderate CG Hp-positive; F, severe CG Hp-positive. The levels of NLRP6, Caspase-1, IL-1β and IL-18 in the gastric tissues of the six groups were compared, and the relationship between NLRP6, Caspase-1, IL-1β and IL-18 in the gastric tissues of the children and the severity of chronic gastritis and *Helicobacter pylori* was analysed.

Results The Hp positive rate was lower in mild CG but higher in moderate and severe CG compared with negative CG, with a statistically significant difference ($\chi 2 = 8.897$, $P = 0.012 < 0.05$). Under the same Hp condition, NLRP6 expression was higher in the mild CG group than in the moderate CG group, while the expression levels of Caspase-1, IL-1β and IL-18 were lower than in the moderate CG group. NLRP6 expression was higher in the moderate CG group than in the severe CG group, while the expression levels of Caspase-1, IL-1β and IL-18 were lower than in the severe CG group, with statistically significant differences ($P < 0.05$). In groups with the same degree of gastric mucosal damage, NLRP6 expression was higher in the Hp-negative group than in the Hp-positive group, while Caspase-1, IL-1β and IL-18 were lower than in the Hp-positive group, with statistically significant differences ($P < 0.05$).

Conclusion Under the same Hp condition, greater gastric mucosal damage was associated with lower NLRP6 expression. In cases with the same degree of mucosal damage, NLRP6 expression level was significantly higher in the Hp-negative group than in the Hp-positive group. These findings suggests that NLRP6 inhibits inflammation in CG and preserves the integrity of epithelial cells, and our data suggest an inverse association between Hp infection and NLRP6 expression.

Keywords NLRP6 inflammasome, *Helicobacter pylori*, Children, Chronic gastritis

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Introduction

Chronic gastritis in children refers to chronic inflammatory lesions of the gastric mucosa caused by physical, chemical, or biological factors [1]. The exact cause remains unclear, but recent research suggests that it may be related to *Helicobacter pylori* (Hp) infection, bile reflux, dietary habits, and other factors [2]. Children mainly present with recurrent epigastric pain, loss of appetite, nausea, vomiting, abdominal distension, and other symptoms when seeking medical attention [3]. Treatment primarily involves medication and dietary adjustments, and most children respond successfully when managed effectively. However, if left unattended and untreated for an extended period, it will affect the normal development of the child, and some children may develop complications such as peptic ulcers, perforation, and bleeding, endangering their lives [4, 5].

The nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 6 (NLRP6) is a relatively unique member of the nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family [6]. Unlike other NLR family members that activate immune responses, NLRP6 is the only known member that can function in both monomeric and multimeric forms [7]. Studies have confirmed that Hp is involved in the expression of NLRP6; however, there are few reports on the involvement of NLRP6 in chronic gastritis, especially regarding changes in NLRP6 expression in Hp-induced chronic gastritis [8]. This study aims to investigate the expression and significance of NLRP6 in Hp-induced chronic gastritis in children. As a component of inflammasomes, NLRP6 can recruit adaptor protein ASC through its PYD domain and activate Caspase-1, thereby catalyzing the cleavage, maturation, and secretion of IL-1 β and IL-18 precursors. These cytokines are key effector molecules mediating inflammatory responses. Previous studies have reported that the NLRP6 inflammasome Caspase-1 axis play important roles in various infectious and inflammatory diseases, and Hp infection can regulate the expression of the above inflammatory factors. Therefore, by detecting the expression levels of Caspase-1, IL-1 β, and IL-18, the activation status of NLRP6 inflammasome can be directly reflected, which helps to clarify the specific mechanism of NLRP6's involvement in inflammation regulation in children with chronic gastritis, especially in the context of Hp infection.

Materials and methods

General information

A total of 160 patients with chronic gastritis who visited the First People's Hospital of Lianyungang from May 2023 to February 2024 were selected as observation subjects. According to the pathological diagnosis and degree of gastric mucosal damage under endoscopy, 83 cases were diagnosed with mild chronic gastritis, 60 cases with moderate chronic gastritis, and 17 cases with severe chronic gastritis. Subsequently, within each of these three severity groups, patients were further divided based on their Helicobacter pylori (Hp) infection status (detected by rapid urease test): Hp-negative subgroup or Hp-positive subgroup. This two-step stratification process resulted in the following six final comparison groups: Group A: Mild CG, Hp-negative (n = 47); Group B: Moderate CG, Hp-negative (n = 19); Group C: Severe CG, Hp-negative (n = 7); Group D: Mild CG, Hp-positive (n = 36); Group E: Moderate CG, Hp-positive (n = 41); Group F: Severe CG, Hp-positive (n = 10). The diagnostic criteria for mild, moderate, and severe chronic gastritis were based on the Sydney System [9] and the Chinese consensus on chronic gastritis [10]. The rapid urease test was used to detect Hp, with a positive result indicating Hp infection. Inclusion criteria: (1) presence of digestive symptoms such as abdominal pain, acid reflux, vomiting, or oral malodour; (2) diagnosis with mild, moderate, or severe chronic gastritis by gastroscopy and pathological examination; (3) no anti-Hp treatment within the past 4 weeks. Exclusion criteria: (1) reciept of antibiotic, proton pump inhibitor, or other drug treatments within the past 2 weeks; (2) long-term use of glucocorticoids and non-steroidal anti-inflammatory drugs; (3) presence of other systemic diseases. The severity of gastritis was assessed based on histopathological examination of gastric mucosal biopsy samples obtained during endoscopy. The pathological assessment was performed independently by two experienced pathologists according to the updated Sydney System and the Chinese Consensus on Chronic Gastritis. These criteria evaluate the degree of inflammatory cell infiltration, glandular atrophy, intestinal metaplasia, and Helicobacter pylori density. The final grading (mild, moderate, or severe) was determined by consensus between the pathologists, ensuring objective and standardized classification. This study was approved by the Ethics Committee of the First People's Hospital of Lianyungang. Informed consent forms were signed by the guardians of all participating children.

