ORIGINAL ARTICLE

The predictive value of ischemia-modified albumin in the diagnosis of acute appendicitis: A prospective case–control study

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ABSTRACT

BACKGROUND: Surgical planning is critical for ongoing treatment and prognosis of the disease's course after an appendicitis diagnosis. Ischemia-modified albumin (IMA) has been used as a biomarker for a variety of ischemia-related disorders in the past. The aim of this study is to determine the IMA level in patients with AA and to evaluate its predictive significance.

METHODS: A total of 139 participants were enrolled in the trial. After diagnosis and before surgery, the amount of plasma IMA was tested. Patients diagnosed with appendicitis in Group 1 (n=97) and volunteer surgical patients not diagnosed with appendicitis in Group 2 (n=42) were compared as the final diagnostic criterion.

RESULTS: The data of 139 patients with a mean age of 36.15 were evaluated statistically. IMA values were analyzed in both groups. The mean IMA of all patients was 0.74±0.16 AbsU. When the two groups were compared, it was seen that IMA was statistically higher in Group 1 than in the control group. While the area under the curve for IMA was 0.670, the sensitivity for the cutoff value of 0.715 was 68%, the specificity was 62%.

CONCLUSION: Our study shows that IMA values provide significant results in predicting acute appendicitis.

Keywords: Appendicitis; ischemia-modified albumin; predictive value.

INTRODUCTION

Acute appendicitis (AA) is a disease characterized by the sudden inflammation of appendix vermiformis. It is among the most common causes of lower abdominal pain that causes patients to present to the emergency department, and it is the most common diagnosis in young patients presented to the hospital with the complaint of acute abdomen.[1] Diagnosis of AA is still challenging, and there is still some controversy regarding its management among different settings and practice patterns worldwide. The lifetime risk of appendicitis is one in 15 in society.[2] AA is caused by the obstruction of the appendiceal lumen. The obstruction leads to secretion accumulation in the appendix, causing distension. Bacterial translocation occurs as a result of impaired lymphatic and venous drainage. Delayed diagnosis may lead to complicated conditions such as perforation, abscess, and peritonitis.[3]

Albumin (also called human albumin), found in blood plasma, is a type of protein synthesized by the liver, approximately 12–14 grams/day. Albumin is the most abundant of the three major types of proteins in human blood. Transition metals such as cobalt, copper, and nickel are attached to the final amino terminus of the albumin molecule. In the case of ischemia, hy-

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poxia, acidosis, free radical damage, and membrane breakdown diminish the binding of these transition metals to the N-terminal of albumin, causing alterations in the albumin structure. Ischemia-modified albumin (IMA) is the consequence, and it can be tested using the albumin-cobalt binding test.[4,5]

IMA levels measured in conditions such as peptic ulcer, pulmonary embolism, and mesenteric ischemia were found above normal.[6–8] IMA is recognized by the Food and Drug Administration as a biomarker of myocardial ischemia.[9] Today, it is used as an early indicator of myocardial ischemia.[10]

Decreased blood flow in the hypoxic-ischemic area leads to anaerobic metabolism with the formation of free oxygen radicals by the reduction of free metals and the catalyst effect of the superoxide dismutase enzyme. This results in increased IMA levels in blood. Considering that AA has a similar pathophysiology, IMA is also expected to increase.

The aim of this study is to determine the IMA level in patients with AA and to evaluate its predictive significance.

MATERIALS AND METHODS

This prospective case–control study was carried out in a training and research hospital's general surgery department. After the approval of the ethics committee (number: 2012-KAEK-15/2295, dated April 27, 2021) was obtained, those presenting to the emergency department (18–65 years of age) with suspected AA (clinical, laboratory, and imaging) between May 1, 2021, and October 1, 2021, and 50 volunteer patients who presented with the right lower quadrant pain without a suspected appendicitis were included in the study.

