

Value of Leucine-rich alpha-2-glycoprotein-1 (LRG-1) on diagnosis of acute appendicitis in female patients with right lower-quadrant abdominal pain

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Abstract

Objective: To assess the diagnostic usefulness of leucine-rich alpha-2-glycoprotein-1 in female patients with acute abdominal pain on right lower-quadrant and acute appendicitis.

Methods: The prospective, cross-sectional study was conducted at the emergency department of Kecioren Training and Research Hospital, Ankara, Turkey, during a two-month period in 2014, and comprised patients with acute abdominal pain in right lower-quadrant, and control subjects. Female patients aged 18-60 years who were admitted to the hospital's emergency department were included. The control group consisted of healthy females without acute or chronic diseases. Venous blood was obtained from all the subjects to measure leucine-rich alpha-2-glycoprotein-1.

Results: Of the 160 participants, 80(50%) were patients and 80(50%) were control subjects. The mean value of leucine-rich alpha-2-glycoprotein-1 level in the patient and control groups were $6.78 \pm 2.21 \mu\text{g/ml}$ and $6.59 \pm 2.37 \mu\text{g/ml}$, respectively ($p > 0.05$). Among the cases, 32(40%) patients were diagnosed with acute appendicitis, whereas 48(60%) with non-acute appendicitis. The mean leucine-rich alpha-2-glycoprotein-1 level was $6.96 \pm 2.76 \mu\text{g/ml}$ in patients diagnosed with acute appendicitis, and $6.66 \pm 1.78 \mu\text{g/ml}$ in those diagnosed with non-acute appendicitis ($p > 0.05$).

Conclusion: Plasma leucine-rich alpha-2-glycoprotein-1 levels were not useful in diagnosing acute appendicitis in female patients with acute abdominal pain in right lower-quadrant.

Keywords: Acute appendicitis, Leucine-rich alpha-2-glycoprotein-1, Abdominal pain, Emergency department. (JPMA 67: 1383; 2017)

Introduction

Acute appendicitis (AA) is one of the most common causes of non-traumatic acute abdominal pain requiring surgical treatment. Notwithstanding advances in laboratory testing and radiological examination, preoperative diagnosis of AA remains a challenge.^{1,2} In female patients, diagnosing AA may be more difficult due to the presence of a larger number of differential diagnoses, including pelvic inflammatory disease, ovarian torsion, ovarian cyst rupture, pregnancy complications, endometriosis, and ectopic pregnancy. In reply to the difficulty of making an accurate diagnosis, several novel biomarkers, including serum amyloid A, riboleukograms, granulocyte colony stimulating factor (GCSF), calprotectin, and interleukin 6 have been studied in patients admitted with acute right lower-quadrant abdominal pain and AA.³⁻⁹ However, it has shown that these markers have very limited diagnostic utility.¹⁰

Therefore, the need remains for a novel utility marker to diagnose AA.

Leucine-rich alpha-2-glycoprotein-1 (LRG-1) is a part of leucine-rich repeat (LRR) family of proteins and contains repetitive sequences via leucine-rich motif.^{11,12} Although LRG-1's function in the human body is not precisely known, it is thought to be involved in cell adhesion.^{11,12} It has been reported that LRG-1 was expressed by particularly liver cells and the neutrophils. In addition, previously studies have revealed that plasma LRG-1 levels increase in some types of cancer and in the inflammatory diseases.¹³⁻¹⁸ To our knowledge, in the literature there are only a few studies, which have evaluated the relationship between LRG-1 and AA.

The current study was planned to assess the diagnostic usefulness of LRG-1 in female patients with acute abdominal pain in right lower-quadrant and AA.

Patients and Methods

This prospective, cross-sectional study was performed at the emergency department (ED) of Kecioren Training and Research Hospital, Ankara, Turkey, during a two-month period in 2014, and comprised patients with abdominal pain and controls. The study was approved by the

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institutional ethics committee, and written informed consent was obtained from the subjects or from their legally authorised relatives.

