# *Evaluation of Serum Interleukin-6 Levels in Correlation to the Severity of Acute Appendicitis*

***Dr. Imad Wajeh Al-Shahwany,** *CABS,* ****Dr. Zuhair Bashir Kamal,** *CABS,*
***** Dr. Laith Naief Hindoosh,** *CABS*

## Abstract

**Background**: Acute appendicitis is a common surgical problem that is associated with an acute-phase reaction. Previous studies have shown that cytokines and acute-phase proteins are activated and may serve as indicators for appendicitis.

**Objective:** The aim of this study was to evaluate of serum levels of interleukin-6 (IL-6) in correlation to the severity to acute appendicitis.

**Methods**: This is a prospective study from December 2008 to March 2009 of patients who had appendectomy in Department of Surgery, Al-Kindy Teaching Hospital - Baghdad/Iraq. Serum for estimating levels of interleukin-6 (IL-6) was taken pre-operatively. Depending on the macroscopic evidence during the operation and the histopathological examination of the specimens, the patients were separated into 3 groups, group one with negative appendectomy (normal appendix), group two with non-complicated acute appendicitis (catarrhal, suppurative), and group three with complicated acute appendicitis (perforated, gangrenous). The histopathological results were correlated with IL-6 values statistically.

**Results:** A total number of 50 patients were included in this study, 28 male (56%) and 22 female (44%). The mean age was 23 year (ranged from 7 to 50). On histological examination, there were 7 patients (14%) in group one, 29 patients (58%) in group two, and 14 patients (28%) in group three. Serum IL-6 values gave true negative results in 6%, true positive results in 82%, false negative results in 4%, and false positive results in 8%. We determined, therefore, in the present study the sensitivity, specificity, and accuracy of serum IL-6 were calculated as 95.34%, 42.85%, and 88% respectively.

**Conclusion:** Laboratory results should be considered to be integrated within the clinical assessment. If used critically, IL-6 can provide surgeons with complementary information in discerning the necessity for urgent operation.

**Key Words:** Interleukin-6 (IL-6), - mediators of inflammation, acute appendicitis

***Al*- Kindy Col Med J 2009; Vol .5 (1):p 63-68**

## Introduction

Appendicitis remains one of the most common acute surgical diseases.[1,2] The average rate of normal appendectomy is 16 percent, with female comprising 68 percent of those patients found to have inflamed appendix at exploration.[1] Diagnosis of acute appendicitis in young children and elderly is more difficult than in adults and also perforation rate is higher.[1] In elderly the causes of abdominal emergency are greater and differential diagnosis is more problematic.[3] Acute appendicitis is a clinical diagnosis and no laboratory or radiological tests are 100% accurate.[4] The methods for diagnosing acute appendicitis have significantly not changed over the past few decades. Clinical examination and laboratory parameters, such as white blood cell, differential counts (percentage of neutrophil granulocytes), and C-reactive protein were the only diagnostic tools for many years. Perforation rate was high, as well as the number of negative appendectomies. Mild leukocytosis, ranging from 10,000 to 18,000 is usually present in patients with acute, uncomplicated appendicitis and is often accompanied by a moderate polymorphonuclear predominance.[1,2,3,4,5] Sensitivity of leukocytosis is from 52% to 96% and sensitivity of shift to left is from 39% to 96%[6]. Following the introduction of ultrasonography in the last two decades and computed tomography (CT) in the last decade, the rate of negative appendectomies and perforations has decreased.

Appendicitis is a common surgical problem associated with a systemic inflammatory response. Several studies have focused on the diagnostic value of the Interleukin-6 (IL-6) concentration in suspected appendicitis. Comparing with patients of a normal control group, cytokine levels of IL-1β, IL-2, IL-6, IL-8, and IL-10 were elevated in patients with acute appendicitis [7]. IL-6 can be detected in patients with suspected acute appendicitis, and the highest concentratio ns are found in patients with perforation.[8, 9]

Interleukin-6 is a mediator of the inflammatory response and an early marker of tissue damage. It plays a role in the induction of an acute-phase response, and it has anti inflammatory activity. The acute phase response is illness - fever, malaise, anorexia, leukocytosis, negative nitrogen balance - which forms a cardinal response of the body to infection and trauma, and may be the result of many immunological reactions and inflammatory processes.[10, 11, 12]

The cytokines IL-1, IL-6, and IL-11; tumor necrosis factor-a (TNF-α); and transforming growth factor-, (TGF-3) all have a role in stimulating transcription of the genes controlling hepatic acute phase production.[13,14,15,16,17,18] The generation of these cytokines by macrophages is an extremely early event in the response to infection or trauma - for example

one of the strongest stimuli to macrophage production is bacterial lipopolysaccharide.