Exclusion Criteria

Patients with cancer, diabetes, and concomitant diseases related to the cardiovascular system, patients with diseases that may affect serum albumin, patients with high bilirubin levels, and patients who refused to participate in the study were excluded from the study.

A total of 181 patients with suspected AA presented to the emergency department, and 50 patients with the right lower quadrant pain and no suspected AA were included in the study. Of the patients meeting the study criteria, those diagnosed with appendicitis as the final diagnostic criterion (patient group, n=97) were identified as Group 1, while voluntary surgical patients with no diagnosis of appendicitis were identified as Group 2 (control group, n=42) (Fig. 1).

Patients diagnosed with appendicitis based on pathological examination were divided according to their subtypes (AA and perforated appendicitis). Definitive diagnosis of appendicitis was determined by pathology and operation reports.

Demographic data of the patients, white blood cell (WBC), neutrophil, lymphocyte, CRP, neutrophil/lymphocyte ratios, and pathology results were obtained from digital records and patient files. Leukocytosis was defined as a WBC level greater than 10.0 × 10^3/μL. Serum IMA level was measured after diagnosis and before AP treatment.

All patients gave written informed consent before participating in the trial, which was done in accordance with the Helsinki Declaration principles.

Calculation of Serum IMA

Serum IMA was analyzed using the albumin-cobalt principle. This procedure involved mixing 50 μL of cobalt dichloride reagent with 200 μL of serum and incubating for 10 min. Cobalt attaches to the N-terminal of unaltered albumin

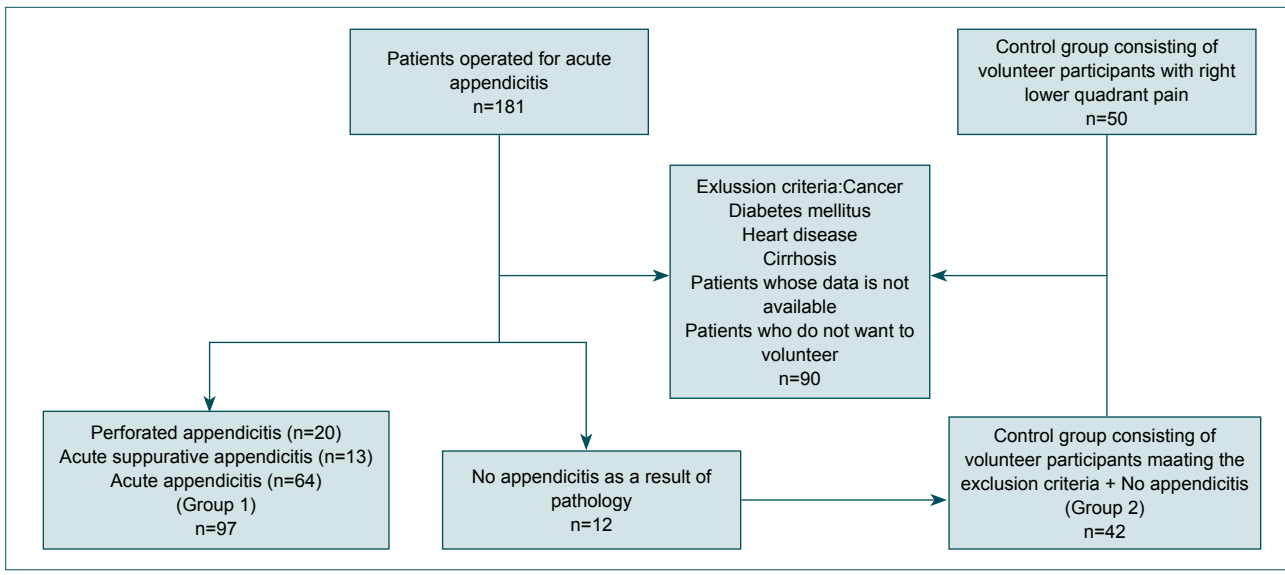

Figure 1. Flowchart of the patient group (Group 1) and control group (Group 2).

throughout the incubation period. With the addition of 50 µL of dithiothreitol (DTT) reagent, DTT combines with unbound cobalt, resulting in color development. The colored product was measured at 470 nm and reported in absorbance units.

