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RESEARCH ARTICLE

Diagnostic Accuracy of Ischemia-Modified Albumin In Complicated Appendicitis And Its Comparison with the Conventional Markers: An Observational Study

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Abstract: ***Background:*** Acute appendicitis is a common emergency, with one-third presenting as complicated cases. Differentiating Complicated Appendicitis (CA) from Uncomplicated Appendicitis (UA) is important to decide on intervention. Conventional markers have limitations, while Ischemia-Modified Albumin (IMA), released during ischemia and oxidative stress, may aid in diagnosis. ***Aim:*** This study evaluates the utility of IMA in distinguishing CA from UA. Setting and Design: Observational cross-sectional study conducted in a tertiary care hospital in South India during 2023 to 2024 ***Materials and Methods***: 85 patients over 13 years with acute appendicitis were further divided into CA (n=42) and UA (n=43) based on ultrasonography and histopathology findings. Total Leucocyte count (TLC), Differential Count (DC), Neutrophil Lymphocyte Ratio (NLR), Modified Alvarado Scoring (MAS), and IMA were estimated and compared between the groups ***Statistical Analysis:*** Sensitivity, specificity, and cut-off were established for the parameters using the Area Under the Curve for the diagnosis of CA. Pearson's correlation analysis of IMA with the parameters was also done. ***Results:*** IMA levels were significantly higher in CA 10.90±5.65 µg/mL compared to UA 6.00±2.93 µg/mL (p-value 0.001). It showed a high sensitivity (82.35%), and specificity (95.56%) with high AUC (0.904) at a cut-off of 8.50 µg/mL in a 95% confidence interval of 0.8871-0.9219 compared to other parameters like TLC, NLR and MAS scoring. Also, a significant positive correlation with TLC (r=0.7463), and a moderate correlation with NLR (r=0.5266) suggest that elevated IMA levels parallel inflammation in CA. ***Conclusion:*** Ischemia-modified albumin (IMA) is an ideal parameter to identify complicated appendicitis

Keywords: Appendicitis, Ischemia Modified Albumin, Serum, Biomarkers, Neutrophil Lymphocyte Ratio

INTRODUCTION

Acute appendicitis is a common non-traumatic surgical emergency worldwide.[1] Nearly 25-30% of the total presentation is complicated appendicitis.[2] Distinguishing Complicated (CA) and uncomplicated appendicitis (UA) is crucial as it impacts clinical decision-making, surgical planning, and patient outcomes. Complicated appendicitis, such as gangrenous, perforated, and abscess, requires immediate surgical intervention, whereas some cases of uncomplicated appendicitis may be managed conservatively with antibiotics.

Conventional diagnostic approaches include clinical examination and investigations like Total Leukocyte Count (TLC), Differential Count (DC), Neutrophil-Lymphocyte Ratio (NLR), and C-reactive protein (CRP). However, these markers lack specificity as they may be elevated in other inflammatory conditions or may be normal in one-third of the acute appendicitis patients.[3],[4] Imaging modalities that aid in the diagnosis, such as ultrasonography (USG) and computed tomography (CT), have several limitations. USG is operator-dependent and may be challenging in obese patients or those with peritonitis who cannot tolerate compression. CT scans, though more reliable, are not always readily available, and involve radiation exposure. Sometimes, atypical presentations and unusual presentations are commonly seen in extremes of age.[5],[6],

Given these challenges, there is a growing need for a single, reliable biomarker that can accurately differentiate CA and UA without the limitations of conventional markers and imaging. Ischemia-modified albumin (IMA) is a promising biomarker in this regard, whose levels rise in hypoxia, oxidative stress, and tissue ischemia, which are hallmarks of CA due to the vascular compromise associated with gangrene and perforation.[7],[8]

Thus, this study aims to evaluate IMA levels in patients with CA and UA, establish a diagnostic cutoff value to differentiate both, and assess its sensitivity and specificity. Additionally, the study seeks to correlate IMA with conventional markers like TLC, NLR, and MAS to determine its clinical utility as a superior diagnostic tool.

