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The importance of leucine-rich α-2 glycoprotein-1 in acute calculus cholecystitis

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Abstract. – OBJECTIVE: The aim of this prospective, single-center cohort study was to analyze serum leucine-rich α-2-glycoprotein-1 (LRG1) expression in patients with acute cholecystitis (AC) and to investigate its variation depending on symptom duration.

PATIENTS AND METHODS: Participants were divided into patients with AC and a healthy control group. At the time of diagnosis, blood samples were collected, and symptom onset times were questioned. Collected serum LRG1 levels were measured.

RESULTS: 30 patients and 30 healthy volunteers were included in the study. LRG1 (p=0.008), white blood cells (WBC) (p<0.001), platelet (p=0.003), neutrophil (p<0.001), lymphocyte (p=0.001), and CRP (p=0.014) were significantly different in AC patients *vs.* the control group. When the correlations of serum laboratory values with the time of onset of symptoms were compared, LRG1 (p<0.001) was significantly correlated, while no significant correlation was observed in C-reactive protein (CRP) (p=0.572), WBC (p=0.155), and neutrophil (p=0.155).

CONCLUSIONS: LRG1 expression increases after 24 hours in AC patients. Due to its correlation with symptom duration, we believe it can be helpful for timing cholecystectomy.

Key Words:
Acute cholecystitis, Cholecystectomy, Leucine, LRG1, Timing.

Introduction

Acute cholecystitis (AC) is a condition characterized by distention, infection, inflammation, and ischemia in the gallbladder, usually as a result of cystic duct obstruction by gallstones. Only 5% of patients who develop AC do not have gallstones[1]. Although the prevalence of gallstones varies between countries, it is 10-15% in the general population[2]. The diagnosis of AC is made by physical examination, laboratory, and radiological parameters. There is no single clinical or laboratory finding for differential or definitive diagnosis. The main biomarkers used in diagnosis are C-reactive protein (CRP), white blood cells (WBC), and neutrophils. When used to diagnose AC, WBC has low sensitivity and specificity, so this parameter alone has no diagnostic value[3]. CRP is produced by the stimulation of interleukin-6 in the liver. AC cases with normal CRP levels have also been reported, as CRP expression is elevated in serum 6-12 hours after inflammation[3]. However, these are insufficient to measure AC's severity and predict surgical risks[4]. Although the gold standard in treatment is laparoscopic cholecystectomy (LC), medical treatment, percutaneous cholecystostomy, and endoscopic interventions are available for unsuitable patients[2]. The timing for LC has been the subject of debate in the literature. Some studies recommend LC in the first 72 hours, while others suggest up to 7-10 days[2,5-7]. However, it is generally known that as time passes, cholecystectomy becomes more complex, and the risk of complications increases. There is a need for a time-dependent biomarker that increases with the onset of symptoms in AC and guides the timing of LC.

Leucine-rich α-2-glycoprotein-1 (LRG1) consists of leucine-rich motifs most involved in protein-protein interactions, signal transduction, and cell adhesion. It has been reported[8] that LRG-1 is expressed primarily by liver cells and neutrophils. It is thought[8] to play a role in the activation and chemotaxis of neutrophils. LGR1 was first identified[9] as an inflammatory biomarker for immune-mediated diseases such as rheuma-

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toid arthritis and inflammatory bowel disease. Subsequent studies[10-13] have reported that LRG1 is increased in inflammatory diseases such as colorectal cancer, gastric cancer, acute appendicitis, ulcerative colitis, and Crohn. In addition, even when CRP is normal in ulcerative colitis and Crohn's, it is an indicator of the severity of the disease and has been defined as a biomarker showing mucosal healing[12,13]. Some studies[14,15] have stated that LRG1 may be a biomarker in diagnosing acute appendicitis.

This study aimed to measure LGR1 expression in AC and evaluate the change of LGR1 level with AC symptom onset time.

Patients and Methods

This study was approved by the Faculty of Medicine, Sakarya University Ethics Committee (No. 16214662-050.01.04-208325-151, date: 08.01.2023). The study was designed as a prospective cohort. The data of patients aged 18-70 diagnosed with acute calculous cholecystitis at Sakarya University Training and Research Hospital between January 2023 and February 2023 were recorded prospectively, as well as physical examination findings and laboratory and radiological results at the time of diagnosis. Pregnant, chronic renal failure, malignancy, autoimmune disease, hematological disease, another focus of infection, and a history of acute cholecystitis attack were not included in the study. A control group was formed from 30 volunteers between the ages of 18 and 70 who came to the polyclinic for check-ups, had no comorbidities, and had no complaints.

