

Diagnostic value of GCP-2/CXCL-6 and hs-CRP in the diagnosis of acute appendicitis

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ABSTRACT

BACKGROUND: Acute appendicitis (AA) is one of the major causes of acute abdomen pain. Various laboratory markers have been studied for diagnosis of AA, but none of them have shown superiority to physical examination or imaging. GCP-2/CXCL6 is a chemokine expressed by macrophages and epithelial and mesenchymal cells during inflammation. The present study aims to investigate the diagnostic role of GCP-2/CXCL6 in AA patients.

METHODS: In this cross-sectional study, the serum level of GCP-2/CXCL6 was measured in 56 AA patients and 32 healthy control subjects. Also, hs-CRP and white blood cell count (WBC) levels of the patient and control groups were evaluated.

RESULTS: GCP-2/CXCL-6, hs-CRP and WBC levels of the AA group were significantly higher than the control group ($p < 0.05$ for all comparisons). Among AA group, GCP-2/CXCL6 levels were higher in complex AA (gangrenous, abscess and perforation) ones when compared to non-complex AA ($p < 0.05$). A strong positive correlation was found between GCP-2/CXCL6 levels and hs-CRP levels ($r = 0.756$, $p = 0.003$) and a moderate positive correlation between GCP-2/CXCL6 levels and WBC count ($r = 0.468$, $p = 0.003$).

CONCLUSION: GCP-2/CXCL6 can be a useful marker in AA diagnosis and discrimination of complex cases, especially if combined with other laboratory markers and imaging techniques.

Keywords: Acute appendicitis; chemokine; complication; diagnosis; prognosis.

INTRODUCTION

Acute appendicitis (AA) is one of the major causes of urgent abdominal operations. Although the signs and symptoms of AA are well-known, sometimes, it is challenging to diagnose AA. Delayed diagnosis leads to complex AA (gangrenous, abscess and perforation).^[1]

To distinguish between non-specific abdominal pain and AA is of critical importance. Physical examination, clinical symptoms and radiological findings can be inefficient sometimes to determine the extent of the disease. Twenty percent of neg-

ative laparotomy rates in AA have empowered the need for laboratory tests in the diagnosis of the acute disease. Several biochemical parameters like white blood cell count (WBC), C-reactive protein, procalcitonin, erythrocyte sedimentation rate (ESR) have been used as potential predictors of complicated appendicitis. Also, members of the interleukin family (IL-6, IL-10, IL-4, IL-5 and IL-12), tumor necrosis factor (TNF) alpha, fibrinogen and alpha-1 antitrypsin have been studied for the diagnosis of AA. However, none of these parameters were found to be superior to clinical history, physical examination, and usual laboratory parameters in predicting appendiceal perforation, and therefore, are not used widely in clinical practice.^[2-5]

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Chemokines are a large family of peptides characterized by four conserved cysteine residues in their NH₂ terminus.^[5] These molecules are responsible for leukocyte trafficking and activation in both health and disease. They are important for host defense, but they also have fundamental roles in the development and homeostasis of the immune system.^[6-8] The CXC chemokines contain one amino acid between the two NH₂ terminal cysteines. A subfamily of the CXC chemokines is further categorized by the presence of an ELR (glutamic acid- leucine-arginine) motif immediately before the CXC sequence. These ELR+CXC chemokines are potent neutrophil chemo-attractants. In humans, seven ELR+CXC chemokines have been identified. Human granulocyte chemotactic protein-2 (GCP-2)/CXCL6 is a 77 amino acid protein that belongs to this family.^[9,10] In the case of leukocytes, GCP-2/CXCL6 binding to receptors causes cellular activation, chemotaxis and sometimes cytotoxic effector functions.^[5] GCP-2/CXCL6 is expressed by macrophages and epithelial and mesenchymal cells during inflammation. Up to date, this chemokine has shown to be activated in lung pathologies, arthritis, colitis and ischemia-reperfusion injury.^[11-14]