Female patients aged between 18 and 60 years who were admitted to the ED with acute abdominal pain in right lower-quadrant, which was defined as new onset pain within 12 hours, were included. Patients with the following conditions were excluded: known pregnancy; any chronic inflammatory intestinal disease; current use of any anti-inflammatory drugs; presence or history of trauma in abdomen; history of an invasive abdominal medical procedure within the last 14 days; active cancer; appendectomy history; or extra-abdominal infection such as pneumonia, cellulites, and upper respiratory tract infections. Healthy females without acute or chronic medical disease were defined as control subjects.

After initial physical examination, patients' demographic data and physical examination findings were recorded on study forms. For all patients, complete blood count, β -human chorionic gonadotropin hormone, renal and liver function tests, and urinalyses were obtained routinely. In addition, 5cc of venous blood was obtained from each patient and control subject to measure LRG-1. Blood samples were decomposed by centrifugal force at 2,200 revolutions per minute (rpm) for 10 minutes at 4°C, and serum was decanted, aliquoted, and stored at -80°C. All blood samples were washed by Rayto (RT-2600+ Microplate Washer) and measured by using serum enzyme-linked immune sorbent assay (ELISA).

The treating physicians had made decisions regarding imaging requirements, including computed tomography (CT) and ultrasonography. Patients, including those with AA, whose final diagnoses required surgical intervention were sent to general surgery, where laparotomy was performed. Then, patients whose pathology results did not indicate AA were excluded from the study. Patients whose final diagnoses were non-surgical and non-emergent were discharged with plans for out-of-hospital treatment, such as antibiotic treatment for pelvic inflammatory disease. Discharged patients were telephoned after one week to determine whether symptoms had persisted, and those whose symptoms had persisted for one week were called back for controlled examination and excluded from the study.

Statistical analyses were carried out by using SPSS 15. Shapiro-Wilk test was used to evaluate the normal distribution of all parameters related to the subjects.

Parametric data was expressed as mean values and standard deviation (SD). Non-parametric data was expressed as number, percentage, median values, and interquartile range (IQR) (25%-75%). Parametric values were analysed using the Student's t-test, and non-parametric values were analysed using the Mann-Whitney U test. The 95% confidence interval (95% CI) was calculated whenever appropriate. $P < 0.05$ was considered statistically significant.

The sample size was estimated with G-Power for Mac OS X (version 3.1.9.2; Universitat Dusseldorf, Germany). Our goal was power to detect a 3 μ g/ml difference between AA and non-AA groups. And also, we considered that SD of LRG-1 value as 6.5 in line with literature.¹⁵ Thus, assuming a two-sided $\alpha = 0.05$, we anticipated a sample size of 74 patients for each group to achieve 80% power.

Results

Of the 160 participants, 80(50%) each were cases and controls. The mean age of the patients was 30.54 $\pm$ 9.6 years, and that of the controls was 31.25 $\pm$ 8.4 years. This difference in age between the two groups was insignificant (95% CI -4.4 - 4.2). Table shows patients' demographic data, presenting symptoms and findings, and laboratory results. The mean values of LRG-1 level in the patient and control groups were 6.78 $\pm$ 2.21 μ g/ml and 6.59 $\pm$ 2.37 μ g/ml, respectively. This difference in LRG-1 level between groups was insignificant (95% CI -0.53 - 0.90).

In the patient group, 32(40%) patients were diagnosed with acute appendicitis, 17(21.3%) with non-specific abdominal pain, 14(17.5%) with ovarian cysts not requiring surgical intervention, 7(8.8%) with urinary-tract infections, and 10(12.4%) with other pathologies. While the mean value of LRG-1 level in patients diagnosed with AA was found to be 6.96 $\pm$ 2.76 μ g/ml, it was 6.66 $\pm$ 1.78 μ g/ml in those diagnosed with other pathologies; but this difference in LRG-1 levels between patients with and without AA was

Table: Patients' demographic data, presenting symptoms and findings, laboratory results.

