RELATIONSHIP BETWEEN SERUM GHRELIN AND NLRP3 INFLAMMASOME LEVELS AND ACTIVITY IN PATIENTS WITH ULCERATIVE COLITIS AND THEIR DIAGNOSTIC VALUE

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ABSTRACT

Objective: *To investigate the relationship between serum ghrelin and Nod-like receptor pyrin domain-related protein 3 (NLRP3) inflammasome levels and activity in patients with ulcerative colitis (UC) and their diagnostic value.*

Methods: *A total of 90 UC patients who were diagnosed and treated in our hospital from January 2020 to May 2022 were selected as the UC group, and 40 healthy people who received physical examination in our hospital during the same period were selected as the healthy control group. Serum ghrelin and NLRP3 inflammation were compared between the two groups. body level. In addition, according to the Raclmilewitz UC disease activity index evaluation system (CAI score), the patients in the UC group were divided into active group (CAI score ≥4 points, n=37), remission group (CAI score <4 points, n=53), The differences in serum ghrelin and NLRP3 inflammasome levels in patients with different UC activities were compared. The Spearman method was used to analyze the correlation between serum ghrelin and NLRP3 inflammasome levels and disease activity in UC patients. In addition, the area under the receiver operating characteristic (ROC) curve (AUC) was used to evaluate the diagnostic value of serum ghrelin and NLRP3 inflammasome levels in evaluating the activity of UC.*

Results: *The serum ghrelin level in the UC group was significantly lower than that in the healthy control group, and the serum NLRP3 inflammasome level was significantly higher than that in the healthy control group (P<0.05). The serum ghrelin level in the active group was significantly lower than that in the healthy control group and the remission group, and the serum NLRP3 inflammasome level was significantly higher than that in the healthy control group and the remission group (P<0.05). The CAI and Mayo scores of UC patients in the active group were significantly higher than those in the remission group (P<0.05). Serum ghrelin level in UC patients was negatively correlated with CAI score and Mayo score (P<0.05), and serum NLRP3 inflammasome level in UC patients was positively correlated with CAI score and Mayo score (P<0.05). The AUC values of serum NLRP3 inflammasome and ghrelin detection alone and combined detection for diagnosing UC activity were 0.788, 0.820, and 0.880, respectively.*

Conclusion: *The detection of serum ghrelin and NLRP3 inflammasome levels has good clinical application value in evaluating the disease activity of UC patients.*

Keywords: *Ulcerative colitis, ghrelin, nod-like receptor pyrin domain-associated protein 3 inflammasome, activity, diagnostic value.*

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Introduction

Ulcerative colitis (UC) is a chronic non-specific inflammatory disease of the colon and rectum, which is limited to the mucosa and submucosa of the sigmoid colon and rectum. It has the characteristics of long course and easy recurrence[(1)]. Under colonoscopy, UC in active phase shows multiple superficial ulcers of intestinal mucosa, accompanied by congestion, edema, etc. the clinical symptoms of patients are mainly abdominal pain, diarrhea, mucus and bloody stool excretion, etc.[(2)]. Clinical studies have shown that[(3)], UC has a slow onset, a wide range of lesions, a greater tendency to canceration, and as a disease prone to recurrent attacks, it will seriously affect the normal work, study and life of patients. Therefore, early detection, early diagnosis and early treatment of UC, timely and accurate evaluation of UC disease activity, and selection of a reasonable treatment plan according to the patient's condition, in order to effectively alleviate the patient's clinical symptoms and avoid further deterioration of the condition is very important. In recent years, with the development of clinical testing technology, the use of serological indicators for the diagnosis and prediction of UC patients has gradually become a

research trend. According to clinical research, UC patients have abnormal expression of immune-inflammatory factors[4].

Nod like receptor thermoprotein domain associated protein 3 (NLRP3) inflammatory corpuscles are an important part of the body's natural immune system. They can be activated after endogenous stimulation and play an important role in the body's immune response and disease occurrence. They have been proved to be closely related to the occurrence and development of inflammatory diseases[5]. Ghrelin is an endogenous gastrointestinal peptide hormone in the body, which can promote the release of growth hormone, participate in feeding and energy metabolism. Its role and value in inflammatory bowel disease have gradually attracted attention in recent years[6].

This study explored the relationship between serum ghrelin and NLRP3 levels and the activity of UC patients and its diagnostic value, in order to provide reference for clinical diagnosis and treatment.

Materials and methods

General information

90 patients with UC who received treatment in our hospital from January 2020 to may 2022 were selected as UC group, and 40 healthy people who underwent physical examination in our hospital during the same period were selected as a healthy control group. In addition, patients in the UC group were divided into active group (CAI score ≥4, n=37) and remission group (CAI score <4, n=53) according to raclmilewitz UC disease activity index evaluation system (CAI score).