C-reactive protein (CRP) as an acute phase reactant increases in appendicitis, and its serum and probably salivary level may be helpful in early diagnosis. CRP is a commonly used tool in emergency medicine, especially in febrile and equivocal infections. CRP was classified as an “acute phase protein” in the 1930s, and since then, CRP is considered as a screening test for tissue inflammation, a biomarker of disease activity and a predictive tool in many acute and chronic infections.^[15-17] CRP is also a helpful reference for decision making in patients with abdominal pain, with its specificity of 89% and a positive predictive value of 88%. CRP has found a place as a biochemical marker in AA diagnosis along with WBC count, and serial CRP measurements in AA among clinical examination is a tool of reference in predicting appendicitis and perforated appendicitis.^[18,19] High-sensitivity CRP (hs-CRP) is more precise than standard CRP when measuring baseline (i.e. normal) concentrations and enables a measure of inflammation.^[20]

In this study, we aimed to determine whether especially serum GCP-2/CXCL6 can be used as a biochemical indicator of AA and its complications.

MATERIALS AND METHODS

Fifty-six patients who were operated emergently for diagnosis of Ankara Numune Training and Research Hospital during the period between October 2016 and January 2017 were included in this prospective study. This study was performed in accordance with the “Declaration of Helsinki guidelines,” and informed consent was obtained from all the participants. This study was approved by the local ethical committee with the number 993/2016.

Patients having any other acute or chronic inflammatory disease and/or malignancy, diabetes mellitus, chronic renal/hepatic failure and cirrhosis and patients younger than 18 years old were excluded from this study. The control group was composed of 32 healthy individuals with no signs of acute or chronic illness.

The demographic features of all participants were recorded. Ultrasonographic (US) and computerized tomography (CT) evaluation of patients was made by expert radiologists. The patients were characterized into two groups according to the histopathological results of the resected appendices: non-complexAA and complexAA. Ten mL of venous blood samples were collected from the participants. Blood samples collected for hs-CRP and GCP-2/CXCL6 analysis were centrifuged at 4000 rpm for 10 minutes. Separated sera were aliquoted into Eppendorf tubes and stored at -80 °C until the time of analysis. R&D Systems Quantikine ELISA Human GCP-2 Elisa kit was used for Human GCP-2/CXCL6 detection. High-sensitivity CRP was studied with the immunoturbidimetric method, while WBC analysis was carried out by VCS (volume, conductivity, and scatter) technique.

Statistical Analysis

Data analysis was carried out using SPSS for Windows 15 programme. The normality tests were performed using the Kolmogorov-Smirnov test. Descriptive statistics of normally distributed continuous variables are illustrated as mean \pm standard deviation (SD). Descriptive statistics of non-normally distributed continuous variables are illustrated as median (interquartile range) values. Among group differences of normally distributed variables were analyzed with Student's t-test, while non-normally distributed variables were analyzed using Mann-Whitney U test. Kruskal Wallis test was used for comparison of more than two groups. Correlation analyses were performed using Pearson's test. P<0.05 was accepted as statistically significant for all tests.

RESULTS

Patient group consisted of 56 cases; 26 males (46.4%), 30 females (53.6%), while the control group consisted of 32 individuals, 17 females (52.4%) and 15 males (47.6 %). Mean age of the patient and control groups were 30.8 \pm 0.95 and 32.7 \pm 1.1 years, respectively. When compared with the control group, GCP-2, hs-CRP and WBC levels of the patient group were significantly higher p<0.001 (Table 1). GCP-2/CXCL6 levels were compared between control, perforated and non-perforated groups. Statistically significant differences were found among groups hs-CRP levels were also significantly different among three groups. A statistically significant difference was found between WBC levels when perforated and non-perforated groups were compared with the control group. No significant difference was found between perforated and non-perforated groups concerning WBC count (Table 2). Also,

Table 1. Clinicopathological characteristics of the acute appendicitis patients

Characteristics	n	%	Mean±SD
Age (y)			30.8±0.95
White blood cell (µL)			14.9±0.73
Gender			
Male	26	46.4	
Female	30	53.6	
Ultrasonographic (n=56)			
Normal	8	14.2	
Appendicitis without perforation	22	39.2	
Appendicitis with perforation	26	46.4	
Computerized tomography			
Normal	0	0	
Appendicitis without perforation	10	47.6	
Appendicitis with perforation	11	52.4	
Pathological diagnosis			
Acute appendicitis	27	48.2	
Gangrenous/perforated appendicitis	29	51.8	

SD: Standard deviation.

