

# Using of calprotectin and lactoferrin levels in early detection of acute uncomplicated appendicitis in children

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**Abstract:** This study was conducted in Kirkuk city for the period from December, 2019 to June 2020, and included 50 children with acute appendicitis and 50 children without any other disease, where the ages of children in both groups ranged from 3-12 years. The study included collecting information from children in both groups, including gender, age, living situation, number of family members, standard of living and social as well as the number of family members, number of rooms in the house. Amount of 5 ml of blood samples were collected from all the children included in this study, where the blood samples were separated by the centrifuge device and the serum samples were separated from them and kept in the refrigerator until the tests for determination of calprotectin and lactoferrin by ELISA and CRP by immunofluorescence. In this study, there were no significant difference between the 2 groups regarding age, sex and residence. The study found that, 95% of children with appendicitis was suffered from abdominal pain, 50% with fever and weight loss, 48% with nausea and 40% with vomiting. The study revealed a significant elevated level of calprotectin in children with appendicitis as compared with healthy children ( $18.54 \pm 4.25$  vs  $11.37 \pm 3.28$  ng/ml) ( $P < 0.001$ ). The study also exposed a significant higher level of lactoferrin in children with appendicitis as compared with healthy children ( $10.51 \pm 1.52$  vs  $2.18 \pm 0.19$  ng/ml) ( $P < 0.001$ ). The study showed that the maximum means of calprotectin and lactoferrin in children with appendicitis were observed in those with abdominal pain, followed by vomiting. The study revealed a significant positive correlation between calprotectin and lactoferrin level among children with acute appendicitis. The mean serum level of CRP was significantly elevated in children with acute appendicitis compared to control group ( $27.57 \pm 4.22$  and  $5.57 \pm 3.9$  mg/dl) respectively at a  $P$  value  $< 0.001$ . **Conclusions:** The study showed a significant relation of calprotectin and lactoferrin with appendicitis

**Keywords:** Calprotectin; lactoferrin; CRP; Appendicitis; Children

## 1. Introduction

Despite rapid increases in computed tomography (CT) utilization, pediatric appendicitis remains a challenging diagnosis, with 5% to 25% negative appendectomy and 10% to 45% perforation rates. Novel biologic markers for appendicitis represent a potential method to improve diagnostic accuracy<sup>(1)</sup>. Through the use of advanced molecular

techniques, several recent publications have identified proteins that are differentially expressed in the diseased appendix<sup>(2)</sup>. Diagnosis of acute appendicitis (AA) remains a surgical dilemma, with negative appendectomy rates of 5% to 40% and perforation suggestive for late operative intervention in 5% to 30%<sup>(3)</sup>. Calprotectin (CP) is a calcium- and zinc-binding protein of the S100/calgranulin family<sup>(4)</sup>. It is also referred to in the literature as S100A8/A9, MRP8/14 (myeloid-related protein), calgranulin A/B, L1 protein, 27E10 antigen, cystic fibrosis antigen, myeloid-histiocyte antigen, and CP-10 (the last refers to the light chain only)<sup>(5)</sup>. CP is mainly exhibited in the cytoplasm of neutrophils (about 5% of their total protein contents and 30–60% of their cytosolic protein<sup>(6)</sup>). It is not only expressed on activated monocytes and macrophages (about 1% of all monocyte cytosol protein) but can also be produced by bone marrow cells, squamous epithelium (keratinizing and nonkeratinizing), some mucosal epithelial cells, microvascular endothelial cells, fibroblasts, generally as a result of activation<sup>(1)</sup>. The two S-100 proteins are found within the cytoplasm of neutrophils and are released by neutrophils that are degranulating. Calprotectin is thought to have antimicrobial activity, likely through zinc chelation<sup>(7)</sup>. LRG is a protein secreted by liver cells and by neutrophils undergoing differentiation. Although its exact function is not known, it is up-regulated in patients with acute inflammatory and bacterial conditions. Investigators recently described favorable test performance characteristics for serum calprotectin for diagnosing appendicitis in adult patients<sup>(5)</sup>. Other investigators identified LRG as being selectively enriched in the urine of pediatric patients with appendicitis. This study was conducted to determine calprotectin and lactoferrin levels in children with acute uncomplicated appendicitis.

## 2. Materials and methods

This study was conducted in Kirkuk city for the period from December, 2019 to June 2020, and included 50 children with acute appendicitis and 50 children without any other disease, where the ages of children in both groups ranged from 3-12 years. The study included collecting information from children in both groups, including gender, age, living situation, number of family members, standard of living and social as well as the number of family members, number of rooms in the house. Amount of 5 ml of blood samples were collected from all the children included in this study, where the blood samples were separated by the centrifuge device and the serum samples were separated from them and kept in the refrigerator until the tests for determination of calprotectin and lactoferrin by ELISA and CRP by immunofluorescence.

