

# Evaluate the Cytokine IL-6 as an Inflammatory Indicator Against Isolated Parasites in Appendicitis Patients

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## Abstract

Appendicitis is one of most common abdominal pains requiring emergency surgery, Acute appendicitis is acute condition happened as a result of obstruction from parasites present. 114 blood sample were collected from patients who had undergone appendectomy operation at periods from 21-12-2010 to 1-4-2011 in General Teaching Hilla Hospital.

This study is done to determine pathogenesis of parasites and determine the type of immune statue for appendicitis caused by parasites by estimating concentrations of IL-6 as an immune marker . The rate of samples that gave positive for parasites isolate was 29.82% , while 70.14% parasitically negative , also the total number of isolated parasites was 7 genera.

The common isolated parasites were *Entamebae histolotica* 37.8% , *Entrobis vermocalaris* 35.1% , *Schistosoma haematobium* , *Blantidium coli* , *Trichurus trichora*, *Giardia lamblia* and *Schistosoma mansoni* in rate 5.4% for each one . Immunological tests to sera of patients with appendicitis refer to significance increasing in the concentration of IL-6 for all patients at different age groups, compared to control and the highest concentration of them in the age group 40-49 years (  $5.944 \pm 0.004$  pg/ml ) , the present study shows absence of parasite as causative for appendix infection in  $\leq 10$  age group , This study refers to the role of cellular immune response through appendix infection with different parasites.

incidence of appendicitis appear in both sex at same rate 50% . Appendicitis appear in all age groups the age period 10-19 show highly infection rate with parasite.

**Keyword:** cytokine IL-6, parasites in appendicitis, inflammatory indicator with appendicitis.

## الخلاصة

ان التهاب الزائدة الدودية من الامراض المفاجئة ومن اكثر آلام البطن شيوعا ويحدث الالتهاب نتيجة انسداد القناة الهضمية لتواجد بعض الطفيليات . تم في هذه الدراسة جمع عينات الدم وفصل المصل منها من المصابين اثناء خضوعهم لعملية الاستئصال الجراحي للزائدة في مستشفى الحلة التعليمي العام وكانت عدد العينات 114 في الفترة الممتدة ما بين 21\ 12\ 2010 الى 1\ 4\ 2011 في مستشفى الحلة التعليمي الجراحي .اجريت هذه الدراسة لتحديد نوع الاستجابة المناعية لالتهاب الزائدة الدودية من خلال تقدير تراكيز IL-6 ، وكانت نسبة العينات التي اعطيت نتيجة موجبة لوجود الطفيليات 29.82% في حين 70.14 % كانت سلبية.

تم عزل البكتريا 7 انواع طفيليات من العينات وتشمل

*Entamebae histolotica* 37.8% , *Entrobis vermocalaris* 35.1% , *Schistosoma haematobium* , *Blantidium coli* , *Trichurus trichora*, *Giardia lamblia* and *Schistosoma mansoni*. بمعدل 5.4% لكل نوع.

كشفت الاختبارات المناعية على امصال دم المصابين ان لالتهاب الزائدة الدودية تأثيرا معنويا في زيادة تركيز السايوتوكين IL-6 بفارق معنوي ولمختلف الفئات العمرية وان اعلى تركيز وجد للفئة العمرية 40-49 عام ( $5.944 \pm 0.004$  بيكوغرام /مل ) كما اشارت الدراسة الحالية الى انعدام وجود الطفيليات في الفئة العمرية دون 10 سنوات، وقد اتضح خلال هذه الدراسة وجود استجابة مناعية خلوية للاصابة الناجمة عن وجود الطفيليات. يظهر توزيع الاصابة لدى كلا الجنسين بمعدل متساوي بواقع 50% ، كذلك تحصل الاصابة في جميع الفئات العمرية وان الفئة العمرية من 10-19 سنة هي الفئة الأكثر اصابة.

## Introduction

Appendicitis is the most common abdominal emergency case and the most common cause of acute surgical abdomen associated with systemic inflammatory responses.(Smpso and Scholefield,2008). Appendicitis exhibits various clinical manifestation and therefore difficulty in diagnosis (Fisher, 2000 ; Pasupati et al,2008 ) , and also it occurs in approximately 7% of the population(Mowlavi et al, 2004).