GCP-2/CXCL6 levels of the perforated group were higher than the non-perforated group. GCP-2/CXCL6 levels of the patient and control groups are shown in a Box-Whiskarplot (Fig. 1). The pathological diagnosis was acute appendicitis in 27 (48.2%), and gangrenous/perforated appendicitis in 29 (51.8%) patients. The clinical, radiological and pathological characteristics of the patient group are summarized in Table 3. When levels of GCP-2/CXCL-6, hs-CRP and WBC were compared between perforated and non-perforated appendicitis cases, the findings showed that levels of GCP-2/CXCL-6 and hs-CRP was higher in the perforated group when compared to non-perforated group (p=0.003 and p=0.018, respectively). WBC levels of perforated and non-perforated patients did not show a statistically significant difference (p=0.062) (Table 4).

The correlation between GCP-2/CXCL6 levels and hs-CRP and WBC levels and were statistically evaluated. It was found that there was a strong positive correlation between GCP-2/

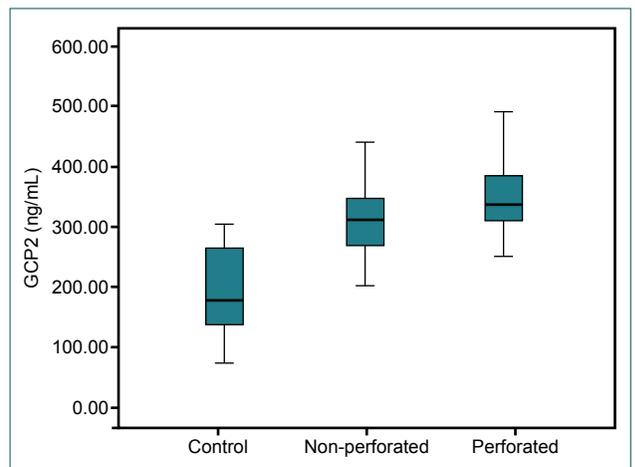


Figure 1. Box-Whiskar Plot for GCP-2 levels in patient and control groups.

CXCL6 levels and hs-CRP levels (r=0.756, p=0.003) and also a moderately positive correlation between GCP-2/CXCL6 levels and WBC count (r=0.468, p=0.003).

DISCUSSION

Acute appendicitis is the most common indication for emergency surgery, affecting patients at any age group. Appendiceal perforation is especially the initial clinical presentation in a significant number of patients with AA in the elderly. Despite the presence of various imaging modalities, biochemical markers, and scoring systems, the negative appendectomy rate remains high.^[21] Laboratory studies, along with the clinical presentation, can help in the diagnosis of AA. However, all clinical and laboratory variables are weak discriminators individually although they can achieve a high discriminatory power when combined. Complex AA is associated with increased morbidity and mortality. Thus, it is important to identify patients with complex AA for surgical planning, for further treatments and for the decision of non-operative therapy, which can be an option in non-complex AA cases.^[22,23]

Many attempts have been made to determine ways of decreasing the negative laparotomy rate in clinically suspected AA. Unfortunately, there is no specific diagnostic test for the determination of AA. In medical practice, WBC count is commonly used for the diagnosis of AA. Although rises

Table 2. GCP-2/CXCL6, hs-CRP and WBC levels of patient and control groups

Variable	Control	Non-perforated	Perforated	p
GCP-2/CXCL6 (ng/mL)	194 (157.4–275.5) ^{a,c}	268 (257–351.1) ^{a,b}	354 (312.1–393.7) ^{b,c}	<0.05
hs-CRP (ng/L)	1.07 (0.60–2.16) ^{a,c}	26.64 (19.26–50.78) ^{a,b}	65.08 (22.49–125.04) ^{b,c}	<0.05
WBC (10 ³ /µL)	6.5 (5.10–7.40) ^{a,c}	12.8 (11.60–15.80) ^a	13.8 (7.40–19.30) ^c	<0.05

GCP-2: Granulocyte chemotactic protein-2; CXCL6: Chemokine Ligand-6; hs-CRP: High-sensitivity C-reactive protein; WBC: White blood cell.

a: Statistically significant difference between perforated and control group (p<0.05). b: Statistically significant difference between perforated and non-perforated group (p<0.05). c: Statistically significant difference between non-perforated and control group (p<0.05).