MATERIAL AND METHODS

An observational cross-sectional study was conducted in a tertiary care hospital from June 2023 to December 2024. 85 adult male and female patients with a clinical diagnosis of acute appendicitis, managed both conservatively and surgically were included. Patients with a recent history of acute coronary syndrome, stroke, Known cases of Peripheral vascular diseases, chronic kidney disease, liver diseases, and Pregnancy were excluded. The sample size of 85 was determined based on an assumed standard deviation of 0.13 and an expected mean difference of 0.08 in IMA levels between the CA and UA groups. A total of 42 participants per group was required to achieve 80% power at a 5% level of significance.[9]

Demographic details, clinical history, and examination findings with Modified Alvarado scoring (MAS) were recorded. A 3 ml of venous blood was collected separately for IMA assay. Other baseline investigations like Hemoglobin, hematocrit, RBC count, TLC, DC, urea, and creatinine were done. Routine ultrasonography, or computed tomography findings were also recorded.

Based on the radiological and operative findings the participants were divided into two groups of Complicated Appendicitis (CA) and Uncomplicated Appendicitis (UA). Radiological findings showing peri-appendiceal fluid, fat stranding, and loss of vascularity or mass were also considered CA, and Operative findings like mass, abscess, gangrene, perforation, or peritonitis were considered CA. If the patient is managed conservatively as a part of CA, based on the USG findings/ CT findings, the patient is included in the appropriate group. The diagnosis of CA and UA was also confirmed using the histopathological report, which followed the surgery. Following this, the patient was managed appropriately and discharged. Progress of the patient, development of any complications, duration of the hospital stay were also recorded .

Ischemia-modified albumin levels in the serum were assayed by Enzyme-linked immunosorbent assay (Bioassay Technology Laboratory). This kit has a diagnostic range of 2ng/ml to 600ng/ml. Positive and negative controls were used as a part of quality control. A standard graph was created using serial dilutions of the standard.

Statistical analysis:

Categorical variables were represented in frequencies and percentages and continuous variables were mean ± Standard Deviation (SD) Student t-test was employed to compare the means of normally distributed parameters.

To establish a cutoff value for IMA, a Receiver Operating Characteristic (ROC) curve analysis, Area Under the Curve (AUC), and identifying the optimal cutoff using the Youden Index was done. Sensitivity, specificity, positive predictive value, and negative predictive value were determined based on this cutoff.

The correlation of IMA with total leucocyte count, neutrophil count, and the Modified Alvarado Score was analyzed using Pearson's or Spearman's correlation coefficients, depending on data distribution. Multivariable logistic regression will evaluate whether IMA can independently differentiate complicated from uncomplicated appendicitis when adjusted for other markers. Additionally, the diagnostic performance of IMA as a single marker was assessed by comparing its AUC with that of combined models. A p-value less than 0.05 was considered significant.

Ethical considerations:

The study was approved by the Institutional Ethics Committee. Reference number RC/2022/45. The study had minimal risk to the participants. The nature of the study was explained to the participants in their own language. Written informed consent was collected from all the participants.

RESULTS AND OBSERVATIONS:

A total of 85 patients with the definitive diagnosis of acute appendicitis were included in the study. They were divided into two groups namely Uncomplicated appendicitis (UA) with n=43 and Complicated appendicitis (CA) with n= 42 based on the operative and histopathological findings.

Table 1 shows the demographic details and the laboratory parameters in the groups. The CA group participants were older compared to UA group and age difference was statistically significant. (31.54 ± 12.73 vs 37.72 ± 12.89 years p = 0.0288). The majority of cases in both groups belonged to the 20-40 years age group. But, there was a noticeable increase

in the number of CA in the age range 40-60 years. Both groups had a higher number of males compared to females, but the difference was not statistically significant.