Statistical Analysis

For power analysis, the G* Power program version 3.1 was utilized. The sample size is calculated using a big Cohen's effect size (0.8). With a power of 95% and a significance threshold of 5%, at least 35 participants per group were necessary to identify the difference between appendicitis patients and control groups.

The SPSS 22.0 (IBM Corp., Armonk, NY, USA) program was used for statistical analysis. Interquartile range and median with 25–75 percentiles are used to present the data. Histogram was used to evaluate whether the distributions were normal. Kruskal–Wallis test was used for the analysis of IMA values between groups. Mann–Whitney U-test was used to make a statistical comparison between two groups. For group ratios, the Chi-square test was utilized. Differences between groups with p=0.05 were deemed statistically significant. Receiver operating curve (ROC) analysis was used to determine optimal cutoff values to distinguish patients with appendicitis from suspected cases. ROC analysis performance was evaluated in a statistically significant way.

RESULTS

A total of 139 patients were included in the study. Two patients from Group I were excluded from the study because the pathology results revealed adenocarcinoma. Moreover, 12 patients from Group I whose final pathology result did not show AA were included in Group II.

In Group I, 69 of the patients (n=109) were operated by laparoscopic surgery and 50 by open surgery.

While the number of patients with the right lower quadrant pain and no suspected appendicitis was 30, a total of 12 patients who underwent surgery and got final pathology were identified as Group 2. Of the 30 patients in the control group with the right lower quadrant pain, 12 were diagnosed as ovarian cyst rupture, eight as urinary tract infection, five as ureteral stones, and five as terminal ileitis.

The 139 patients had an average age of 36.15 (11.7). In terms of age and gender, there was no significant difference between Groups I and II (p=0.874 and p=0.264, respectively) (Table 1).

The mean WBC count was 13.47±4.38 µL. While this value was 14.20±4.34 µL for Group 1, it was 11.78±4.05 µL for Group 2. Comparison between the two groups showed statistically higher WBC count in patients with appendicitis (p=0.02). While neutrophil and lymphocyte values were 11.23±4.17 K/ml and 2.10±0.95 K/ml for Group 1, respectively, and 9.32±3.61 K/ml and 2.21±0.83 K/ml for Group 2. No statistical difference was found in the comparison between groups. In addition, comparison of calculated neutrophil/lymphocyte ratios revealed significantly higher results in Group 1 (p=0.02).

IMA values were analyzed in both groups. The mean IMA of all patients was 0.74±0.16 AbsU. Comparison of two groups revealed that IMA was statistically higher in Group 1 compared to the control group (p=0.001).

Patients diagnosed with appendicitis were evaluated as a subgroup. None of the laboratory parameters were statistically significant in differentiating AA, acute suppurative appendicitis, and perforated appendicitis (Table 2). Although IMA values were higher in perforated appendicitis, the difference was not statistically significant (Fig. 2).

The ROC analysis produced the following results for IMA;

Table 1. Comparison of demographic characteristics and laboratory findings

	Total (n=139)	Grup 1 (n=97)	Grup 2 (n=42)	p
Age (years)	36.15±11.71	35.75±12.13	35.93±10.07	0.874
Sex				
Male	77	53	24	0.356
Female	62	44	18	0.264
White blood cell (µL)	13.47±4.38	14.20±4.34	11.78±4.05	**0.02**
Neutrophil (K/ml)	10.65±4.09	11.23±4.17	9.32±3.61	0.08
Lymphocyte (K/ml)	2.14±0.92	2.10±0.95	2.21±0.83	0.5
NLR	6.56±5.79	7.16±6.52	5.19±3.29	**0.02**
CRP (mg/L)	61.16±60.65	62.27±60.25	58.60±62.24	0.748
IMA, AbsU	0.74±0.16	0.77±0.14	0.693±0.16	**0.001**

CRP: C-reactive protein; NLR: Neutrophil/Lymphocyte ratio; AbsU: Absorbance unit. Statistically significant values shown in bold.