Table 3. The sex distribution, mean ages, WBC, hs-CRP and GCP-2 levels of the acute appendicitis and control groups

	Acute appendicitis (n=56)	Control (n=32)	p
Age (mean±SD)	30.8±0.95	32.7±1.16	0.266
Gender (%) (Female/Male)	53.6/46.4	52.4/47.6	0.945
WBC (10 ³ /μL) (mean±SD)	14.9±0.73	6.5±0.24	0.000*
hs-CRP (ng/L) (mean±SD)	41 (2.1–188) [^]	1.25 (0.19–4.18) [^]	0.000*
GCP-2/CXCL6 (ng/mL) (mean±SD)	310±13.0	220±13.7	0.000*

GCP-2: Granulocyte chemotactic protein-2; CXCL6: Chemokine Ligand-6; hs-CRP: High-sensitivity C-reactive protein; WBC: White blood cell; SD: Standard deviation. [^]: Continuous variables that do not show normal distribution; *: Statistically significant.

Table 4. GCP-2/CXCL6, hs-CRP and WBC levels of the perforated and non-perforated patients

Variable	Non-perforated	Perforated	p
GCP-2/CXCL6 (ng/mL)	268 (257–351.1)	354 (312.1–393.7)	0.003*
hs-CRP (ng/L)	26.64 (19.26–50.78)	65.08 (22.49–125.04)	0.018*
WBC (10 ³ /μL)	12.8 (11.60–15.80)	13.8 (7.40–19.30)	0.622

GCP-2: Granulocyte chemotactic protein-2; CXCL6: Chemokine Ligand-6; hs-CRP: High-sensitivity C-reactive protein; WBC: White blood cell.

in WBC count is associated with the severity of AA, it is not absolute and not reliable in predicting the extent of the disease.^[22,24] In our study, WBC levels of the AA group were significantly higher than the control group. This is consistent with the literature.

Previous studies have shown that elevated CRP levels, along with leukocytosis, improve the diagnostic value in AA. In AA, especially in 12–24 hours, CRP seems to be useful, especially when serial levels show an increase.^[18,25] Although CRP, especially hs-CRP, is a useful marker in the management of acute abdominal pain, it should be combined with CT results and clinical examinations. A meta-analysis exploring the diagnostic accuracy of CRP revealed a very wide range of sensitivity and specificity (47–74%, and 55–89%, respectively).^[25] In our study, consistent with literature knowledge, hs-CRP levels in the patient group were significantly higher than that of the control group. The level of C-reactive protein alone does not influence the decision to confirm or exclude the diagnosis of AA safely or sufficiently. Thus, a stronger parameter of confidence in doubtful cases is needed.

As stated in the literature, inflammatory markers may be useful when deciding operative management.^[26] In the field of cardiology, hs-CRP has been a research topic in assessing heart failure.^[27] Also, the use of hs-CRP to detect the severity of preeclampsia has been the subject of intensive research in particular.^[28] The usefulness of inflammatory markers has also been investigated in the differentiation of complicated and uncomplicated acute diverticulitis recently.^[29] In our study,

especially in the complicated appendicitis, although GCP-2/CXCL-6 and hs-CRP levels were statistically significant, the significance value of GCP-2/CXCL-6 level was found to be higher. Here, the increase in GCP-2/CXCL-6 level has shown that it can be used as a potential marker, especially in the diagnosis of complicated AA.

The value of various biochemical/hematologic parameters other than WBC and CRP (Mean platelet volume, lymphocyte/leucocyte ratio, interleukin (IL)-6, IL-10, IL-4, IL-5, IL-12, tumour necrosis factor alpha (TNF-α), endotoxin, erythrocyte sedimentation rate, procalcitonin, fibrinogen, alpha 2-macroglobulin, alpha 1- antitrypsin, D-lactate) have been studied for diagnosis of AA. However, none of them has found place in the routine clinical practice.^[2] For the prevention of negative laparotomy rates and also delayed diagnosis of AA, new biochemical markers are still a topic of interest. In our study, there was a significant difference between GCP-2/CXCL6 levels of patient and control groups. This data can provide us with a positive opinion especially about the discriminatory power of this chemokine in the diagnosis of AA.