The concentrations of IL-6 in blood increase after surgery in correlation with the degree of surgical stress. It has been reported that post operative serum levels of IL-6 are higher for open colectomy and cholecystectomy than for laparoscopic surgery.[19]

Improving the diagnosis of acute appendicitis in order to prevent unnecessary surgery is crucial. To reduce the incidence of normal appendectomies, many studies have been published on quantitative analysis of interleukin-6 (IL-6). [19]

## Methods

This is a prospective study of 50 appendectomised patients in Department of Surgery, Al-Kindy Teaching Hospital- Baghdad/Iraq, from December 2008 to March 2009. Clinical signs of acute appendicitis were determined by the surgeon and the duration of symptoms were documented on admission. All the patients were operated on for a clinical suspicion of acute appendicitis.

Whole blood sample for analysis of IL-6 was centrifuged; the serum was separated and stored frozen at -20 $^{o}$C for later IL-6 measurements. The serum IL-6 concentration was measured by IL-6 enzyme linked immunosorbent assay (ELSA). An IL-6 concentration of 12.5pg/ml. was taken as the reference.

The final diagnosis of the specimens was established by histopathological examination. Removed appendices were fixed in formalin and analyzed histopathologically. The patients were grouped, according to the macroscopic evidence during operation and the histopathology reports, into three groups; group one with negative appendectomy (normal appendix), group two with non-complicated acute appendicitis (catarrhal, suppurative), and group three with complicated acute appendicitis (perforated, gangrenous).

**Statistical analysis:**

We compared serum IL-6 concentrations with the histopathological results to detect the sensitivity, specificity, and accuracy of IL-6 in diagnosis of acute appendicitis. The calculation was done as follows:

Sensitivity = TP/TP + FN

Specificity = TN/TN+FP

Accuracy = TP+TN/TP+TN+FP+FN

(FP=false positive, TP=true positive, TN=true negative, FN=false negative).

Statistical analysis of IL-6 values for P-value and Odds ratio were estimated in relation to histopathology results.

## Results

Fifty patients with the preliminary diagnosis of acute appendicitis were recruited in this study within the period of December 2008 to March 2009. The mean age was 23 year (ranged from 7 to 50). **(Table-1)** shows the distribution of the age groups of the patients.

There were 28 male (56%) and 22 female (44%) with a male to female ratio 1.27:1, this ratio changed from 0.4:1 in group one to 1.2:1 in group two to 1.8:1 in group three.

Depending on the macroscopic evidence of the appendix during the operation and later on the histopathological examination of the specimens, the patients were separated into three groups: Group one of patients who did not have acute appendicitis as the cause for acute abdomen (normal appendix) 7 patients (14%), group two who had non-complicated acute appendicitis (catarrhal, suppurative) 29 patients (58%), and group three with complicated appendicitis (perforated, gangrenous) 14 patients (28%).

**(Table-2)** shows the patients characteristics in relation to histopathological findings.

IL-6 levels, when compared to histopathology, gave true positive (TP) results in 41 patients (82%), true negative results (TN) in 3 patients (6%), false positive results (FP) in 4 patients (8%), and false negative results (FN) in 2 patients (4%). **(Table -3)** illustrates TP, TN, FP, and FN results of IL-6.

Statistically the sensitivity of IL-6 values was calculated as 95.34%, specificity as 42.85%, and the accuracy as 88%. **Fig. 1**

The median IL-6 levels (pg/ml) assay in group one with normal appendix was 24.7376 pg/ml., in group two with non-complicated acute appendicitis was 53.7294 pg/ml, and in group three with complicated acute appendicitis was 84.2622 pg/ml., all the above results were statistically significant (P-value<0.001). (**Table-4)** shows the statistical analysis (P-values and odds ratio) of median IL-6 (pg/ml) in relation to histopathology. **Fig.2** demonstrates the median IL-6 levels according to histopathology.