Appendicitis is triggered by a rise in the intraluminal pressure consequent upon obstruction (Wangensteen and Dennis,1985), microorganism, luminal mucus elaboration and inflammatory mediators all have roles in appendix inflammation , the

enteric parasites are one cause associated with appendix obstruction especially helminthes of common injury appendicitis (Martin and Gustafson, 1985), and the rate increases of intestinal parasites in the developing world, as observed in Nigeria, there is correlation between parasites and some appendicitis cases recorded a *Schistosoma spp.* a major cause in addition to the presence of some Protozoa (Pasupati *et al*, 2008; Oguntola *et al*, 2010).

The diagnosis of appendicitis is most commonly by detecting of immunological state of patients by evaluating some of cytokines concentration as a cellular immune response. Interleukine-6 one an important pro-inflammatory factor to acute appendicitis diagnosis through highest in its concentration and affected on other inflammatory factors as a systematic immune response and tissue damage in early time, as well as IL-6 have ability to estimated another cytokines secretion (Gurleyik *et al*, 2000; Almagor *et al*, 2005).

### Materials and methods

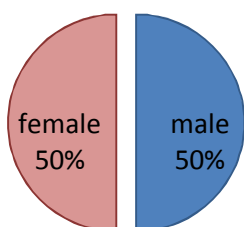
- 1- The study included 114 patients with different ages undergoing appendicectomy at the General Teaching Hilla Hospital from 21-12-2010 to 1-4-2011, after recording information about patients.
- 2- Blood samples: 5ml of blood were taken from patient and isolated serum for immunological study. Swabs for inner surface of appendix were used for microscopic analysis and parasite diagnosis, after observing the appendix cavity macroscopically to ensure some helminthes infection .
- 3- Immunological test: The study contributed also measuring the concentration of interleukin-6 of infected and healthy by ELISA method according to the manufacturer's instructions.

### Statistical Analysis:

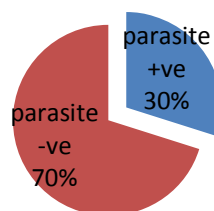
Use the U.S. Census (spss 11) to perform statistical analysis, as analyzed the results using the design random full-scale analysis of variance and adopted the test less significant differences Least significant difference test (LSD) and table analysis of variance (ANOVA Table) below the level of significance 0.05 (Niazi, 2004).

### Results:

Between December 2010 and April 2011 our study was carried out on 114 human patients with different ages and sexes, appendicitis appear in rate 50% for both sex (Figer 1). Also the study showed that the rate of samples that gave positive for parasites isolate 29.82% , while 70.14% piratically negative (figer 2).

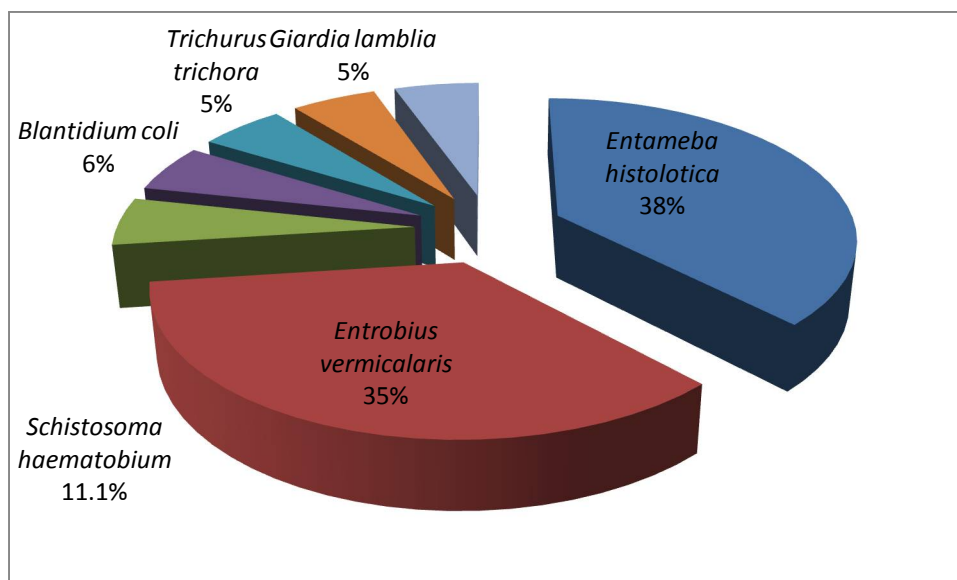


figer 1: Distribution of infection according to the sex



figer 2: percentage of parasites that diagnostic

The total number of isolated parasites were 7 genera, was time, the common isolated parasites were *Entameba histolotica* 37.8% , *Entrobilus vermocalaris* 35.1% , *Schistosoma haematobium* , *Blantidium coli*, *Trichurus trichora*, *Giardia lamblia* and *Schistosoma mansoni* (figer 3).