Hp detection

Hp was detected by detected by rapid urease test (Haidewei Biotechnology Co., Ltd, Guangzhou, China). After gastroscopy, one piece of gastric mucosal tissue was taken and placed in a reagent. The change in the reagent was observed; if the phenol red changed from yellow to red, indicating an increase in pH, the test result was positive, suggesting the presence of Hp infection in the biopsy tissue.

Immunohistochemistry

Paraffin-embedded gastric tissue sections fixed with formaldehyde were sectioned, and the sections were placed in a 60 °C oven for 3 h, then dewaxed with xylene, and antigen retrieval was performed by microwave

boiling in sodium citrate buffer for 5 min. The sections were incubated in 3% H_2O_2 at room temperature for 10 min to remove endogenous peroxidase, and incubated overnight at 4 °C with anti-NLRP6 (ab314498, 1:1000, Abcam, MA, USA), anti-Caspase-1 (ab207802, 1:1000, Abcam), anti-IL-1β (ab216995, 1:1000, Abcam), and anti-IL-18 (ab207323, 1:1000, Abcam) antibodies. The sections were then incubated with the EnVision horseradish peroxidase system at room temperature for 50 min, stained with DAB for 2 min, restrained with haematoxylin, dehydrated, and mounted. The staining results were scored in a double-blind method by two pathologists. The criteria for staining intensity scores were: no staining, 0; light yellow, 1; brown, 2; dark brown, 3. The criteria for positive staining range scores were: no positive cells, 0; <10% positive cells, 1; 10%–50%, 2; 51%–80%, 3; >80%, 4. Five random fields (400×) were scored for each section, and the average was calculated. The product of the staining intensity and range scores was the immunoreactive score (IRS), with IRS ≥ 6 defined as high expression and IRS < 6 as low expression. After independent scoring by two pathologists under double-blind conditions, we calculated the intra-group correlation coefficient (ICC) to evaluate the consistency of the continuous variable (immune response score IRS). The results showed an ICC value of 0.89, indicating good consistency between the two raters. In addition, for categorical variables, we also calculated Cohen's kappa coefficient, which is 0.82, indicating a high degree of consistency between the two pathologists.

RNA extraction and real-time quantitative reverse transcription polymerase chain reaction (RT-PCR)

Total RNA was extracted following the instructions of the tissue RNA rapid extraction kit (Yeasen, Shanghai, China), and RNA concentration was measured using a NanoDrop 2000 (Thermo Fisher, CA, USA). cDNA was synthesized using 500 ng total RNA as a template in a 20 μL total reaction volume using HiScript III All-in-One RT SuperMix (Vazyme, Nanjing, China). RT-qPCR was performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China). The primer sequences used are shown in Table 1.

Table 1 Primer sequences for NLRP6, Caspase-1, IL-1β, IL-18, β-actin

Primer Name	Primer Sequences (5′ to 3′)
NLRP6-F	ACTGTGCCATCTGAGCAGCCTC
NLRP6-R	TCACTGAGCCTGTTGTGGAGGA
Caspase-1-F	GCTGAGGTTGACATCACAGGCA
Caspase-1-R	TGCTGTCAGAGGTCTTGTGCTC
IL-1β-F	CCACAGACCTTCCAGGAGAATG
IL-1β-R	GTGCAGTTCAGTGATCGTACAGG
IL-18-F	GATAGCCAGCCTAGAGGTATGG
IL-18-R	CCTTGATGTTATCAGGAGGATTCA
β-actin-F	CACCATTGGCAATGAGCGGTTC
β-actin-R	AGGTCTTTGCGGATGTCCACGT

Western blotting

Gastric tissue samples were weighed, and proteins were extracted by adding RIPA lysis buffer (Beyotime) and protease inhibitors proportionally. After centrifugation at 4 °C, the supernatant was collected, and protein concentration was determined using the bicinchoninic acid protein quantitation kit (Beyotime). Samples were loaded onto SDS-polyacrylamide gels for electrophoresis. The separated proteins were then electro transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked in 5% non-fat milk for 1.5 h, then incubated with the corresponding primary antibodies (All from Abcam) overnight at 4 °C. After washing with PBST buffer thrice for 15 min each, the membranes were incubated with secondary antibodies (Abcam, ab205718, 1:5000) at room temperature for 1.5 h on a shaker. The membranes were then washed with PBST buffer 3 times for 15 min each, and the bands were visualized using a sensitive ECL kit (PK10002, Proteintech).