Healthy control group:

• Male 22, female 18, 20-63 years old, average age (39.85±10.47);

• Active group: 21 males and 16 females, aged from 18 to 65 years, with an average age of (40.34±11.20);

• Remission group: 30 males and 23 females, aged 19-64 years, with an average age of (39.33±10.21) years.

There was no significant difference in the general data of each group ($p>0.05$), which was comparable. This study was reviewed and approved by the ethics committee of the Academy, and all subjects signed the informed consent.

Inclusion criteria:

• UC was definitely diagnosed by endoscopy and pathological examination[7], the clinical data were complete, and the informed consent was signed.

Exclusion criteria:

• Patients with severe impairment of heart, brain, liver, kidney and other organ functions, psychiatric patients, patients with digestive tract diseases such as severe intestinal obstruction and rectal polyps, patients with malignant tumors, lactating or pregnant women.

Method

Take 5 ml of fasting peripheral venous blood from all subjects in the morning, centrifuge at the rate of 3500 r/min for 10 minutes to separate the serum, and put it statically in the refrigerator at -80° C for testing. NLRP3 inflammatory corpuscles and ghrelin levels were measured by enzyme-linked immunosorbent assay with varioskan lux automatic microplate reader (Thermo Fisher Scientific, USA). The kits were purchased from Beijing Leadman Biochemical Co., Ltd. and operated in strict accordance with the instructions.

Observation index

Raclmilewitz UC disease activity index evaluation system[8]

Raclmilewitz UC disease activity index evaluation system (CAI score) was used to evaluate the activity of UC patients and stage the disease. To evaluate the activity of the disease: Cai score ≥4 points for the active period, Cai score <4 points for the remission period.

Mayo score[9]

Mayo score was used to evaluate the disease activity of patients in the two groups, including stool frequency, rectal bleeding and endoscopic mucosal appearance.

According to the four grades of no, light, medium and heavy, the patients were divided into 0-3 points, with a total score of ≤ 2 points and no single subscore >1 points for clinical remission, 3-5 points for mild activity, 6-10 points for moderate activity, and 11-12 points for severe activity.

Statistical method

Spss20.0 was used for statistical analysis. Chi-square test was used for counting data, and mean ± standard deviation ($\bar{x}\pm s$) was used for measurement data. T-test was used for comparison between

two groups, analysis of variance was used for comparison between multiple groups, LSD test or tamhane test was used for comparison between two groups, Spearman method was used for correlation analysis, and the diagnostic value of each parameter was analyzed by area under receiver operating characteristic (ROC) curve (AUC), The difference was statistically significant with $p<0.05$.

Results

Comparison of serum NLRP3 inflammatory corpuscles and ghrelin levels between the two groups

The serum ghrelin level in UC group was significantly lower than that in healthy control group, and the serum NLRP3 inflammatory corpuscle level was significantly higher than that in healthy control group ($p<0.05$), see Table 1.

Group	Number of cases	NLRP3 inflammatory corpuscle (pg/mL)	Ghrelin (ng/mL)
UC Group	90	389.87±62.82	5.42±1.76
Healthy control group	40	256.92±42.12	7.25±2.14
t		12.208	-5.112
P		0.000	0.000

Table 1: Comparison of serum NLRP3 inflammatory corpuscles and ghrelin levels between the two groups.

Comparison of serum NLRP3 inflammatory corpuscles and ghrelin levels in patients with UC with different degrees of activity

The serum ghrelin level in the remission group was significantly lower than that in the healthy control group, and the serum NLRP3 inflammatory corpuscle level was significantly higher than that in the healthy control group ($p<0.05$).

Group	Number of cases	NLRP3 inflammatory corpuscle (pg/mL)	Ghrelin (ng/mL)
Healthy control group	40	256.92±42.12	7.25±2.14
Remission group	53	323.98±56.00[a]	6.50±2.02[a]
Active period group	47	440.19±69.25[ab]	5.03±1.38[ab]
F		114.872	16.202
P		0.000	0.000

Table 2: Comparison of serum NLRP3 inflammatory corpuscles and ghrelin levels in patients with UC with different degrees of activity.

Notes: Compared with the healthy control group: [a]P<0.05; Compared with remission group: [b]P<0.05.

The serum ghrelin level in the active group was significantly lower than that in the healthy control group and the remission group, and the serum NLRP3 inflammatory corpuscle level was significantly higher than that in the healthy control group and the remission group ($p<0.05$), see Table 2.

Comparison of CAI and Mayo scores of UC patients with different degrees of activity

The CAI and Mayo scores of UC patients in the active phase group were significantly higher than those in the remission phase group ($p<0.05$), as shown in Table 3.