## 3. Results

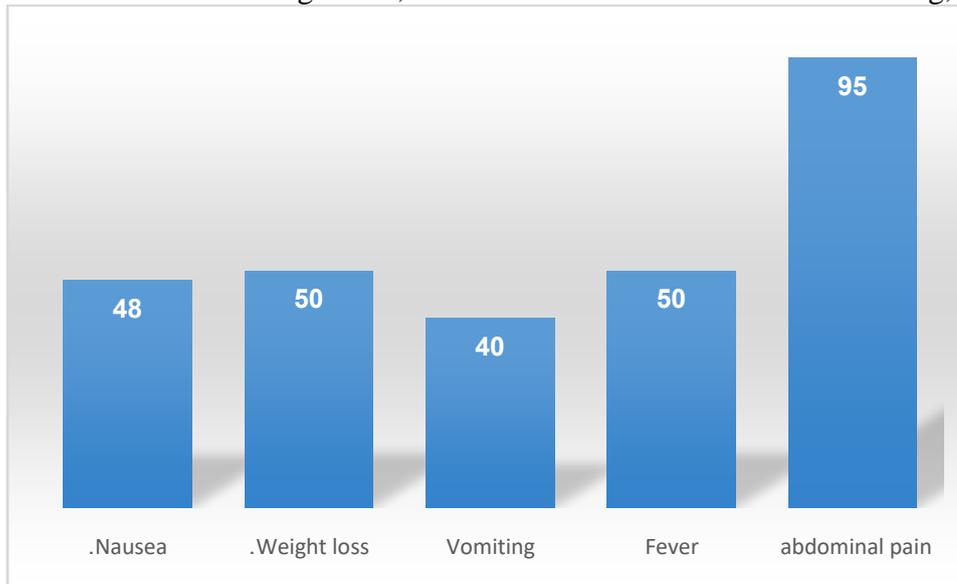
In this study, there were no significant difference between the 2 groups regarding age, sex and residence. (Table 1).

**Table 1: general characteristics of studied children**

Variables	Cases (n:50)		Control (n:50)		P. value
	N	%	No.	%	
<b>Age</b>					
<b>3-4 year</b>	5	10	5	10	<b>&gt;0.05</b>
<b>5-6 year</b>	7	14	7	14	
<b>7-8 year</b>	1	2	13	26	
<b>9-10 year</b>	1	2	12	24	

	4				
<b>11-12 year</b>	2	1	24	13	26
<b>Residence (urban)</b>	2	3	64	33	66
<b>Sex (male)</b>	4	2	48	25	50

The study found that, 95% of children with appendicitis was suffered from abdominal pain , 50% with fever and weight loss, 48% with nausea and 40% with vomiting, Figure 1.



**Figure 1: Clinical features of children with appendicitis**

The study revealed a significant elevated level of calprotectinin children with appendicitis as compared with healthy children(18.54±4.25 vs 11.37±3.28 ng/ml) (P<0.001), Table 2.

**Table 2: Level of calprotectinin the studied children**

Parameter (ng/ml)	N	Mean	SD
<b>calprotectin (cases)</b>	<b>50</b>	<b>18.54</b>	<b>4.25</b>
<b>calprotectin (control)</b>	<b>50</b>	<b>11.37</b>	<b>3.28</b>

**P<0.001**

The study also exposed a significant higher level of lactoferrinin children with appendicitis as compared with healthy children(10.51±1.52 vs2.18±0.19 ng/ml) (P<0.001), Table 3.

**Table 3: Level of lactoferrinin the studied children**

Sample	N	Mean	SD
<b>Lactoferrincases</b>	<b>50</b>	<b>10.51</b>	<b>1.53</b>

<b>Calprotectincontrol</b>	<b>50</b>	<b>2.18</b>	<b>0.19</b>
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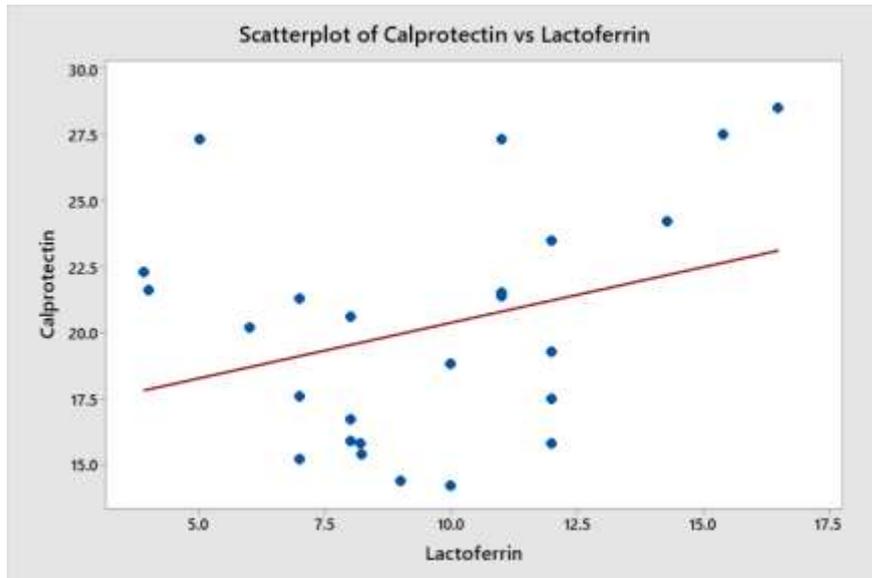
**P<0.001**