Statistical analysis

Data analysis was performed using SPSS 22.0 software. For quantitative data following a normal distribution, results were expressed as mean ± standard deviation ($\bar{x}\pm s$). All quantitative data were first subjected to Shapiro-Wilk test for normality test, and the results showed that the data followed a normal distribution (all P values > 0.05). Two-way analysis of variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) test was used for multiple group comparisons. For categorical data, rates (%) were presented, and the $\chi 2$ test was used for inter-group comparisons. A P-value < 0.05 was considered statistically significant.

Results

Comparison of general data

There were no statistically significant differences in age, gender, etc. among the mild, moderate, and severe chronic gastritis groups divided by the degree of gastric mucosal damage ($P > 0.05$), indicating comparability (Table 2).

Comparison of Hp positive rates among the three groups

The Hp positive rates among mild chronic gastritis, moderate chronic gastritis and severe chronic gastritis groups were compared. There was a statistically significant difference in Hp positivity rate among the three groups ($\chi^2 = 8.897$, $P = 0.012$, Table 3).

Table 2 Comparison of clinical data among the mild, moderate, and severe chronic gastritis groups

Group	Number of cases	Age (years, $\bar{x}\pm s$)	Sex (cases)	
			Male	Female
Mild chronic gastritis group	83	10.13 ± 2.45	43	40
Moderate chronic gastritis group	60	10.00 ± 2.41	22	38
Severe chronic gastritis group	17	10.18 ± 2.48	11	6
Statistical value		$F = 0.064$	$\chi 2 = 5.459$	
P value		0.938	0.065	

Table 3 Comparison of Hp infection status among children with mild, moderate and severe chronic gastritis

Group	Mild chronic gastritis (*n* = 83)	Moderate chronic gastritis (*n* = 60)	Severe chronic gastritis (*n* = 17)	Total (*n* = 160)
Hp negative group	47(64.4%)	19(26.0%)	7(9.6%)	73(45.6%)
Hp positive group	36(41.1%)	41(47.1%)	10(11.5%)	87(54.4%)
χ^2 Value	8.897			
P value	0.012			

Immunohistochemical results for NLRP6, Caspase-1, IL-1β, and IL-18 in the gastric tissues of the 6 groups

The brownish-yellow areas indicated positive expression of each indicator. The immunohistochemical results for NLRP6, Caspase-1, IL-1β, and IL-18 in the gastric tissues of the six groups were as follows: Under the same Hp condition, NLRP6 expression was higher in the mild chronic gastritis group than in the moderate chronic gastritis group, while the expression levels of Caspase-1, IL-1β, and IL-18 were lower than in the moderate chronic gastritis group. NLRP6 expression was higher in the moderate chronic gastritis group than in the severe chronic gastritis group, while the expression levels of Caspase-1, IL-1β, and IL-18 were lower than in the severe chronic gastritis group, with statistically significant differences ($P < 0.05$). In cases with the same degree of gastric mucosal damage, NLRP6 expression was higher in the Hp-negative group than in the Hp-positive group, while Caspase-1, IL-1β, and IL-18 were lower than in the Hp-positive group, with statistically significant differences ($P < 0.05$) (Fig. 1; Table 4).