Table 2. CqLaboratory data of acute appendicitis, acute suppurative appendicitis and perforated appendicitis

	Acute appendicitis (n=64)	Acute suppurative appendicitis (n=13)	Perforated appendicitis (n=20)	p
White blood cell (µL)	13.63±3.92	14.20±5.44	16.01±4.45	0.063
Neutrophil (K/ml)	10.82±4.07	10.56±4.74	12.99±3.83	0.334
Lymphocyte (K/ml)	2.06±0.89	2.52±1.20	1.97±0.95	0.228
NLR	6.37±4.7	7.49±4.41	10.51±13.2	0.846
CRP (mg/L)	54.70±55.31	66.75±60.76	92.69±76.12	0.109
IMA, AbsU	0.78±0.12	0.78±0.14	0.79±0.12	0.234

CRP: C-reactive protein; NLR: Neutrophil/Lymphocyte ratio; AbsU: Absorbance unit.

AUC: 0.670, cutoff: 0.715, sensitivity: 68%, and specificity: 62% (Fig. 3). Other laboratory parameters are shown in Table 3.

DISCUSSION

In this prospective case–control study, we investigated the predictive value of serum IMA for appendicitis in patients who presented to the emergency department. To the best of our knowledge, our study is the first prospective case–control study examining the relationship between IMA and appendicitis. Patients in the AA group had greater IMA levels than in the control group. The regression analysis confirmed high sensitivity and specificity.

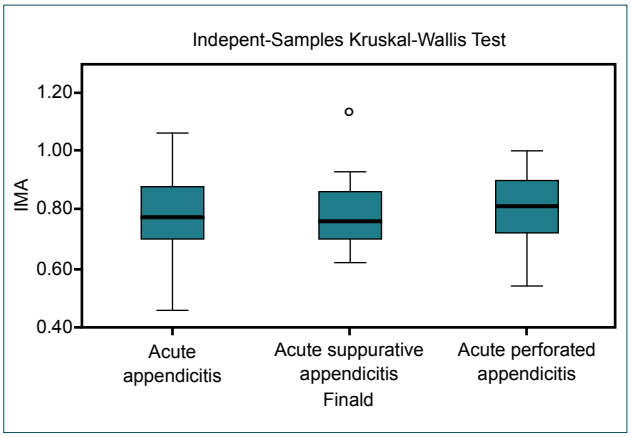

Figure 2. Ischemia-modified albumin (IMA) levels in patients with acute appendicitis, acute suppurative appendicitis, and perforated appendicitis (0.78±0.12 AbsU, 0.78±0.14 AbsU, and 0.79±0.12 AbsU, p=0.234).

The most prevalent intra-abdominal pathology requiring emergency surgery in general surgery clinics is AA. According to available research, males have a 16.33% lifetime risk of appendicitis and females have a 16.34% lifetime risk of appendectomy, and males have a 9.89% lifetime risk of appendectomy and females have a 9.61% lifetime risk of appendectomy.[11] Despite the development of advanced scoring systems and clinical, laboratory, and imaging methods along with the correct diagnosis rate approaching nearly 90%, negative appendectomy rates still remain to be undesirably high. In recent years, studies have been conducted on various biochemical markers that may be of help to the clinician in the diagnosis of AA.[12–14]

Ischemia and necrosis due to lumen obstruction, fluid accumulation, and distension play a role in the pathophysiology of AA. IMA, a recent prominent marker, is a molecule formed as a result of the structural changes of albumin induced by cell modifications in the ischemic environment and increased free radicals.[15] IMA was first introduced in 1990 by Bar-Or et al.[16] These studies examined hypoxic cardiac tissue and demonstrated the change of circulating albumin in blood. The diagnosis of IMA is based on reduced cobalt-binding affinity to albumin's modified N-terminus. IMA rises within minutes