Each patient whose AC diagnosis was confirmed and met the inclusion criteria was given information about the study and a consent form. The same consent form was also given to the control group. Blood samples were collected for LRG1 at the time of diagnosis, and demographic characteristics, biochemical blood analysis, and time from the onset of symptoms were recorded.

Each sample taken from the patient was stored at -80°C straight after being centrifuged at 2,000-3,000 RPM for 20 minutes. A commercially available Human LRG1 ELISA kit (Catalog No. EA0065Hu, Bioassay Technology Laboratory, Shanghai, China) was used to determine LRG1 levels according to the manufacturer's instruction manual. All reagents were brought to room temperature before use. It was reconstituted in one standard vial with 150 µl of standard/sample diluent (Bioassay Technology Laboratory, Shanghai, China) to generate a 96 µg/mL standard stock solution, which should be used within 24 hours. The standard was allowed to sit for 15 minutes with gentle agitation before making dilutions. It was prepared duplicate or triplicate standard points by serially diluting the standard stock solution 1:2 with diluent to produce 48 µg/mL, 24 µg/mL, 12 µg/mL, 6 µg/mL, and 3 µg/mL solutions. It was added standard/sample diluent (Bioassay Technology Laboratory, Shanghai, China) only as the zero standard (0 µg/mL).

The assay was performed at room temperature. Blank wells: Only substrate solution A, substrate solution B, and stop solution (Bioassay Technology Laboratory, Shanghai, China) were added as blank controls. 50 µl diluted standard was added to the standard well, 50 µl sample (sample recommended dilution: 2-5 times when necessary) was added to the sample well, and 50 µl biotinylated antigen was added to each well. After mixing, the plate was covered with a sealer and incubated for 60 minutes at 37°C. The sealer and the liquid were removed from the well and washed five times with 300 µl wash buffer manually. The plate was inverted each time, and the contents were decanted and hit 4-5 times on absorbent material to completely remove the liquid. For automated washing, all wells were aspirated and washed five times with wash buffer, overfilling wells with wash buffer. The plate was blotted on absorbent material. 50 µl avidin-HRP was added to the standard well and sample well, covered the plate with a sealer, and incubated for 60 minutes at 37°C. The sealer and wash were removed as described above. 50 µl substrate solution A was added to each well, and then 50 µl substrate solution B was also added to each well, then incubated in a plate covered with a new sealer for 10 minutes at 37°C in the dark. 50 µl Stop Solution was added to each well, and the blue color changed into yellow immediately. The optical density (OD value) of each well was determined immediately using a microplate reader (BioTek® ELx800, Winooski, VT, USA) set to 450 nm within 10 minutes after adding the stop solution.

Statistical Analysis

Analytical examinations were conducted to provide insights into the general characteristics of the study population. The Kolmogorov-Smirnov test was utilized to assess whether the distributions of

Table I. Comparison of AC and control group.

	Acute calculus cholecystitis (n=30)	Control group (n=30)	*p*-value
Age	50.22±14.17	58.56±12.77	0.22*
Gender			0.789**
Female	12 (40%)	10 (33.3%)	
Male	18 (60%)	20 (66.7%)	
LRG1 (µg/mL)	7.60±2.66	6.06±1.52	0.008*
WBC (K/µL)	14.30±4.54	7.63±1.68	<0.001*
Platelet (K/µL)	277.26±77.52	208.26±95.08	0.003*
Neutrophil (K/µL)	12.58±4.58	4.63±1.32	<0.001*
MPV (fl)	10.30±1.08	10.30±1.13	0.973*
Lymphocyte (K/µL)	1.56±0.85	2.30±0.68	0.001*
RDW (%)	13.40 (12.4-18.5)	13.45 (12.2-33.9)	0.836***
CRP (mg/L)	53.45 (2.17-426.0)	6.57 (3.10-22.0)	0.014***

*Independent samples *t*-test, **Chi-Square Test, ***Mann-Whitney-U Test. LRG1: leucine-rich α-2-glycoprotein-1, WBC: white blood cell, MPV: mean platelet volume, RDW: red cell distribution width, CRP: C-reactive protein.

numerical variables were normal. Consequently, the independent sample *t*-test, Mann-Whitney U test, and the Kruskal-Wallis test were employed to compare the numerical variables among different groups. The Mann-Whitney U test was applied to examine the significance of pairwise distinctions while using Bonferroni correction to account for multiple comparisons. The numerical variables were presented as either mean ± standard deviation or median [minimum-maximum]. Categorical variables underwent comparison through the Chi-Square test and were expressed as count and percentage. The findings were evaluated with a 95% confidence interval, with $p<0.05$ values considered statistically significant. Receiver operator characteristic (ROC) curve analysis was employed to determine the optimal cut-off value and evaluate the performance of the test score for diagnosing AC. Analyses were performed using SPSS statistical software (Version 23.0, IBM Corp., Armonk, NY, USA).