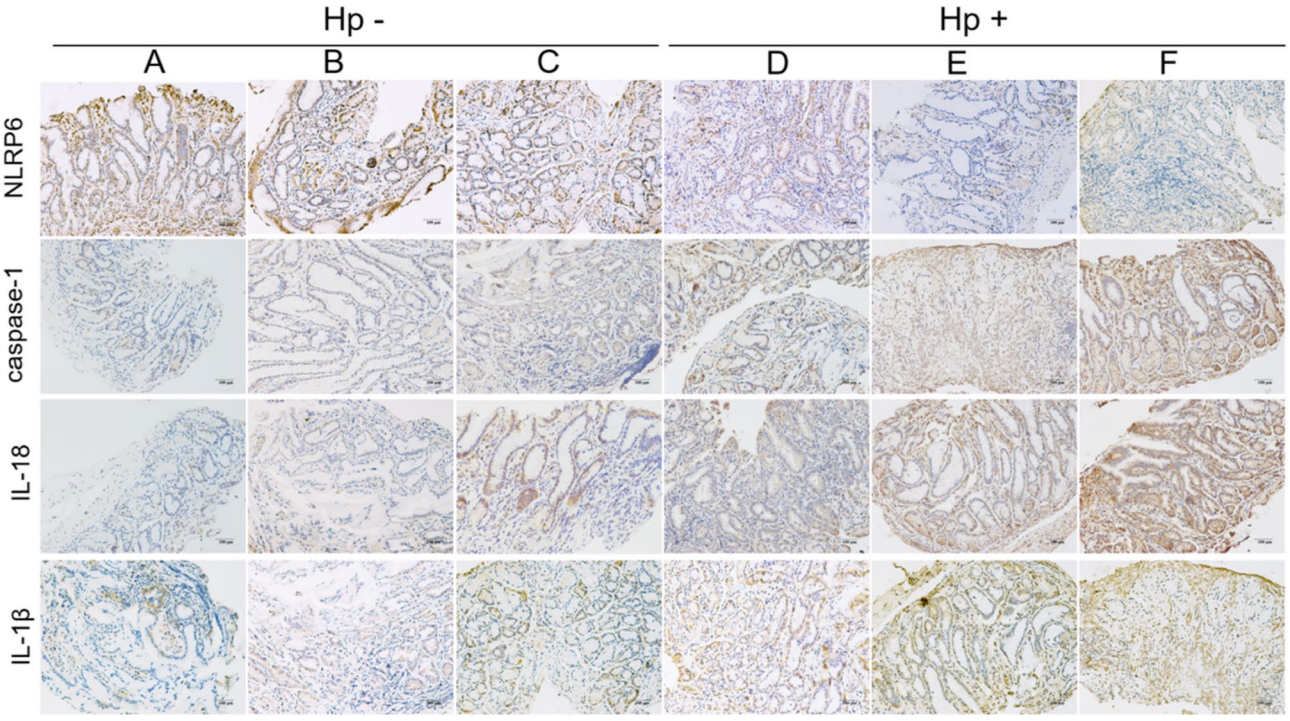

Fig. 1 Expression levels of NLRP6, Caspase-1, IL-1β, and IL-18 in gastric tissues of each group detected by immunohistochemistry (magnification ×400). Group A: mild chronic gastritis, Hp-negative (*n* = 47); Group B: moderate chronic gastritis, Hp-negative(*n* = 19); Group C: severe chronic gastritis, Hp-negative (*n* = 7); Group D: mild chronic gastritis, Hp-positive(*n* = 36); Group E: moderate chronic gastritis, Hp-positive (*n* = 41); Group F: severe chronic gastritis, Hp-positive(*n* = 10). Scale bar: 50 μm

Table 4 Two-way ANOVA results showing significant effects of Hp infection, gastritis severity, and their potential interaction on inflammasome markers (NLRP6, Caspase-1, IL-1β, IL-18)

Dependent Variable	Source of Variation	F value	*P* value
NLRP6	Hp infection	15.79	<0.001
	Gastritis severity	65.80	<0.001
	Interaction	2.49	0.087
Caspase-1	Hp infection	11.76	<0.001
	Gastritis severity	72.50	<0.001
	Interaction	3.31	0.036
IL-1β	Hp infection	38.90	<0.001
	Gastritis severity	31.06	<0.001
	Interaction	1.24	0.291
IL-18	Hp infection	58.23	<0.001
	Gastritis severity	71.24	<0.001
	Interaction	27.07	<0.001

PCR results for NLRP6, Caspase-1, IL-1β, and IL-18 in the gastric tissues of the 6 groups

For NLRP6, Caspase-1, IL-1β, and IL-18 in the gastric tissues of the six groups, ANOVA showed that under the same Hp condition, NLRP6 expression was higher in the mild chronic gastritis group than in the moderate chronic gastritis group, while the expression levels of Caspase-1, IL-1β, and IL-18 were lower than in the moderate chronic gastritis group. NLRP6 expression was higher in the moderate chronic gastritis group than in the severe chronic gastritis group, while the expression levels of Caspase-1, IL-1β, and IL-18 were lower than in the severe chronic gastritis group, with statistically significant differences ($P<0.05$). In cases with the same degree of gastric mucosal damage, NLRP6 expression was higher in the Hp-negative group than in the Hp-positive group, while Caspase-1, IL-1β, and IL-18 were lower than in the Hp-positive group, with statistically significant differences ($P<0.05$) (Fig. 2).

Western blot detection of NLRP6, Caspase-1, IL-1β, and IL-18 expression levels in gastric tissues of the 6 groups

As shown in Fig. 3, under the same Hp positive condition, NLRP6 expression was higher in the mild chronic gastritis group than in the moderate chronic gastritis group, while the expression levels of Caspase-1, IL-1β, and IL-18 were lower than in the moderate chronic gastritis group ($P<0.05$). NLRP6 expression was higher in the moderate chronic gastritis group than in the severe chronic gastritis group($P<0.001$), while the expression levels of Caspase-1, IL-1β, and IL-18 were lower than in the severe chronic gastritis groups, with statistically significant differences ($P<0.05$). In cases with the same