A statistically significant difference was observed in the distribution of comorbidities between the groups. 31% of CA patients had comorbidities, whereas only 5% of UA patients had comorbidities. Out of the 31%, the most prevalent comorbidities were Diabetes mellitus (16.67%), hypertension (9.52%), and hypothyroidism (4.76%)..The average duration of hospital stay, though higher in CA, was not statistically significant.

Random glucose levels were significantly higher in CA (127.35±58.41 mg/dL) than in UA (107.08±22.72 mg/dL) (p=0.035). The mean TLC was higher in the study population and significantly higher in the CA group (10.87±4.41 vs 14.00±4.16 ×1000/µL p value=0.031).

In differential count, the study population showed a slightly higher neutrophil count 71.35±12.23% similarly the CA group showed a significantly higher neutrophil percentage and low lymphocyte percentage compared to UA. Neutrophil percentage in UA is 67.26±13.27% and in CA is 75.45±9.61% with p-value 0.023. Whereas the lymphocyte count was higher 22.57±11.00 × 1000µL in UA compared to 15.33±7.85× 1000µL in CA with a p-value of 0.028. The CA group showed a higher platelet count (288.35±85.20× 1000µL) compared to UA group(253.14±73.02× 1000µL) (p=0.0074).

The study group showed a higher NLR ratio mean being 4.07±4.00 opposed to the reference range of 1.5-2.5 for adults. Similarly, NLR showed a marked increase in CA (6.37±4.67) compared to UA (1.76±0.39) which was statistically significant (p<0.0001).Modified Alvarado scores (MAS) were significantly higher in CA (p=0.0021).

Ischemia Modified Albumin (IMA) levels were notably elevated in CA (10.90±5.65 µg/mL) compared to UA (6.00±2.93 µg/mL) (p=0.0001).

Figure 1 shows the ROC analysis for the various parameters. IMA achieved an Area Under the curve (AUC) of 0.9045, reflecting outstanding discrimination between outcomes. NLR had an AUC of 0.9524, also suggesting excellent performance. TLC demonstrated acceptable discrimination with an AUC of 0.716. The MAS however, showed poor discriminatory power with an AUC of 0.6851.

Table 2 summarizes the diagnostic performance of three parameters—TLC, NLR, IMA, and MAS in differentiating CA and UA. NLR and IMA are excellent diagnostic markers for differentiating CA and UA, with both having high AUCs (0.952 and 0.9045) and significant p-values (<0.001). NLR demonstrates good sensitivity, while IMA exhibits a strong positive predictive capability.

TLC, although moderately useful, has a lower AUC of 0.716, indicating it is less reliable than NLR and IMA. The p-value of 0.05 is on the borderline, and the CI suggests that TLC's diagnostic accuracy is more uncertain. The Modified Alvarado scoring with a cutoff of 7 yielded an AUC of 0.6851 (95% CI: 0.783–0.905), PLR of 4.33, NLR of 0.24, and a p-value <0.001, indicating moderate diagnostic value.

Table 3 shows the sensitivity and specificity of various inflammatory markers used in our study. TLC shows good sensitivity (88.89%) and moderate specificity (74.42%), making it a fairly balanced diagnostic parameter. NLR demonstrates very high sensitivity (97.62%) but extremely low specificity (16.28%), leading to many false positives and limiting its usefulness as a sole diagnostic tool. The sensitivity and specificity of IMA at the cut-off value of 8.50 were calculated to assess its diagnostic accuracy in differentiating complicated from uncomplicated appendicitis. The sensitivity was found to be 82.35%, indicating that IMA correctly identified 82.35% of the complicated appendicitis cases. The specificity was 95.56%, reflecting its ability to correctly classify 95.56% of the uncomplicated appendicitis cases. The MAS demonstrated a sensitivity of 79.1% and specificity of 72.1% at a cut-off of 7, suggesting its moderate diagnostic accuracy.