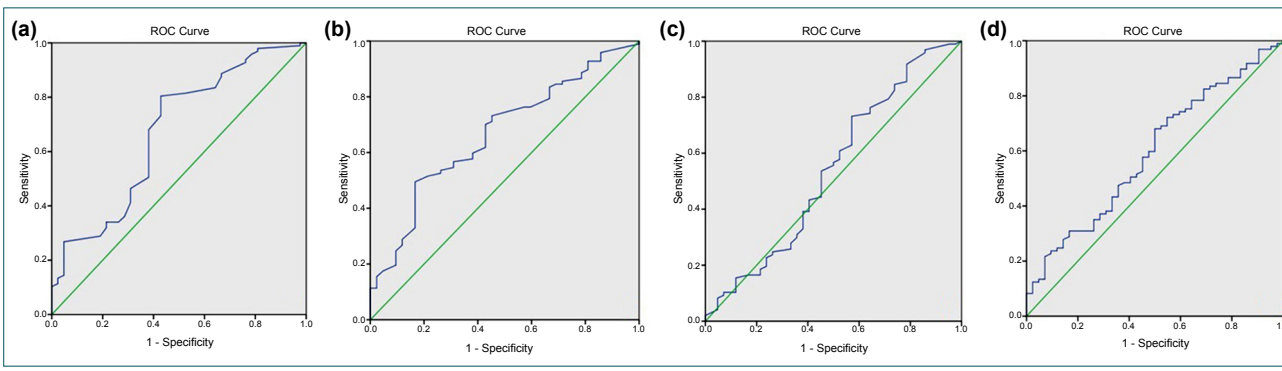

Figure 3. ROC curve analysis for parameters (**[a]** IMA, **[b]** WBC, **[c]** CRP, and **[d]** NLR).

Table 3. ROC Curve analisis for WBC, NLR, CRP and IMA

	AUC	Cut-off value	Sensitivity (%)	Spesifity (%)
White blood cell (μL)	0.667	12.350	70	57
NLR	0.592	4.766	60	52
CRP (mg/L)	0.541	17	73	42
IMA, AbsU	0.670	0.715	68	62

AUC: Area under the curve; NLR: Neutrophil/Lymphocyte ratio; CRP: C-reactive protein; IMA: Ischemia-modified albumin.

of the beginning of ischemia, remains increased for 6–12 h, and recovers to baseline after 24 h, according to clinical trials.[17] In our study, blood samples for IMA were taken in the early pre-operative period.

Our results show higher IMA values in pathologically confirmed patients who were operated on with the diagnosis of AA compared to healthy individuals. Dumlu et al.[18] investigated both serum and tissue levels of various oxidative metabolism markers, including IMA, and concluded that IMA levels were statistically higher in patients with AA. Nazik et al.[19] concluded that IMA was determined to have the highest area under the curve (0.991) when compared with other laboratory parameters in the diagnosis of AA in children. Parallel to our study, IMA was significantly higher in patients with AA than in healthy individuals.

In this study, the rate of complicated AA was 23%. Two different studies found this rate 24.1% and 17.7%, respectively.[20,21] There are also some studies with large case series reporting as low as 8.3%.[22] Yildirim et al.[20] reported the rate of complicated cases in AA as 20% after the first 24 h due to delayed diagnosis. Kılıç et al.[23] investigated IMA in determining complications in patients with AA and concluded that IMA values were higher in patients with complicated appendicitis. In our study, no difference was found between those with complicated appendicitis and those without. We believe that the prospective design of our study with a larger number of patients helps us produce more accurate results.

In their clinical study, Bostancı et al.[24] concluded that although IMA values were higher in cases with AA compared to normal patient population, the predictive value was low because there was no statistically significant difference while observed statistically higher in complicated appendicitis. Our results show higher IMA values compared to normal population. Furthermore, no statistical difference was found between complicated and uncomplicated cases. This was because they excluded patients with cancer, diabetes, and comorbidities related to the cardiovascular system from the study. However, it is seen that those with concomitant diseases that would affect serum bilirubin and albumin levels were not excluded from the study. We think that high bilirubin levels may partly explain the differences in IMA between the study groups, thus causing this difference. Patients with hyperbilirubinemia were excluded from our study.