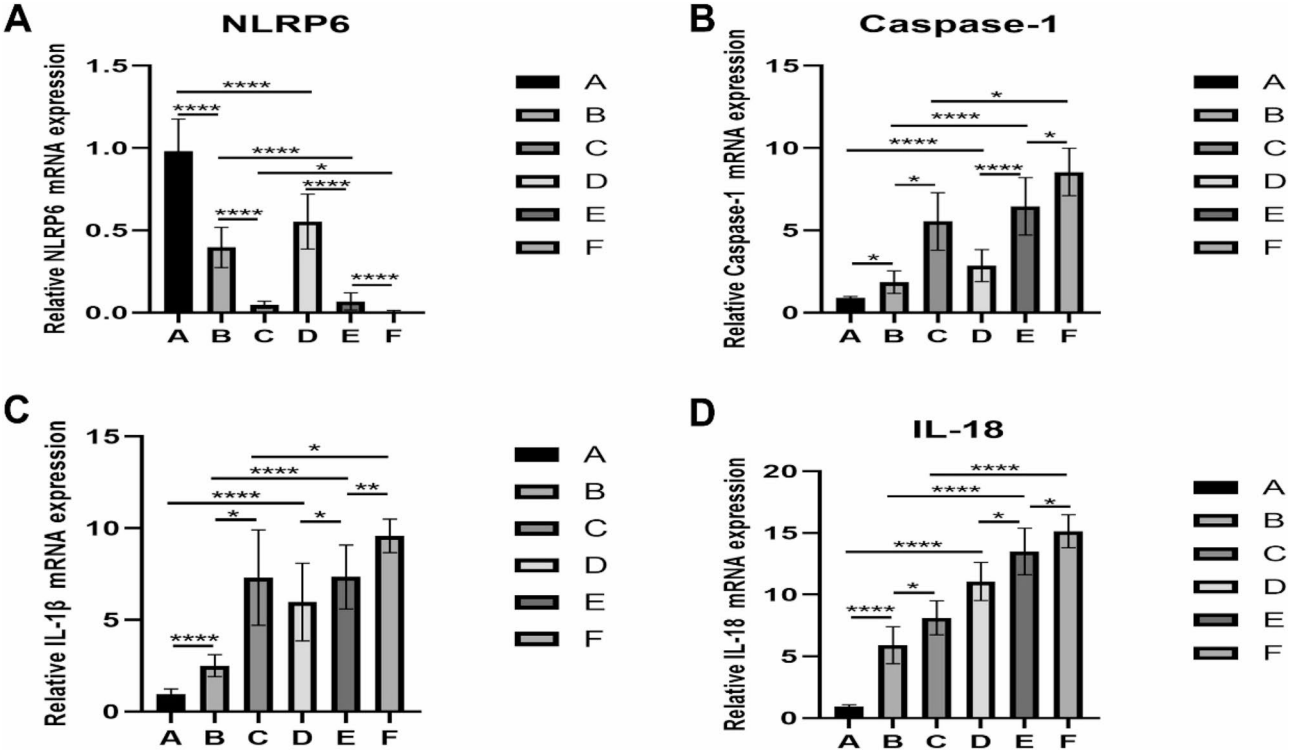

Fig. 2 Inflammatory factor expression levels in gastric tissues of the 6 observation groups Comparison of NLRP6(**A**), Caspase-1(**B**), IL-1β(**C**), IL-18(**D**) expression levels in gastric tissues of the 6 observation groups. Group A: mild chronic gastritis, Hp-negative ($n=47$); Group B: moderate chronic gastritis, Hp-negative($n=19$); Group C: severe chronic gastritis, Hp-negative ($n=7$); Group D: mild chronic gastritis, Hp-positive($n=36$); Group E: moderate chronic gastritis, Hp-positive ($n=41$); Group F: severe chronic gastritis, Hp-positive($n=10$). *$P<0.05$, **$P<0.01$, ****$P<0.0001$