Table 4 shows the correlation analysis between IMA and other revealed a strong positive correlation between IMA levels and TLC (r=0.7463, p=0.032), indicating that higher IMA levels are significantly associated with increased TLC. This suggests that IMA and TLC may jointly reflect disease severity in complicated appendicitis. A moderate positive correlation was seen between IMA and NLR (r=0.5266, p=0.044p), which is statistically significant. This indicates that as IMA levels increase, NLR also tends to rise, further supporting the role of inflammatory markers in distinguishing complicated appendicitis. A weak positive correlation (r=0.311) was noted between IMA levels and the MAS. However,

this relationship was not statistically significant (p=0.821), suggesting that IMA levels do not directly correlate with this scoring system in predicting disease severity.

Table 5 indicates the logistic regression analysis for the three parameters for predicting CA. The odds ratios for all three parameters are greater than 1, indicating that higher values of these parameters are associated with an increased risk of complicated appendicitis. Overall, NLR and TLC demonstrated the strongest predictability, IMA showed significance with a smaller effect size, and MAS exhibited the weakest predictive utility

Table 1: Baseline Characteristics of the Total Participants and Study Groups

PARAMETER MEAN± SD	**TOTAL n=85**	**UA n=43**	**CA n=42**	**p-value**
Age in years	34.45±13.07	31.54±12.73	37.72±12.89	0.0288
Gender Males Females	 51 34	 26 17	 25 17	0.9294
Associated comorbidities	15	2	13	0.0003
Duration of hospital stay (days)	6.54±3.82	5.69±2.59	7.40±4.62	0.071
Random glucose (mg/dL)	119.06±44.96	107.08±22.72	127.35±58.41	0.035
Haemoglobin (mg/dL)	12.80±2.02	12.20±2.21	12.48±1.64	0.646
TLC (× 1000µL)	12.44±4.41	10.87±4.41	14.00±4.16	0.031
Differential count Neutrophils(%) Lymphocytes(%) Monocytes(%)	 71.35±12.23 18.95±10.17 6.83±2.98	 67.26±13.27 22.57±11.00 6.42±2.48	 75.45±9.61 15.33±7.85 7.23±3.35	 0.023 0.028 0.437
Platelet count(× 1000µL)	270.78±80.83	253.14±73.02	288.35±85.20	0.0074
NLR	4.07±4.00	1.76±0.39	6.37±4.67	<0.0001
PLR	24.57±10.59	26.38±11.04	22.77±10.05	0.1190
MAS	7.01±1.12	6.64±1.12	7.38±1.03	0.0021
IMA (µg/mL)	7.09±5.76	6.00±2.93	10.90±5.65	0.0001

P<0.05 significant, <0.01 highly significant. SD : Standard deviation, UA: Uncomplicated appendiciti, CA : Complicated appendicitis, TLC : Total leucocyte count, NLR : Neurophil Lymphocyte ratio, PLR : Platelet lymphocyte ratio, MAS: Modified alvarado score, IMA: Ischemia Modified albumin.

Table 2: Diagnostic Performance of Various Biomarkers in Differentiating Complicated and Uncomplicated Appendicitis

PARAMETER	CUT OFF	PLR	NLR	AUC	95%CI	P VALUE
TLC	12.66	3.42	0.15	0.716	0.5002,0.9318	0.05
NLR	2.59	0.3136	1.742	0.952	0.9068,0.9972	<0.001
IMA	8.50	5.2	0.18	0.904	0.8871,0.9219	<0.001
MAS	7	4.33	0.24	0.685	0.783, 0.905	<0.001

P<0.05 significant, <0.01 highly significant. TLC : Total leucocyte count, NLR : Neurophil Lymphocyte ratio, PLR : Platelet lymphocyte ratio, MAS: Modified alvarado score, IMA: Ischemia Modified albumin, AUC : Area under the curve, CI : Confidence interval.

Table 3: Sensitivity and Specificity of Diagnostic Parameters

PARAMETER	CUT OFF	SENSITIVITY %	SPECIFICITY %
TLC	12.66	88.89	74.42
NLR	2.59	97.62	76.28
IMA	8.50	82.35	95.56
MAS	7	79.1%	72.1%.