The accuracy of IL-6 in relation to histopathology is illustrated in **Fig.3**.

## Discussion

The mean age of our patients in this study was 23 year, and the main age group was 20-29 years (44%), this is comparable to median age and age groups in other studies, Fernando A.et al. [20], Alshahwany I. [21], Goodwin AT. *et al.* [22], and Khan MN.*et al*.[23]

The over all male to female ratio in our results was 1.27:1, though this ratio changed to 0.4:1 in the group one with normal appendix. This can be explained by the fact that about one third of females operated upon for acute appendicitis turned to have gynaecological disorders with a normal appendix,[21] this increases the females numbers having normal appendices relative to males and therefore changing the ratio.

Seven patients (14%) of our patients had normal appendices macroscopically on exploration and later on proved by histopathology; this rate of negative

appendectomy is an acceptable result as the rate of normal appendices removed is still about 15-30%. [24] We believe that the origin of pain in these cases could be related to gynaecological disorders, mesenteric lymphadenitis, meckel's diverticulitis, regional ileitis, peritonitis of different causes, urinary tract infection, ureteric colic, gastroenteritis or other medical causes.

The diagnostic accuracy of IL-6 in adult population is controversial. In our study it was 88% but there are several studies in adults were unable to confirm the usefulness of IL-6 for diagnosing acute appendicitis, [25, 26] while others, including studies in children, found it a useful marker.[27, 28, 29]The differences in the studies could be attributed to different study populations and designs.

In our study, serum IL-6 concentrations gave 50% diagnostic accuracy results in group one of patients with histopathologically confirmed normal appendices. The median IL-6 level in this group was 24.7376 pg/ml., which is considered double the control laboratory value (12.5 pg./ml), this true negative result can be explained by the probability that these patients had inflammatory process else where in the body which stimulates the production of IL-6, such as gastroenteritis, urinary tract infection, mesenteric lymphadenitis, or any other hidden inflammatory process, thus, a mild elevated serum IL-6 concentration is of poor specificity to the early process of acute appendicitis and serum IL-6 concentration is not useful for preventing negative laparotomies in the majority of patients with right iliac fossa pain.

In group two, the median IL-6 concentration was more than four times (53.7294pg/ml) the laboratory control value and it correlate well (accuracy rate was 94.44%) with the histopathology results of non-complicated acute appendicitis.

In group three, with complicated appendicitis, the median IL-6 concentration was more than six times (84.2622pg.ml) the laboratory control value and it gave 100% accuracy rate and correlated very well with complicated appendicitis, thus high levels of serum IL-6 concentrations are very useful to discriminate between uncomplicated and complicated appendicitis.

Gurleyik G.*et al* [25] and Paajanen H.*et al*[27] found that preoperative high increased IL-6 concentrations were clearly correlated with perforation and poor postoperative conditions in adults. Our data agrees with the results of these studies.

## Conclusion

The data in this study would support the conclusion that elevated levels of inflammatory markers IL-6 are supportive of the diagnosis of appendicitis during the assessment of patients felt clinically to require laparotomy for acute appendicitis.

Serum IL-6 level is a good laboratory marker of acute appendicitis with particular reference to the discrimination between uncomplicated and complicated appendicitis.

## References

**1.** Kozar RA, Roslyn JJ. The Appendix. In: Schwartz SI, Shires GT, Spencer FC. Principles of Surgery. McGraw Hill, 7th ed. 1999; 1383-93.

**2.** Lally KP, Cox CS, Andrassy RJ. Appendix. In: Beauchamp RD, Evers BM, Mattox KL: Sabiston Text-book of Surgery. Saunders, 16th ed. 2001; 16: 917-27.

**3.** Ellis H, Nathunson LK. Appendix and Appendectomy. In: Zinner MJ, Schwartz SI, Ellis H. Maingot's Abdominal Operation. Appleton & Lange, 10th ed.1997; 1191-225.

**4.** Field S, Marrison L. Acute Abdomen. In: Sutton D. Textbook of Radiology and Imaging. Churchill Livingston, 7th ed. 2003; 685.