Recently, the treatment of appendicitis with antibiotics instead of surgery has been a topic of interest all over the world. In their study, Hansson et al.^[30] proposed a model to identify patients with phlegmonous appendicitis, as these patients had an 80% probability of recovering with antibiotic therapy. Furthermore, Di Saverio et al.^[31] provide further evidence that antibiotic treatment can be safe and effective in selected patients with suspected acute appendicitis.

In summary, it seems unclear at which stage of disease progression antibiotic therapy is still feasible. Therefore, the suspected acute appendicitis is the one that requires markers to distinguish between cases with diagnosed or complicated appendicitis. In particular, a marker that can support the diagnosis of complicated acute appendicitis can help us. Here, GCP-2/CXCL-6 and hs-CRP may be useful markers in this respect.

When we compared AA and complex AA groups, we found that GCP-2/CXCL6 levels were higher in complicated than in non-complex AA cases. This chemokine is not routinely used in clinical settings, so automated kits are not found yet for quick analysis. This may limit the usage for now in emergency settings, but our study can be accepted as a pioneer study as, to our knowledge, this is the first study in the literature to show the correlation between GCP-2/CXCL6 levels and AA. Another shortcoming of our study may be the limited patient number.

Conclusion

This study showed that GCP-2/CXCL6 could be a candidate biochemical marker in the diagnosis of AA. Also, it can be helpful in distinguishing complex AA from noncomplex AA. An additional study can be planned to discuss the difference in a larger study group. This can be accepted as a pioneer study in understanding the role of GCP-2/CXCL6 in AA. More detailed studies are needed to be carried out about protein expressions, immunohistochemistry and kinetics of GCP-2/CXCL6 production for further understanding of the nature of this chemokine and mechanisms of action in AA.

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ORİJİNAL ÇALIŞMA - ÖZET

Akut apandisit tanısında GCP-2/CXCL-6 ve hs-CRP'nin tanısal değeri

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AMAÇ: Akut apandisit (AA), akut karın ağrısının en önemli nedenlerinden biridir. AA tanısı için çeşitli laboratuvar belirteçleri çalışılmıştır, ancak hiçbiri fizik muayene veya görüntülemeye üstünlük göstermemiştir. GCP-2/CXCL6, enflamasyon sırasında makrofajlar ve epitelyal ve mezenkimal hücreler tarafından eksprese edilen bir kemokindir. Bu çalışmanın amacı AA hastalarında GCP-2/CXCL6'nın tanısal rolünü araştırmaktır.

GEREÇ VE YÖNTEM: Bu kesitsel çalışmada, serum GCP-2/CXCL6 düzeyi 56 AA hastası ve 32 sağlıklı kontrolde ölçülmüştür. Ayrıca, hasta ve kontrol gruplarının hs-CRP ve lökosit (WBC) düzeyleri değerlendirilmiştir.

BULGULAR: Akut apandisit grubunun GCP-2/CXCL-6, hs-CRP ve WBC düzeyleri kontrol grubundan anlamlı derecede yüksekti (tüm karşılaştırmalar için $p < 0.05$). AA grubu arasında ise, GCP-2/CXCL6 seviyeleri kompleks AA'da (gangrenöz, apse ve perforasyon), kompleks olmayan AA'lara göre daha yüksekti ($p < 0.05$). GCP-2/CXCL6 seviyeleri ile hsCRP seviyeleri arasında güçlü bir pozitif korelasyon bulunmuştur ($r = 0.756$, $p = 0.003$) ve GCP-2/CXCL6 seviyeleri ile WBC sayısı arasında orta derecede pozitif korelasyon bulunmuştur ($r = 0.468$, $p = 0.003$).

TARTIŞMA: GCP-2/CXCL6, AA teşhisinde ve karmaşık olguların ayırt edilmesinde, özellikle diğer laboratuvar belirteçleri ve görüntüleme teknikleriyle birleştirildiğinde yararlı bir belirteç olabilir.

Anahtar sözcükler: Akut apandisit; kemokin; komplikasyon; prognoz; tanı.

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