TLC : Total leucocyte count, NLR : Neurophil Lymphocyte ratio, MAS: Modified alvarado score, IMA: Ischemia Modified albumin.

Table 4: Correlation analysis of the IMA with other parameters

PARAMETER	R	P VALUE
TLC	0.7463	0.032
NLR	0.5266	0.044
MAS	0.311	0.821

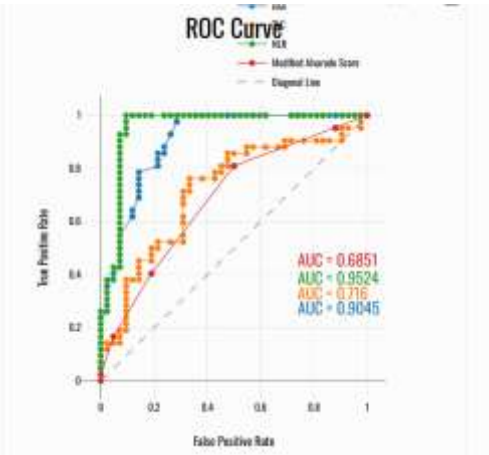
P<0.05 significant, TLC : Total leucocyte count, NLR : Neurophil Lymphocyte ratio, MAS: Modified alvarado score, IMA: Ischemia Modified albumin.

Table 5: Logistic regression analysis for predicting complicated appendicitis

PARAMETER	COEFFICIENT	STANDARD ERROR	Z VALUE	P VALUE	ODDS RATIO	95%CI
IMA	0.0015	0.0002	7.31	<0.001	1.0015	1.0011, 1.0019
TLC	0.23	0.04	5.75	<0.001	1.26	1.16, 1.37
NLR	0.43	0.06	7.31	<0.001	1.54	1.34, 1.76
MAS	0.10	0.042	4.59	<0.001	0.98	0.77, 1.23

P<0.05 significant, TLC : Total leucocyte count, NLR : Neurophil Lymphocyte ratio, MAS: Modified alvarado score, IMA: Ischemia Modified albumin.

Figure 1: Receiver Operating Characteristic (ROC) Curve Analysis of Biomarkers for Differentiating Complicated Appendicitis



DISCUSSION

Acute appendicitis is one of the most common surgical emergencies seen in adults and children.[1] The study aimed to assess the serum levels of IMA and its utility in differentiating CA and UA, alongside analyzing other clinical, demographic, and biochemical parameters. Our findings suggest that IMA is a valuable diagnostic marker with high sensitivity and specificity.

Our study found significantly elevated serum IMA levels among all participants. The normal reference range of IMA in healthy controls is 3-6 µg/mL, for which our study group showed a higher mean than the reference range, which is expected.[10] Multiple studies have found that patients with appendicitis had substantially higher levels of IMA compared to healthy controls.[10],[11],[12] In the study conducted by Dumlu et al in Turkey, the serum IMA levels in patients who underwent appendectomy were markedly higher compared to the control group.[10] In a similar study done by Nazik et al in the same region also had a similar result in patients with acute appendicitis showing high IMA compared to controls. [12]Also, the study done by Sarac et al found a high level of IMA in patients diagnosed with acute appendicitis compared to those of non-specific abdominal pain.[11] These findings are consistent with the underlying pathophysiology of acute appendicitis, where ischemia and oxidative stress contribute to tissue damage and IMA formation.

Our study demonstrated significantly higher serum IMA levels in the CA group compared to the UA group. Such findings were also observed in a few studies around the world.[13],[14] In a study done by Kilic in Turkey serum IMA levels were significantly higher in patients with complicated acute appendicitis compared to those with non-complicated cases.[9] Study done by Hakkoymaz et al found serum IMA levels were significantly elevated in patients with CA compared to UA.[14] But in the above-mentioned study done by Dumulu et al there was no notable difference in values between the complicated cases (which included perforated and phlegmonous appendicitis) and the non-complicated group.[10]