**5.** Ho HS, Appendectomy. In: Wilmore DW, Cheung LY, Harden AL: ACS Surgery, Principle & Practice. Web MD, 2002: 815-23.

**6.** Anderson KD, Parry RL, O'Neill JA, Rowc MI, Grosfeild JL: Appendicitis. Pediatric Surgery. Mosby, 5th ed. 1998; 1369-77.

**7.** Yoon DY, Chu J, Chandler C, Hiyama S, Thompson JE, Hines OJ. Human cytokine levels in nonperforated versus perforated appendicitis: molecular serum markers for extent of disease. *Am Surg* 2002**,** 68:1033-37.

**8.** Eriksson S, Granstrom L, Olander B, Wretlind B: Sensitivity of interleukin- 6 and C-reactive protein concentrations in the diagnosis of acute appendicitis. *Eur J Surg* 1995, 161:41-45.

**9.** Erkasap S, Ates E, Ustuner Z, Sahin A, Yilmaz S, Yasar B, Kiper H: Diagnostic value of interleukin-6 and C-reactive protein in acute appendicitis. *Swiss Surg* 2000, 6:169-72.

**10.** Kushner I. The phenomenon of the acute phase response. Ann N Y Acad Sci 1982; 389: 39-48.

**11.** Gordon AH, Koj A. The acute phase response to injury and infection. Research Monographs in Cell and Tissue Physiology. Vol 10. Amsterdam: Elsevier, 1985.

**12.** Fey GH, Gauldie J. The acute phase response of the liver in inflammation. In: Popper H, Schaffner F, eds. Progress in liver disease. Vol 9. Philadelphia: WB Saunders, 1990.

**13.** Baumann H, Gauldie J. Regulation of hepatic acute phase plasma protein genes by hepatocyte stimulating factors and other mediators of inflammation. *Mol Bio Med* 1990; 7: 147-59.

**14.** Dinarello CA. Interleukin-1 and the pathogenesis of the acute phase response. NEngJMed 1984; 311: 1413-8.

**15.** Gresser I, Delers F, Tran Quangs N, Marion S, Engler R, Maury C, *et al*. Tumor necrosis factor induces acute phase protein in rats. *J Biol Regul Homeost Agents* 1987; 1: 173-6.

**16.** Le J, Vilcek J. Biology of disease. Interleukin 6: a multifunctional cytokine regulating immune reactions and the acute phase protein response .*Lab Invest*1989;61588-602.

**17.** Mackiewicz A, Ganapathi MK, Schultz D, Brabenec A, Weinstein J, Kelley MF, *et al*. Transforming growth factor

PI regulates production of acute phase proteins. Proc Nat Acad Sci USA 1990; 87: 1491-5.

**18.** Baumann H, Schendel P. Interleukin-1 1 regulates the hepatic expression of the same plasma protein genes as interleukin-6.J7 *Biol Chem* 1991; 266: 20424-7.

**19.** Almagor M, Mintz A, Sibirsky O, Durst A. Preoperative and postoperative levels of interleukin-6 in patients with acute appendicitis. *Surg Endosc* 2005; 19: 331–33.

**20.** Fernando A., Rivera-Chavez, Herbert Wheeler, Guy Lindberg, Robert S. Munford, and Grant E. O'Keefe. Regional and Systemic Cytokine Responses to Acute Inflammation of the Vermiform Appendix. Annals of Surgery 2003; 237(3): 408– 16.

**21.** Alshahwany I. The dilemma of incidental gynaecological disorders in the general surgical practice. JABMS 2005; 7(1): 28-32.

**22.** Goodwin AT, Swift RI, Bartlett MJ, Fernando BS, Chadwick SJ. Can serum interleukin-6 levels predict the outcome of patients with right iliac fossa pain? *Ann R Coll Surg Engl.* 1997 Mar; 79(2):130-3.

**23.** Khan MN, Davie E, Irshad K. The role of white cell count and C-reactive protein in the diagnosis of acute appendicitis. *J Ayub Med Coll Abbottabad*. 2004 Jul-Sep; 16(3):17-9.