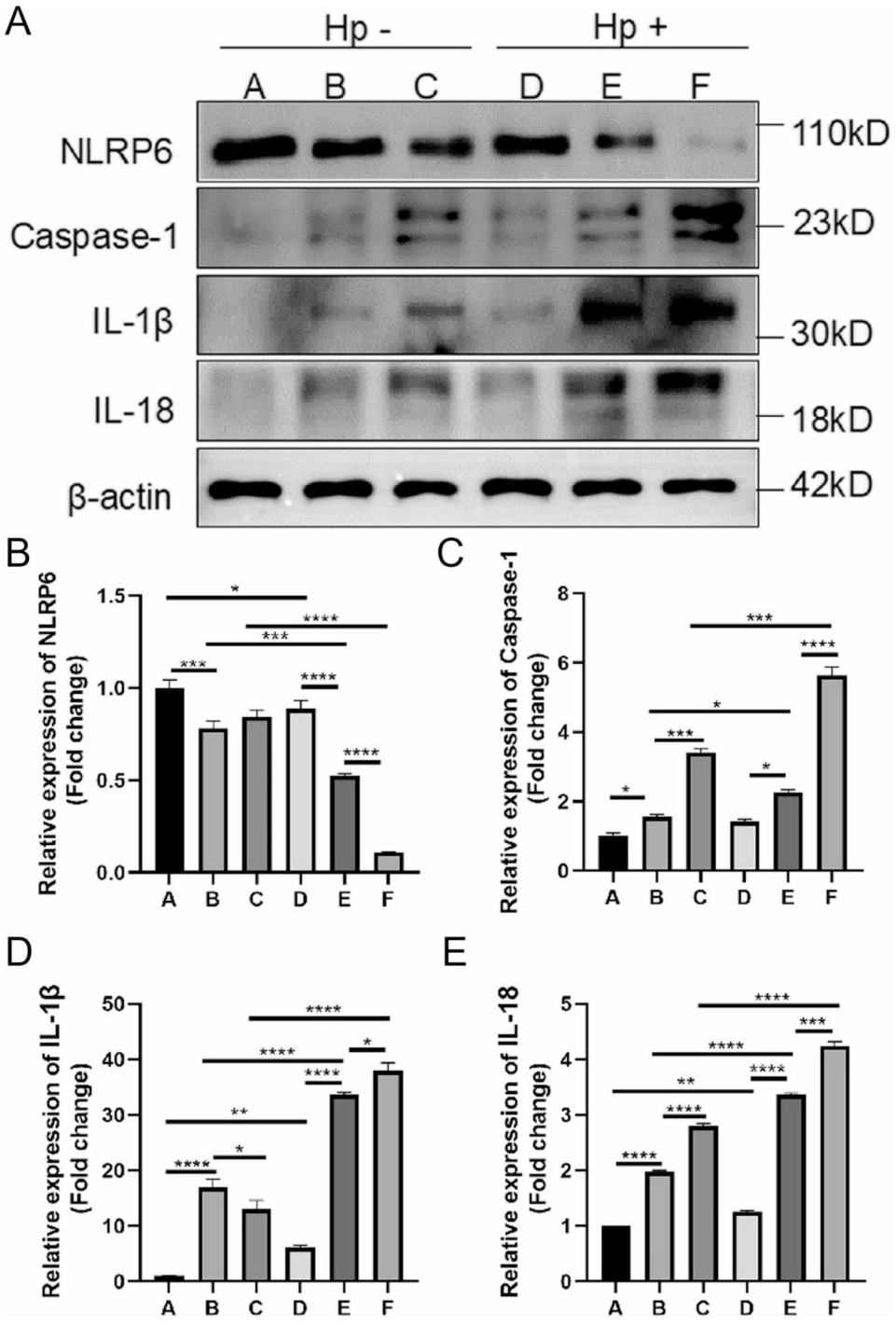

Fig. 3 Comparison of NLRP6, Caspase-1, IL-1β, and IL-18 protein expression levels in gastric tissues of the 6 observation groups(**A**), amd densitometric analysis of the Western blot bands for NLRP6(**B**), Caspase-1(**C**), IL-1β(**D**), and IL-18(**E**). Group A: mild chronic gastritis, Hp-negative; Group B: moderate chronic gastritis, Hp-negative; Group C: severe chronic gastritis, Hp-negative; Group D: mild chronic gastritis, Hp-positive; Group E: moderate chronic gastritis, Hp-positive; Group F: severe chronic gastritis, Hp-positive

degree of gastric mucosal damage, NLRP6 expression was higher in all Hp-negative groups than in Hp-positive groups ($P<0.05$), while Caspase-1 was lower in the moderate and severe Hp-negative groups than in the Hp-positive groups($P<0.05$); IL-1β, and IL-18 were lower than in all Hp-negative groups than in Hp-positive groups, with statistically significant differences ($P<0.05$).

Discussion

Chronic gastritis is a chronic inflammatory disease of the gastric mucosa caused by physical, chemical, and biological harmful factors [11]. It has become one of the most prevalent chronic diseases in children. If not actively prevented and treated, it can significantly impact children's growth and development [3]. Endoscopically, the main changes include mucosal congestion, oedema, erosions, follicular changes, and bleeding spots. Studies have confirmed that more than 90% of chronic gastritis cases involve Hp infection [12]. Hp is a spiral, microaerophilic, gram-negative bacillus that, once acquired, can persistently colonize the surface of the gastric mucosal epithelium, leading to various diseases such as gastritis, gastric ulcers, and gastric cancer. Research has found that the mechanisms by which Hp causes chronic gastritis may be related to the following aspects: ① Hp can produce various harmful enzymes, metabolites, and toxins, causing degeneration and damage to gastric mucosal epithelial cells, and consequently leading to chronic gastritis; ② Hp infection produces IgE, and the IgE antigen complexes can increase the activity of lysosomes in macrophages and participate in macrophage-mediated cytotoxic reactions, causing immunological tissue damage and resulting in gastric mucosal inflammation; ③ Hp can also increase the release of gastrin, which inhibits the contraction of the pyloric sphincter, leading to bile reflux, an important factor in gastric mucosal damage. In 2015, Hp infection was clearly defined as an infectious disease in the Kyoto global consensus report on *H. pylori* gastritis, and the World Health Organization (WHO) has identified it as a Group I carcinogen for gastric cancer [13]. Statistics show that the overall global prevalence of Hp infection in children is about 32.2% [14], with an overall prevalence of 44.2% in mainland China and 28% in children [15]. Most patients are infected during childhood, and if not treated promptly, the majority remain asymptomatic lifelong carriers. However, 15%–20% may develop serious conditions, such as peptic ulcer disease (PUD), gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma, among others. It can also lead to various extra-gastric diseases, including iron deficiency anaemia, growth retardation, and type I diabetes mellitus [16].

The NLR family is a class of cytoplasmic pattern recognition receptors that recognize highly conserved pathogen-associated molecular patterns bound to pathogens and their products, activating a series of cellular defence mechanisms as recognition molecules involved in the immune response [17]. NLRP6 is a member of the NLRP subfamily of NLRs and, unlike other NLRP family proteins, NLRP6 is the only known member that can function in both monomeric and multimeric forms: (1) As a monomer, it inhibits the nuclear factor kB and mitogen-activated protein kinase (MAPK) signalling pathways, affecting the downstream expression of cytokines and chemokines, participating in the negative regulation of intracellular signalling pathways, inhibiting inflammation progression, and thus leading to the establishment of pathogens in vivo [18]. (2) Through its N-terminal pyrin domain, it recruits the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and Caspase-1, forming an inflammasome complex, which then catalyses the maturation and secretion of the cytokines IL-1β and IL-18 [19].