Moreover, we only included patients presenting to the emergency department with the right lower quadrant pain. As far as our research, there is no other study investigating IMA in adults to help diagnose appendicitis in isolated right lower quadrant pain. While high IMA values are expected in this group of patients compared to the normal patient population, extra significant elevation in patients with the right lower quadrant pain implies it as a new predictive marker that can be used in differential diagnosis.

Our study is designed as a prospective case–control study. Therefore, more reliable data have been obtained compared to various retrospective studies. The number of patients was determined by the power analysis method. However, our study has limitations. Because our study was done in a single location, the generalizability of our findings may be limited. Another drawback of our study is the limited sample size, which may limit the interpretation of subgroup findings. Finally, we were unable to control some physiological variables (exercise, hydration, etc.) that could influence serum IMA levels.

Conclusion

Early diagnosis of AA plays an important role in preventing possible complications. Our study shows that IMA values provide significant results in predicting AA. Our results show that serum IMA is high but not statistically significant in differentiating complicated AA. Since our aim is to diagnose AA without complications, we believe that serum IMA is valuable in early period. Besides, when compared with routine blood values, the best AUC value is provided by IMA.

Ethics Committee Approval: This study was approved by the Ankara Kecioren Training and Research Hospital Clinical Research Ethics Committee (Date: 27.04.2021, Decision No: 2012-KAEK-15/2295).

Peer-review: Internally peer-reviewed.

Authorship Contributions: Concept: A.U.; Design: V.B.T.; Supervision: H.B.; Resource: D.O.; Materials: G.F.T.; Data: A.U.; Analysis: O.E.; Literature search: V.B.T.; Writing: V.B.T.; Critical revision: H.B.

Conflict of Interest: None declared.

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ORİJİNAL ÇALIŞMA - *ÖZ*

Akut apandisit tanısında iskemi modifiye albüminin prediktif değeri: İleriye yönelik olgu kontrol çalışması

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AMAÇ: Cerrahi planlama, apandisit tanısından sonra devam eden tedavi ve hastalığın seyrinin prognozu için kritik öneme sahiptir. İskemi ile modifiye edilmiş albümin (IMA), geçmişte çeşitli iskemi ile ilişkili bozukluklar için bir biyobelirteç olarak kullanılmıştır. Bu çalışmanın amacı, AA'lı hastalarda iskemi modifiye albümin düzeyini belirlemek ve prediktif önemini değerlendirmektir.

GEREÇ VE YÖNTEM: Araştırmaya toplam 139 katılımcı alındı. Tanıdan sonra ve ameliyattan önce plazma IMA miktarı test edildi. Grup 1'de (n=97) apandisit tanısı alan hastalar ile grup 2'de (n=42) apandisit tanısı almayan gönüllü cerrahi hastalar son tanı kriteri olarak karşılaştırıldı.

BULGULAR: Yaş ortalaması 36.15 olan 139 hastanın verileri istatistiksel olarak değerlendirildi. IMA değerleri her iki grupta da analiz edildi. Tüm hastaların ortalama IMA'sı 0.74±0.16 AbsU idi. İki grup karşılaştırıldığında, grup 1'de IMA'nın kontrol grubuna göre istatistiksel olarak daha yüksek olduğu görüldü. IMA için eğrinin altında kalan alan 0.670 iken, 0.715 cut-off değeri için duyarlılık %68, özgüllük %62 idi.

TARTIŞMA: Çalışmamız İMA değerlerinin akut apandisit öngörmede anlamlı sonuçlar sağladığını göstermektedir.

Anahtar sözcükler: Apandisit; iskemi modifiye albümin; prediktif değer.

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