**24.** Amalesh T, Shankar M, Shankar R. CRP in acute appendicitis--is it a necessary investigation? Int J Surg. 2004; 2(2):88-9

**25.** Gurleyik G, Gurleyik E, Cetinkaya F, Unalmiser S. Serum interleukin-6 measurement in the diagnosis of acute appendicitis. ANZ J Surg. 2002; 72: 665-7.

**26.** Van den Ende ED, Boellaard WP, Allema JH, Holscher HC, Putter H, Breslau PJ. Diagnostic surplus value of echography in children with acute abdominal pain. Ned Tijdschr Geneeskd. 2003; 147: 1174-7.

**27.** Paajanen H, Mansikka A, Laato M, Ristamaki R, Pulkki K, Kostiainen S. Novel serum inflammatory markers in acute appendicitis. Scand J Clin Lab Invest. 2002; 62:579-84.

**28.** Turkyilmaz Z, Sonmez K, Karabulut R, Elbeg S, Moralioglu S, Demirtola A, et al. Sequential cytokine levels in the diagnosis of appendicitis. Scand J Clin Lab Invest. 2006; 66:723-31.

**29.** Sack U, Biereder B, Elouahidi T, Bauer K, Keller T, Trobs RB. Diagnostic value of blood inflammatory markers for detection of acute appendicitis in children. BMC Surg. 2006; 6:15.

## *(Table -1)*
## *Patients distribution according to age groups.*

| Age groups | No. of patients | Percentage |
|---|---|---|
| 0-9 year | 5 | 10% |
| 10-19 year | 15 | 30% |
| 20-29 year | 22 | 44% |
| 30-39 year | 4 | 8% |
| 40-49 year | 3 | 6% |
| 50-above | 1 | 2% |
| Total | 50 | 100% |

## *(Table-2)*
## *Patients characteristics in relation to histopathology results.*

| | Histopathological groups | | | |
|---|---|---|---|---|
| | Group one: Normal appendix | Group two: Non-complicated appendicitis | Group three: Complicated appendicitis | Total |
| Number (%) | 7 (14%) | 29 (58%) | 14 (28%) | 50 (100%) |
| Male | 2 (4%) | 16 (32%) | 9 (18%) | 27 (54%) |
| Female | 5 (10%) | 13 (26%) | 5 (10%) | 23 (46%) |
| Male/Female ratio (1.27:1) | 0.4:1 | 1.2:1 | 1.8:1 | |
| Mean age (23year) | 30.75year | 20.66 year | 25 year | |

*(Table-3)*

*TP, TN, FP, and FN results of IL-6.*

| Overall serum IL-6 results | IL-6 concentrations | |
|---|---|---|
| | Number | Percentage |
| True positive (TP) | 41 | 82% |
| True negative (TN) | 3 | 6% |
| False positive (FP) | 4 | 8% |
| False negative (FN) | 2 | 4% |
| Total | 50 | 100% |

*(Table-4)*

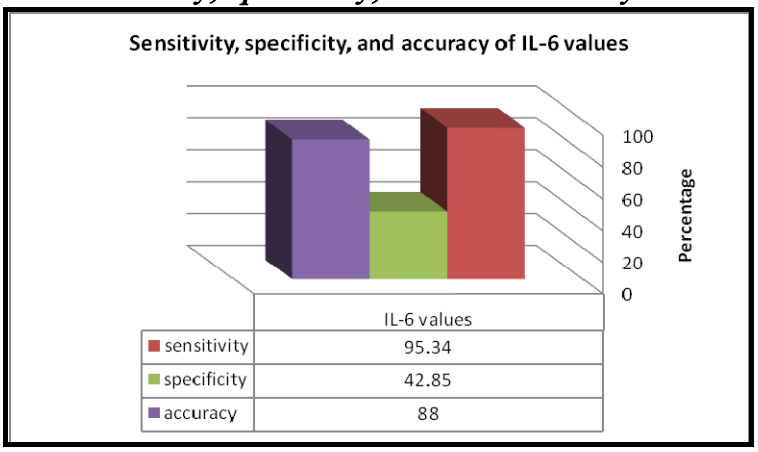
*Statistical values of median IL-6 (pg/ml) in relation to histopathology results.*

| | Histopathological groups | | |
|---|---|---|---|
| | Group one: Normal appendix | Group two: Non-complicated appendicitis | Group three: Complicated appendicitis |
| Median IL-6 (pg/ml.) | 24.7376 | 53.7294 | 84.2622 |
| P-value | <0.001 | <0.001 | <0.001 |
| Odds ratio (range) | 0.07 (0.02-0.27) | 0.14 (0.06-0.36) | 0.04 (0.01-0.12) |