After Hp infection, it can persistently colonize the gastric mucosa, not only cause increased inflammatory reactions, but also potentially leading to gastric ulcers and even gastric cell carcinogenesis. It has been reported that Hp can induce and maintain long-term molecular-mediated mucosal inflammatory responses through the nuclear factor kB pathway, regulating the expression of various inflammatory cytokines and promoting the release of a series of inflammatory factors [20]. On the other hand, research has proven that after Hp infection, it can downregulate the transcription of NLRP6 through the AKT/FOXO3 signalling pathway [21]. Furthermore, NLRP6 can participate in the negative regulation of intracellular signalling pathways by inhibiting the nuclear factor kB and MAPK signalling pathways, thereby inhibiting inflammation progression. Our current research results indicate that the Hp positivity rate was higher in patients with moderate and severe chronic gastritis than the negative patients. Moreover, Hp positivity rate was lower in patients with mild chronic gastritis than the negative patients. In chronic gastritis cases with the same degree of inflammatory infiltration, the NLRP6 level in gastric tissues was significantly lower in the Hp-positive group compared to the Hp-negative group. We speculate that Hp infection inhibits NLRP6 expression, which significantly reduces NLRP6's negative regulation of the nuclear factor kB signalling pathway. At the same time, Hp can induce the expression of various inflammatory factors through the nuclear factor kB pathway, thereby leading to more severe gastric mucosal damage. Current evidence suggests that NLRP6 plays an important negative regulatory role in gastrointestinal inflammation, infection, and tumours. Our research results show that in Hp-negative condition, the expression level of NLRP6 in gastric tissues of mild chronic gastritis was significantly

higher than that of moderate chronic gastritis, and the expression level of NLRP6 in gastric tissues of moderate chronic gastritis was significantly higher than that of severe chronic gastritis. This result further confirms that NLRP6 is involved in maintaining the integrity of the epithelial barrier, and high expression of NLRP6 in gastric tissues can inhibit inflammation and alleviate the degree of gastric mucosal inflammation.

Current research has proven that after sensing external stimuli, NLRP6 can bind to Caspase-1 and ASC to form an inflammasome complex [22], ultimately leading to the maturation of cytokines such as IL-1β and IL-18, which participate in inflammatory immune responses. To investigate whether the NLRP6 inflammasome is involved in the occurrence and development of chronic gastritis and Hp infection in children, we further detected the expression of Caspase-1, IL-1β, and IL-18. The results showed that under the same mucosal damage conditions, the expression of Caspase-1, IL-1β, and IL-18 was significantly higher in the Hp-positive group than in the negative group. Similarly, under the same Hp conditions, the expression of Caspase-1, IL-1β, and IL-18 was higher in the severe chronic gastritis group than in the moderate chronic gastritis group, and higher in the moderate chronic gastritis group than in the mild chronic gastritis group. This result suggests that more inflammasomes are formed after external stimulation. It has been confirmed that Caspase-1 is a special cysteine protease that can be induced and activated by external stimuli, forming an inflammasome complex. Activated Caspase-1 can promote the maturation and secretion of certain inflammatory factors and participate in the apoptosis of inflammatory factors. Studies have shown that Caspase-1 plays an important role in innate immunity, crucially participating in the activation of inflammatory responses and promoting the maturation of IL-1β and IL-18, allowing their release into the interstitial tissues to participate in inflammatory responses and cellular autophagy [23, 24]. The IL-1β secreted by the body is an important pro-inflammatory cytokine, initially defined as an endogenous pyrogen. It can be generated by various cells such as monocytes, endothelial cells, and fibroblasts, stimulating the proliferation and differentiation of B lymphocytes, leading to the generation of immunoglobulins, and further aggravating the inflammatory response [25, 26]. Studies have confirmed that the inflammatory response of the gastric mucosa induced by Hp infection is closely related to IL-1β, and the expression level of IL-1β is associated with the severity of gastric mucosal inflammation [27]. On the other hand, IL-1β can reduce gastric acid secretion by inhibiting the intermediates that promote gastric acid secretion, thus favouring the growth and colonization of Hp [28]. IL-18 is a cytokine involved in infection, inflammation, and autoimmune diseases, playing a dual role in inflammation and internal balance. Many studies have proven that IL-18 is expressed in intestinal epithelial cells, macrophages, neutrophils, and can promote the activity of NK cells, T cells, and interferon-gamma, among others [26, 29]. Furthermore, studies have shown that the expression of IL-18 in the gastric antral mucosa and macrophage infiltration were significantly higher in Hp-infected children than in Hp-negative children, indicating that the IL-18 index plays an important role in the gastric inflammatory response related to Hp in children [30]. Yamauchi K et al. [31] confirmed that the virulence factors of Hp, outer inflammatory protein A (OipA) and the cytotoxin-associated gene pathogenicity island (CagPAI), have different effects on IL-18 induction. The upregulation of IL-18 mRNA/protein in epithelial cells depends on these two virulence factors; however, the upregulation of IL-18 mRNA in monocytes is not affected by these two factors, while IL-18 protein depends on OipA and CagPAI, indicating that OipA and CagPAI regulate the induction of IL-18 in monocytes at the post-transcriptional level. From this, we speculate that the formation of the NLRP6 inflammasome and the activation of the downstream cytokines IL-1β and IL-18 play an important role in Hp-infected chronic gastritis. However, the specific mechanism of action of the NLRP6 inflammasome in Hp-infected chronic gastritis in children still needs further research.