Group	Number of cases	Mayo score (points)	Cai score (points)
Remission group	53	3.16±1.02	2.90±0.74
Active period group	47	8.19±2.25	4.67±1.54
t		-14.671	-7.456
P		0.000	0.000

Table 3: Comparison of CAI and Mayo scores of UC patients with different degrees of activity.

Correlation analysis of serum NLRP3 inflammatory corpuscles, ghrelin levels and disease activity in patients with UC

The serum ghrelin level of UC patients was negatively correlated with CAI score and Mayo score ($p<0.05$), and the serum NLRP3 inflammatory corpuscle level of UC patients was positively correlated with CAI score and Mayo score ($p<0.05$), see Table 4.

Index	NLRP3 inflammatory corpuscle		Ghrelin	
	r	*P*	*r*	*P*
Cai score	0.415	0.000	-0.350	0.000
Mayo score	0.427	0.000	-0.356	0.000

Table 4: Correlation Analysis between serum NLRP3 inflammatory corpuscles, ghrelin levels and disease activity in patients with UC.

ROC curve analysis of the clinical value of serum NLRP3 inflammatory corpuscles and ghrelin in evaluating the activity of UC

The AUC values of serum NLRP3 inflammatory corpuscles and ghrelin levels for the diagnosis of UC activity were 0.788, 0.820 and 0.880, respectively, as shown in Table 5 and Figure 1.

Discussion

With the changes of people's lifestyle and eating habits, the incidence of unhealthy living habits such

as overeating and unclean diet has increased, resulting in a significant upward trend in the incidence rate of UC. UC is mainly in a state of chronic inflammatory reaction, which can involve different parts of the rectum and colon. It is characterized by alternating attack, remission and recurrence[10].

Index	AUC	95% CI	*P*	Susceptibility	Specificity
Ghrelin	0.788	0.690~0.894	0.000	82.35	90.00
NLRP3 inflammatory corpuscle	0.820	0.733~0.919	0.000	87.50	65.90
Joint detection	0.880	0.733~0.919	0.000	93.80	88.60

Table 5: ROC curve analysis of the clinical value of serum NLRP3 inflammatory corpuscles and ghrelin levels in evaluating UC activity.

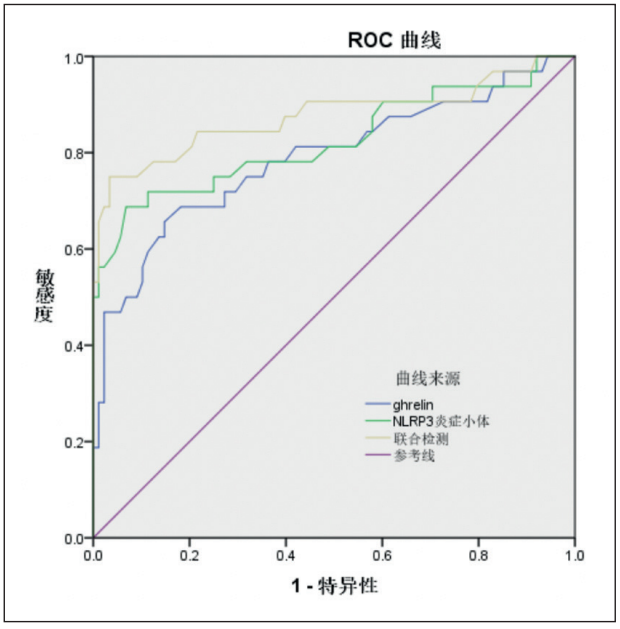

Figure 1: ROC curve analysis of the clinical value of serum NLRP3 inflammatory corpuscles and ghrelin levels in evaluating UC activity.

Under colonoscopy, UC in active phase showed multiple superficial ulcers in intestinal mucosa, accompanied by congestion, edema, etc., and the appearance of intestinal mucosa was rough and uneven, showing fine granular; The clinical symptoms of the patients are mainly abdominal pain, diarrhea, mucus and bloody stool excretion, etc.[11].

At the beginning of UC active phase, the lesions are mucosal basal crypts, showing neutrophil infiltration and crypt abscess. With the progress of the lesions, crypt abscess combines, and the covering epithelium falls off, forming ulcers, and even causing ulcers to deepen, leading to perforation[12]. Therefore, timely and accurate evaluation of the disease activity of UC and the selection of a reasonable treatment plan according to the patient's condition can effectively alleviate the clinical symptoms of patients, which is of great clinical significance to improve the prognosis and quality of life of patients. At present, endoscopy and colonoscopy are mainly used to evaluate the condition of UC patients.