	Appendicitis	Non-appendicitis
Age	30.3 $\pm$ 9.5	30.6 $\pm$ 9.8
Duration of symptoms (hours)	3.03 $\pm$ 1.6	2.8 $\pm$ 1.5
Nausea or vomiting n (%)	30 (93.8)	38 (79.2)
Temperature at admission	37.3 $\pm$ 0.8	37.1 $\pm$ 0.6
White blood cell count	14.5 $\pm$ 3.8	10.7 $\pm$ 3.2
LRG-1 levels μ g/ml	6.96 $\pm$ 2.76	6.66 $\pm$ 1.78
USG imaging n (%)	26 (81.2)	26 (54.2)
CT imaging (%)	12 (37.5)	22 (45.8)

insignificant (95% CI -0.70 - 1.31).

Discussion

This study aimed at finding answers to certain clinical questions. First, are LRG-1 levels in patients who have acute abdominal pain in right lower-quadrant increased compared to those of healthy subjects? Second, if LRG-1 levels are increased in these patients, could LRG-1 level be useful in diagnosing AA? However, the study found that the answer to both questions was in the negative. LRG-1 levels in patients having acute right lower-quadrant abdominal pain or AA were not higher than levels in healthy controls.

This result conflicts with the findings of the limited number of previous studies that have investigated these questions. One such study, by Kentsis et al., used accuracy mass spectrometry to test the effects of several urine markers on diagnosing acute paediatric appendicitis.¹⁹ We found that LRG levels were particularly higher in the patients with appendicitis than in the patients without appendicitis. The area under the curve (AUC) value was reported as 0.97 to distinguish between patients with and without appendicitis. In a similar way, another study conducted by Kentsis et al. studied the usefulness of urine LRG values in children in diagnosing AA.²⁰ In that study, LRG was measured using two methods: LRG ELISA and selected ion monitoring (SIM) mass spectrometry (MS). LRG concentrations in urine were significantly increased in patients with appendicitis compared to those without appendicitis (3.9 µg/ml [IQR; 0.9, 19.3] versus 0.3 µg/ml [IQR; 0.1, 0.8], respectively). To differentiate between patients with appendicitis and those without, the AUC value was reported as 0.80 for the LRG ELISA and as 0.98 for the LRG SIM MS.²⁰ To our knowledge, investigation of the relationship between LRG levels and AA is limited to these two studies.

This is the initial study to evaluate the utility of LRG-1 levels in diagnosing AA in adult patients. In addition, the present study differed from previous studies by including control subjects and using the LRG-1 levels in plasma rather than in urine. Considering the results of the two previous studies mentioned above, it can be argued that LRG levels perform excellently in diagnosing paediatric AA. However, the findings of the present study showed no differences in terms of LRG-1 level, not only between patients with and without AA, but also between all patients with acute abdominal pain in right lower-quadrant and the control subjects. Therefore, we concluded that LRG-1 values are not useful in diagnosing AA in female patients with acute abdominal pain in right lower-quadrant.

In any discipline, the literature tends to contain more studies with positive results than with negative results. Similarly, the majority of studies on LRG-1 levels associated with various diseases, including AA, have reported positive results.¹³⁻²⁰ Since the present study reported negative results, in contrast to those of most published studies on the use of LRG-1 values in clinical practice, we believe that the present study is significant to the conclusion on whether LRG-1 does or does not have value in clinical practice. To clarify this conclusion, further studies with large cohort populations are needed.

The present study had some limitations. First, it had a relatively small patient cohort and was a single-centre study. Second, in diagnosing AA, radiological examination, including computed tomography and ultrasonography, was not performed routinely on all patients because of ethical concerns. To address this limitation, we telephoned one week later to keep track of patients who were discharged from the emergency department. Third, the present study included only female patients because they have a greater number of differential diagnoses than male patients.

Conclusions

Plasma LRG-1 levels were not useful in diagnosing AA in female patients with acute abdominal pain in right lower-quadrant.

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Conflict of Interest: None.

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