After the Warren and Marshall reported isolating Hp from human gastric mucosal biopsy tissues in 1983, Hp was considered the only bacterial species in the stomach. However, high-throughput sequencing technology has since revealed hundreds of other microorganisms in the stomach, some of which can colonize the gastric mucosa and may have potential pathogenicity. Compared with adults, children infected with Hp have a more diverse gastric microbiota, while the gastric microbiota composition of uninfected children and adults shows little difference [32]. Related studies have shown that the NLRP6 inflammasome regulates goblet cell function and epithelial cell secretion of antimicrobial peptides, thereby affecting intestinal mucosal barrier integrity and altering the composition of the intestinal microbiota. The lack of NLRP6 expression leads to an increase in intestinal Prevotella and exacerbates intestinal inflammation [33]. Gálvez et al. confirmed that NLRP6-deficient mice had a significantly increased abundance of harmful Helicobacteraceae in the intestine, while the abundance of symbiotic Lactobacteriaceae and Bacteroides was significantly decreased. Our research results found that under the same mucosal damage conditions, the expression level of NLRP6 in the Hp-positive group was lower than that in the Hp-negative group, and Hp-positive patients with moderate and severe chronic gastritis were significantly higher than Hp-negative patients. In contrast,

Hp-positive patients with mild chronic gastritis were lower than Hp-negative patients. We speculate that this may be because Hp inhibited the expression of NLRP6, causing a decrease in the abundance of some symbiotic probiotics in the stomach and an increase in the abundance of harmful bacteria, thereby leading to more severe gastric mucosal damage in Hp-infected cases compared to non-Hp-infected cases.

Current study has limitations. Frist, we only use the rapid urease test to diagnose Helicobacter pylori infection. Although this method has the advantages of being fast and convenient, and is one of the commonly used clinical methods, its sensitivity (about 80–95%) is lower than the 'gold standard' methods such as histological examination or urea breath test. The limitations of this methodology may lead to misclassification of Hp infection status. Specifically, false negative results and rare false positive results may occur. This misclassification is likely to weaken the differences in NLRP6 and related inflammatory cytokine expression levels observed between the Hp positive and Hp negative groups. Because some true Hp infected individuals are mistakenly classified as negative, the average inflammation level in the negative group increases and the NLRP6 level decreases, thereby narrowing the gap with the positive group. Despite this limitation, the significant association between NLRP6 expression and the severity of gastritis and Hp infection status revealed in our study provides valuable preliminary evidence for the role of NLRP6 in chronic gastritis in children. Future research needs to adopt more precise Hp diagnostic criteria to further confirm these relationships. Moreover, due to the relatively rare cases of severe chronic gastritis diagnosed by endoscopy and pathology in the clinical practice of pediatrics, the sample size of the severe gastritis subgroup (especially Hp negative C group, $n = 6$) in this study was relatively small. Although we used parameter testing methods such as analysis of variance and observed a consistent trend with mild to moderate gastritis in the severe gastritis group (i.e., NLRP6 expression decreased with increasing mucosal damage, while Caspase-1, IL-1 β, and IL-18 expression increased), the insufficient sample size may weaken the statistical testing power for differences between severe gastritis subgroups (such as C and F groups) and increase the risk of false negative results. Therefore, for the data of severe gastritis subgroups, especially the P-values of intra - and inter group comparisons, caution should be exercised when interpreting them. Future research needs to include a larger sample of children with severe gastritis through multi-center collaboration to further validate the exact role of NLRP6 and its inflammasome in disease progression. Lastly, due to the cross-sectional nature of the study design, we could not establish a causal relationship between Hp infection, NLRP6 expression, and the progression of chronic gastritis. Future longitudinal or interventional studies are needed to validate these associations and explore the underlying mechanisms more deeply.

Conclusions

Taken together, the present findings showed that NLRP6 can inhibit the inflammatory response of the gastric mucosa, and our data suggest an inverse association between Hp infection and NLRP6 expression. Additionally, NLRP6 can bind to ASC and Caspase-1 to form an inflammasome complex, which promotes the secretion of IL-1β and IL-18 by participating in the inflammatory response. Hp infection can aggravate this process.

Abbreviations

CG	Chronic gastritis
Hp	Helicobacter pylori
NLRP6	Nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 6
WHO	World Health Organization
PUD	Peptic ulcer disease
MALT	Mucosa-associated lymphoid tissue
NOD	Nucleotide-binding oligomerization domain
NLRs	Nucleotide-binding oligomerization domain (NOD)-like receptors
MAPK	Mitogen-activated protein kinase

Supplementary Information

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Supplementary Material 1

Authors' contributions

Conceptualization: SY.G., Data curation: SY.G., DL.L., and JY. L. Supervision: J.S., and KW.M. Validation: SY.G., J.S. and KW.M. Writing-original draft: SY.G., and DL.L. Writing-review & editing: JY.L., J.S. and MK. M. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Informed consent was provided by all patients, and all aspects of this study were approved by the Ethics Committees of The First People's Hospital of Lianyungang(KY-20240611002-01).Our study adhered to the Declaration of Helsinki to this effect.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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