Results

Twenty-five (83.3%) of the 30 patients in the patient group were operated on. The intraoperative findings of the operated patients and histopathological analysis of the removed specimen were consistent with AC. Percutaneous cholecystostomy was performed in 5 patients (16.7%) who were not operated on.

There was no statistically significant difference between the two groups in terms of age ($p=0.22$), gender ($p=0.789$), mean platelet volume (MPV) ($p=0.973$), and red cell distribution width (RDW) ($p=0.836$) (Table I).

LRG1 ($p=0.008$), WBC ($p<0.001$), platelet ($p=0.003$), neutrophil ($p<0.001$), lymphocyte ($p=0.001$), and CRP ($p=0.014$) were observed to be significantly higher in the AC group (Table I).

In the ROC analysis of serum LRG1 in AC, the cut-off value was 6.46 µg/mL, sensitivity was 0.60, and specificity was 0.63 (Figure 1 and Table II).

When the correlations of serum laboratory values with the onset of symptoms were compared,

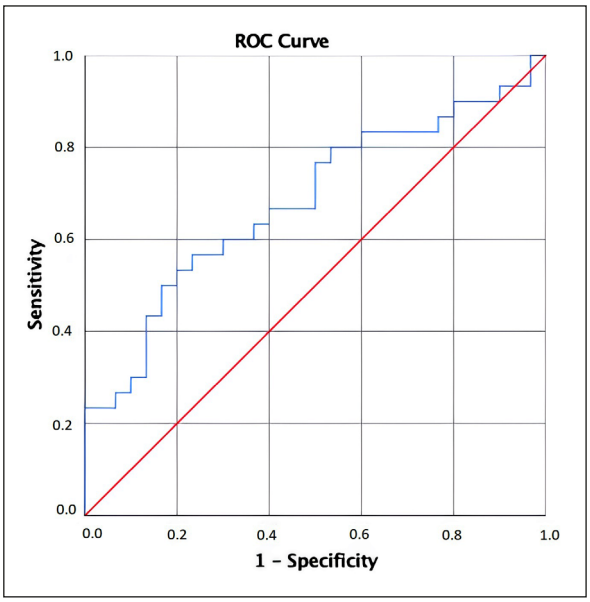

Figure 1. LRG1's ROC curve.

Table II. ROC analysis of LRG1.

	AUC (Area Under Curve)	*p*-value	Sensitivity	Specificity	Cut-off value
LRG1 (μg/mL)	0.681	0.016	0.60	0.633	6.463 μg/mL

LRG1: leucine-rich α-2-glycoprotein-1.

LRG1 (p<0.001) was significantly correlated, while no significant correlation was observed in CRP (p=0.572), WBC (p=0.155), and neutrophil (p=0.155) (Table III).

Discussion

The diagnosis of AC can be made with high sensitivity and specificity in physical examination, laboratory results, and radiological imaging[16]. Inflammatory markers such as WBC and CRP may also increase in AC, as they are elevated in most inflammations. They are insufficient to diagnose AC, but if physical examination and radiological images are appropriate, their height supports the diagnosis of AC. WBC and CRP are not sufficient to show the severity of AC either[17]. During the first 6-12 hours of AC, the CRP level is usually in the normal range[18]. Yuzbasioglu et al[18] found that procalcitonin (PCT) was insufficient to predict AC severity. In our study, it was found that LRG1 was elevated in AC and correlated with symptom duration. This is the first study in the literature reporting the association of LRG1 with AC.

There is no specific biomarker that measures the severity of AC. Centers performing cholecystectomy are based on the duration of symptoms of patients. The timing of cholecystectomy in AC is controversial in the literature. Some studies[4,19,20] suggest LC for the first 72 hours, some for the first 96 hours, and some for up to 7-10 days. Because as time passes, cholecystectomy becomes more complex, and the risk of injury increases. A biomarker is needed to assist in the timing of LC. In our study, it was found that the LRG1 level increased after 24 hours in AC and was correlated with time. We believe that LRG1 can guide the timing of LC.

In the study by Kakar et al[15], serum LRG1 level was found to be a biomarker in the diagnosis of acute appendicitis and also in the differentiation of complicated/uncomplicated acute appendicitis.

Rainer et al[21] determined that LRG1 could be a biomarker in differentiating patients with acute appendicitis and other patients with abdominal pain. It was also determined that a higher LRG1 level could be a stimulator of complicated acute appendicitis.