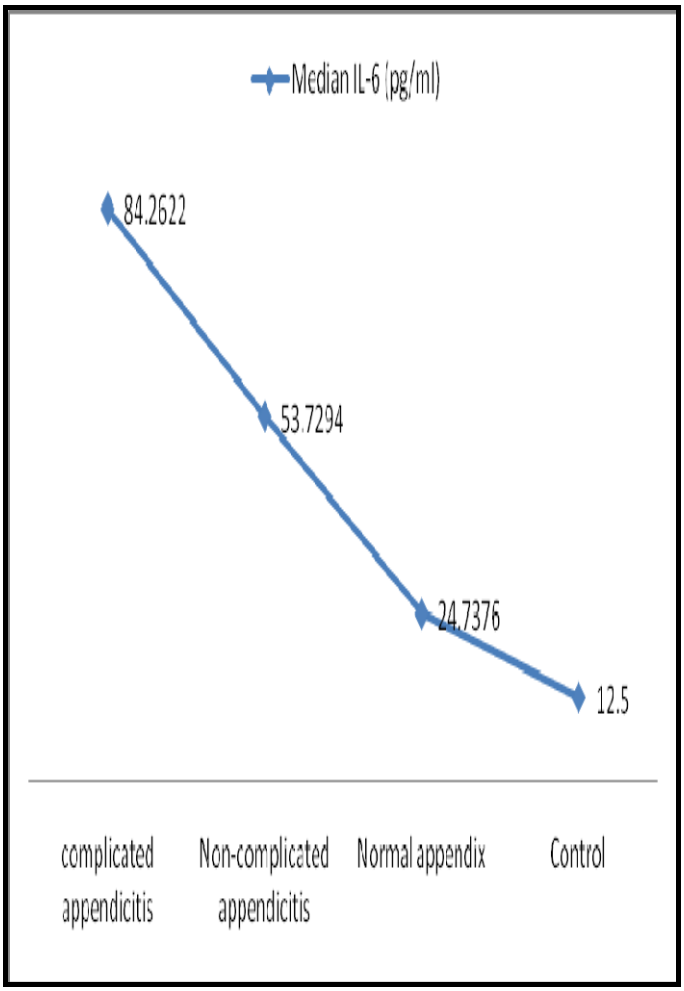
**FIGURES:**

*(Fig.-1)*

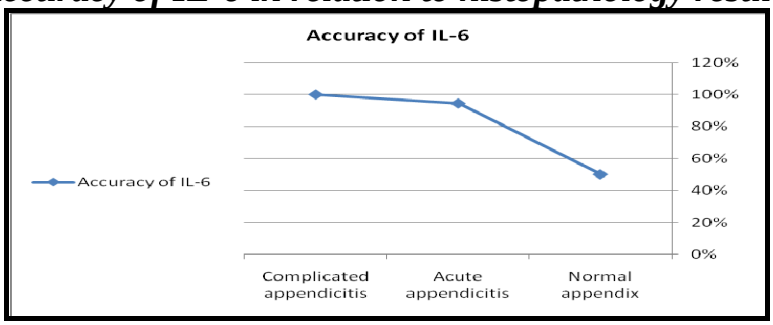
*The sensitivity, specificity, and the accuracy of IL-6.*



*(Fig-.2)*
*The median IL-6 levels in relation to histopathology results.*




*(Fig.-3)*
*Accuracy of IL-6 in relation to histopathology results*




***Al*- Kindy Col Med J 2009; Vol .5 (1):p 68**

***From the department of surgery Al-Kindy College of medical., Baghdad, Iraq.**
****From the department of surgery Al-Kindy College of medical., Baghdad, Iraq.**
*****From the department of surgery Al-Kindy College of medical., Baghdad, Iraq.**

***Correspondence to:*** **Dr** . Imad Wajeh Al-Shahwany
**Received at:** 5-5-2009 **Accepted at:** 3-6-2009