Males exhibited slightly higher IMA values compared to females in both UA and CA groups; the differences were not statistically significant. These findings suggest that gender does not have a significant impact on IMA levels. The lack of significance aligns with previous studies indicating that oxidative stress and tissue ischemia, which drive IMA elevation, are more influenced by disease severity rather than gender differences.[10]

CA patients with comorbidities had higher mean IMA values compared to those without comorbidities, though this difference was not statistically significant. Comorbid conditions such as diabetes and hypertension are known to exacerbate oxidative stress and systemic inflammation, which may contribute to higher IMA levels.[14] However, the absence of statistical significance may be due to the small sample size or variability in comorbidity severity among participants.

While analyzing the utility of IMA with other markers we found that IMA had an AUC of 0.904 with 82.35% sensitivity and 95.56% specificity. In a study done by Nazik et al in children, they found that IMA had a sensitivity of 96.7% and specificity of 99.7%.[12] They compared various parameters like ESR, CRP, WBC, NLR, and PLR out of which IMA had the highest sensitivity and specificity. A comparable study by Hakkoymaz on pediatric acute appendicitis reported AUC values of 0.86 for IMA, 0.83 for TLC, and 0.90 for NLR, closely aligning with the findings of our study.[14] In a study done with another marker, namely immature granulocyte count, showed low sensitivity and specificity for diagnosing complicated

appendicitis.[16]This study highlights the importance of IMA as a marker for oxidative ischemic injury .

TLC showed an AUC of 0.716 with a sensitivity of 88.89% and specificity of 74.42%. Although TLC has been traditionally used as an inflammatory marker, our findings indicate its limited reliability compared to IMA and NLR. This may be due to the non-specific nature of leukocytosis in various inflammatory conditions, leading to overlap between uncomplicated and complicated cases.

The NLR showed a high AUC of 0.952 with a sensitivity of 97.62% and a specificity of 76.28%. This indicates NLR's strong sensitivity, making it a reliable early screening tool for potential complications. However, its lower specificity compared to IMA suggests that while it is excellent at detecting potential cases, it may lead to more false positives. This aligns with previous studies where NLR was highlighted for its role in identifying inflammation severity but lacked precision in differentiating between various clinical conditions.[15] A meta-analysis by Hajibandeh et al. reported that an NLR cut-off value of 8.8 predicted complicated appendicitis with 88.89% sensitivity, 90.91% specificity, and an AUC of 0.96I.[17]

MAS showed the weakest diagnostic accuracy, reflected by its lowest values for AUC, sensitivity, and specificity.This suggests that MAS has limited clinical utility in the differentiation of appendicitis complications. It is possible that MAS is less sensitive to the specific biochemical changes associated with complications or is influenced by a broader range of conditions.

A significant positive correlation between IMA and TLC, suggests that elevated IMA levels parallel leukocytosis in the inflammatory response of CA. Similarly, IMA exhibited a moderate correlation with NLR, further supporting its role in identifying inflammation severity.

These findings suggest that IMA is a robust marker for distinguishing between complicated and uncomplicated appendicitis, offering high specificity with good sensitivity. Levels Its utility may be attributed to its pathophysiological role in oxidative stress and ischemia, which are prevalent in complicated appendicitis.

One limitations of the study are the small sample size which restricts the ability to generalise the findings to a larger population, and being a single-center study there might be biases with respect to the demographics and medical practices. Also, this being a cross-sectional study, IMA values after the management of the patients, including post-surgical or post-treatment levels, were not assessed in this study. The study only evaluated a limited number of biomarkers namely TLC, and NLR in comparison to IMA. The inclusion of other biomarkers like CRP and interleukins could have given more insights into IMA. The study used a specific IMA assay (ELISA), which may have limitations in terms of sensitivity and specificity.

CONCLUSION

Ischemia-modified albumin (IMA) is a valuable biomarker for differentiating complicated and uncomplicated appendicitis. Large-scale multicenter validation study should be conducted to validate the findings and establish the utility of IMA as a diagnostic biomarker for complicated appendicitis

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