The study by Shinzaki et al[12] determined that LRG1 could be a marker of mucosal healing in patients with ulcerative colitis and even in patients with normal CRP. Our study showed that LRG1 could be a more sensitive biomarker than CRP in patients with AC and better correlated with time.

In our study, although the correlation of serum levels of LRG1 with symptom duration was superior to CRP, WBC, and neutrophils, it was superior to CRP in the differential diagnosis of patients with AC from the healthy group but not to WBC and neutrophils. We can attribute this to the fact that LRG1 remains silent in the first 24 hours.

As can be understood from the studies mentioned above and our study, LRG1 can be considered as an acute phase reactant. This is because serum levels rise following microbial infections and other inflammatory stimuli[22]. To determine whether LRG1 is an acute phase reactant, Shirai et al[23] detected A dose-dependent increase in LRG1 expression in the lipopolysaccharide-injected mouse experiment.

In the biliary tract infection model with rabbits, TNF-α increased at 12 hours and IL-6 at 24

Table III. Correlation of LRG1, CRP, WBC, and neutrophil by symptom duration.

	<24 hours (n=10)	24-48 hours (n=9)	48-72 hours (n=6)	>72 hours (n=5)	*p*-value
LRG1 (μg/mL)	5.45 (3.70-6.42)	7.59 (4.25-10.95)	8.07 (7.15-12.63)	11.29 (8.15-14.58)	<0.001*
CRP (mg/L)	186.0 (18.2-426.0)	32.7 (2.17-283.0)	82.3 (3.9-291.0)	49.87 (2.8-416.0)	0.572*
WBC (K/μL)	13.80 (10.89-20.73)	16.7 (8.18-24.94)	13.46 (9.75-21.2)	12.82 (7.2-18.23)	0.155*
Neutrophil (K/μL)	11.81 (7.14-19.61)	14.73 (5.19-22.12)	10.26 (7.3-19.8)	9.15 (4.60-16.69)	0.155*

*Kruskal-Wallis Test. LRG1: leucine-rich α-2-glycoprotein-1, WBC: white blood cell, CRP: C-reactive protein.

hours after induction of inflammation[24]. Wang et al[25] showed that TNF-α is the predominant proinflammatory cytokine inducing the secretion of LRG1. We think that LRG1 expression increases as a result of TNF-α and IL-6 reaction chain in acute cholecystitis.

Emphasis is placed on the role of LRG1 as a vasculopathy factor, disrupting the cellular interactions usually required for the formation and maintenance of vessels, thereby indirectly contributing to the formation of a highly hypoxic and immunosuppressive microenvironment. The studies we mentioned above suggest that the situation in acute appendicitis, Crohn's disease, and ulcerative colitis is similar. Similarly, acute calculous cholecystitis is caused by blockage of the cystic duct, leading to gallbladder swelling. As the gallbladder enlarges, blood flow and lymphatic drainage are impaired, leading to mucosal ischemia and necrosis. This suggests that LRG1 plays as a vasculopathy factor in cholecystitis.

The LRG1 level can predict the duration of infection or the severity of mucosal ischemia. Therefore, LRG1 levels may shed light on surgical, medical, or percutaneous cholecystostomy choices in the management of AC.

Limitations

Although our study is prospective, it has limitations. Due to financial constraints, we could not get more human LRG1 ELISA kits, so we could not work with more patients. Patients were not operated by a single surgeon, and the specimens were not examined by a single pathologist. We did not classify difficult cholecystectomy based on intraoperative findings.

Conclusions

In conclusion, LRG1 expression increases after 24 hours in AC. Because of its time-dependent increasing correlation, we believe it can be a guide for timing cholecystectomy.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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This research did not receive any specific funding from any agencies in the public, commercial, or not-for-profit areas.

Availability of Data and Materials

Data of participants with AC are available upon request.

Informed Consent

The data of patients were obtained from our patients anonymously. Informed consent was obtained from all patients. The data does not contain sensitive data.

Ethics Approval

This study was approved by the Faculty of Medicine, Sakarya University Ethics Committee (No. 16214662-050.01.04-208325-151, date: 08.01.2023).

Authors' Contributions

ATH, EG: Study concept and design, acquisition of data, analysis, and interpretation of data, drafting, and revision of the article, and final approval for the submission. KO: Data acquisition, drafting, and revision of the article, as well as final approval for submission. ED: Data acquisition, drafting, and revision of the article, as well as final approval for submission. NF: Acquisition of data, critical revision of the article, final approval for the submission. BHU: Acquisition of data, revision of the article, final approval for the submission. MO: Data acquisition, article revision, and final approval for submission. AOC: Acquisition of data, revision of the article, final approval for the submission.

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