The study showed that the maximum means of calprotectin and lactoferrin in children with appendicitis were observed in those with abdominal pain, followed by vomiting, Table 4.

**Table 4: relation calprotectin and lactoferrin with symptoms of diarrhea**

Parameter	Nausea.	Weight loss.	Vomiting	Fever	abdominal pain	P. value
calprotectin	18.22	14.6	19.91	18.11	20.16	0.043
Lactoferrin	10.16	9.16	12.13	11.81	14.28	0.09

The study revealed a significant positive correlation between calprotectin and lactoferrin level among children with acute appendicitis, Figure 2.



**Figure 2: Correlation between calprotectin and lactoferrin level among children with acute appendicitis**

As shown in Table 5, the mean serum level of CRP was significantly elevated in children with acute appendicitis compared to control group ( $27.57 \pm 4.22$  and  $5.57 \pm 3.9$  mg/dl) respectively at a P value < 0.001.

**Table 5: The mean and standard deviation (SD) of CRP level in studied groups**

CRP level (mg/dl)	children with acute appendicitis	Control group
No.	50	50

<b>Mean</b>	27.57	5.57
<b>SD.</b>	4.22	3.9

**P<0.001**

#### 4. Discussion

The study found that, 95% of children with appendicitis was suffered from abdominal pain , 50% with fever and weight loss, 48% with nausea and 40% with vomiting. The study revealed a significant elevated level of calprotectin in children with appendicitis as compared with healthy children(18.54±4.25 vs 11.37±3.28 ng/ml) (P<0.001). The study also exposed a significant higher level of lactoferrin in children with appendicitis as compared with healthy children(10.51±1.52 vs 2.18±0.19 ng/ml) (P<0.001).There is currently no evidence for suggesting, that serum Cal is superior to standard inflammatory markers for the exclusion or confirmation of suspected AA (8,9). However, uncomplicated and complicated AA is considered to be two entities. The curability of uncomplicated disease without surgery is proven in studies comparing to antibiotic therapy, whereas complicated AA with necrosis or perforation of the appendix cannot be treated successfully without invasive modality. The preferred surgical approach in complicated AA is more unclear, therefore of lack of evidence in this group (10). In this study has supported, that plasma CRP level increase was nonspecific, as in literature. Another fact that, we wanted to emphasize the identification of Cal was a useful inflammatory parameter. In distinction of complicated and uncomplicated AA, Cal was statistically more effective than the other inflammatory parameters according to imaging and laboratory methods (10). When inflammatory process progresses and peritonitis findings have occurred, Cal level has also increased. Due to thickening of appendix wall, inflammation around fat plans or the liquid amount around the appendix, plasma Cal level has increased, and, therefore, Cal levels were determined USG (+) patients higher than USG (-) patients (7,8). The comparison of these biomarkers showed a significant difference between patients with acute appendicitis and healthy controls. In consistence with our study, another study on new biomarkers for acute appendicitis showed a significant difference of lactoferrin between acute appendicitis and healthy controls (11). In another study, the plasma level of procalcitonin in patients with confirmed acute appendicitis was reported to be more than the control group (0.5 ng/ml serum level for patients) (12). In this regard, a lot of clinical studies have been carried out in order to identify gold markers to reach a better diagnosis. The most accurate clinical biomarkers presented for diagnosis of this disease are CRP and WBC. However, they do not have the ability to discriminate all true patients among suspected subjects (13, 14). Several studies have shown that serum level of procalcitonin (PCT) and lactoferrin (LF) increase during bacterial infections (15,16). PCT, a precursor of calcitonin, is secreted by C cells of thyroid gland under normal conditions, and it has been demonstrated to be one of the most important laboratory markers for the presence of fungal and bacterial diseases. Unlike other clinical biomarkers such as CRP, the level of procalcitonin does not change in patients with non-bacterial and viral infections, which makes it an appropriate biomarker for AA (10, 11). A lot of studies have assessed the value of PCT for diagnosing and determining the severity of acute appendicitis, but the results have been contradictory and the diagnostic value of procalcitonin in appendicitis is still unclear (17,18).

#### 5. Conclusions

The study showed a significant relation of calprotectin and lactoferrin withappendicitis

## 6. References

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