Although they can visually display the degree of intestinal mucosal lesions, endoscopy and colonoscopy belong to invasive examination methods. Patients need to endure a lot of pain, and some patients are intolerant[13]. Therefore, it is of great clinical significance to find a rapid and non-invasive monitoring method that can effectively and accurately evaluate the disease activity of UC patients for early treatment and prognosis evaluation of the disease. NLRP3 inflammatory corpuscles are multi protein complexes assembled by pattern recognition receptors in the cytoplasm, and are an important part of the natural immune system[14]. According to clinical research, NLRP3 inflammatory body dysfunction is closely related to the occurrence and development of inflammatory bowel disease[15]. The results of this study showed that the serum NLRP3 inflammatory corpuscles level in UC group was significantly higher than that in healthy control group. The level of serum NLRP3 inflammatory corpuscles in the remission group was significantly higher than that in the healthy control group; The level of serum NLRP3 inflammatory corpuscles in the active group was significantly higher than that in the healthy control group and the remission group.

The level of serum NLRP3 inflammatory corpuscles in patients with UC was positively correlated with CAI score and Mayo score. It is suggested that the level of NLRP3 inflammatory corpuscles in UC patients is significantly increased, and the level of serum NLRP3 inflammatory corpuscles increases with the aggravation of disease activity, which can be used as an objective index to evaluate the disease activity of UC patients. NLRP3 inflammatory corpuscle is an intracellular pattern recognition receptor, which is mainly activated and expressed in dendritic cells and macrophages. The study found that NLRP3 inflammatory corpuscle recruits and activates the pro-inflammatory protease caspase-1 through a series of mechanisms, thereby producing corresponding mature cytokines, increasing the expression of inflammatory factors, and a large number of inflammatory cells and inflammatory factors invade the lesion, thus mediating the apoptosis of intestinal mucosal

cells and repeated inflammation, Form ulcer[16]. In addition, NLRP3 inflammatory corpuscles can also affect the nucleotide decomposition of intestinal epithelial cells by promoting the self nucleotide metabolism of intestinal epithelial cells and the expression of inflammatory factor interleukin-6, thereby mediating the inflammatory necrosis of intestinal mucosal cells, repeated inflammation, and the formation of ulcers[17].

Ghrelin, also known as growth hormone releasing secretagogue receptor ligand, is a brain-gut peptide composed of 28 amino acid residues synthesized and secreted by A-like cells at the bottom of the stomach and the hypothalamus[18]. Ghrelin exerts physiological effects such as regulating appetite, eating and energy balance by binding with the corresponding receptors on the surface of effector cells. According to clinical studies, ghrelin is involved in the regulation of body feeding, glucose homeostasis, growth hormone release, gastrointestinal activity, gastric acid secretion, energy metabolism and other processes[19]. The results of this study showed that the serum ghrelin level in UC group was significantly lower than that in healthy control group. The serum ghrelin level in the remission group was significantly lower than that in the healthy control group; The serum ghrelin level in the active group was significantly lower than that in healthy control group and remission group. Serum ghrelin level in patients with UC was negatively correlated with CAI score and Mayo score.

It is suggested that the ghrelin level in UC patients is significantly reduced, and the serum ghrelin level shows a downward trend with the aggravation of disease activity, which can be used as an objective index to evaluate the disease activity of UC patients. Ghrelin can be synthesized and secreted by T cells and monocytes, and ghrelin can inhibit interleukin and tumor necrosis factor-α. The activation of some cytokines such as leptin can play a certain role in fighting inflammation and reducing gastrointestinal inflammation; In addition, ghrelin can increase the body's food intake by increasing intestinal movement, thereby promoting the recovery of inflammatory bowel disease[20]. In addition, according to basic experiments, ghrelin can increase colon blood flow and promote the stability of gastrointestinal mucosa[21]. The above studies show that the decline of ghrelin level can affect the disease activity of UC patients through multiple molecular pathways. The structure of this study also found that the AUC values of serum NLRP3 inflammatory corpuscles and ghrelin levels for the diagnosis of UC activity were 0.788, 0.820 and 0.880 respectively, suggesting that the detection of serum ghrelin and NLRP3 inflammatory corpuscles has a good clinical value for the evaluation of disease activity in patients with UC.

To sum up, the serum ghrelin level in patients with UC decreased significantly, and the serum ghrelin level decreased with the aggravation of disease activity. The NLRP3 inflammatory corpuscle level in patients with UC increased significantly, and the serum NLRP3 inflammatory corpuscle level increased with the aggravation of disease activity. The detection of serum ghrelin and NLRP3 inflammatory corpuscle level has good clinical application value for evaluating the disease activity of patients with UC.

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