figer 3: Types of isolated parasites

This study showed that parasites isolate appears in age ranged between 10-49 years, however, the incidence of inflammation increased in the rate 10-19 year, also all the parasite species presented in different shapes trophozoit, cyst or egg and adult (table 1),(figer3).

Table (1): Total number of parasites isolates from appendix

Age group	Number and type of parasites					
	<i>E.vermicalaris</i>	<i>E.histolatica</i>	<i>Sch.haematobium</i>	<i>B.coli</i>	<i>T.trichora</i>	<i>G.lamblia</i>
≤10	0	0	0	0	0	0
10-19	2 adult 3 egg	2 trophozoite 3 cyst	1 egg	0	0	0
20-29	3 egg	2 cyst	1 egg	1 cyst	1 egg	1 trophozoite
30-39	2 adult 3 egg	2 trophozoite 2 cyst	0	0	0	1 trophozoite
40-49	0	2 trophozoite	1 adult+1 egg	1 cyst	1 egg	0
Total	13	14	4	2	2	2

The results of the current study significantly increased ( $P < 0.05$ ) in the level of IL-6 and for all age groups of patients compared to control its subsidiaries through the use

of technology calibration absorbance linked immunoassay Enzyme - Linked Immunosorbent Assay (ELISA) (table 2).

**Table (2):The level of IL-6 in patients with appendicitis:**

age group	Groups	Concentration of IL-6 pg/ml Mean $\pm$ S.D
10-19	Control	3.761 $\pm$ 0.816
	infection	5.942 $\pm$ 0.005 *
20-29	Control	4.449 $\pm$ 0.761
	infection	5.868 $\pm$ 0.179*
30-39	Control	2.578 $\pm$ 0.462
	infection	5.942 $\pm$ 0.001*
40-49	Control	3.276 $\pm$ 0.428
	infection	5.944 $\pm$ .004 *

\*L.S.D under ( $P < 0.05$ ) = 4.348

### Discussion:

Appendicitis, which affects about 10 percent of the population, is the most common reason for general emergency medical surgery. Our study has shown that parasite infection is a possible cause of acute appendicitis. Although the number of positive cases was not large enough to make a significant association, an incidence of 29.82% , while the highest rate was 70.14% act a negative parasitically. Recognizing that appendicitis is a complex disease that involves mechanical obstruction of the lumen, ischemia, thrombosis and bacterial overgrowth (Fernando *et al*, 2004; Preidt, 2010). The present study demonstrated that appendicitis was distributed in both human sexes at same rate 50% comparted to other studies , the same study also demonstrated that there was no large rate for parasite test with appendix infection (Pranesh *et al*, 2006; Pasupati *et al*, 2008 ).

Appendicitis appears in all age groups and the age period 10-19 show highly infection rate with parasite in time that absence of parasite as a causative for appendix infection in  $\leq 10$  years, can be attributed to eating food from variety sources which may be unhealthy while the child food already under housing control.

The prevalence of infection is related to several factors including nutritional habits, contact with soil, age, rural or urban settings and frequency of contact with domestic animals and climatic condition such as humidity (ALmusay, 2004).

Our study shows the parasite examination supported the diagnosis and explain inflammation state in appendix .Intestinal parasite such as worms persist and grow in all intestine sites that lead to full appendix cavity causing damage in its tissue, the high

prevalence of intestinal parasites in the developing world could also account for some cases of appendicitis, as it has been noticed to be initiated by or associated with them (Hill, 2000; Oguntola *et al*, 2010 ), although some study refer to presented parasite in appendicitis condition is very rare especially in developed countries (Terada, 2009).

Although the immune response in some parasite infection has been well characterized, the mechanisms involved in reactivation in humans are still unclear. Knowledge of the anti-parasite immune response is important. We addressed this issue by examining the specific human cellular responses by evaluation IL-6 concentration . Serum levels of IL-6 have a role in discerning the extent of disease in this condition (Yoon *et al*, 2002). The present study appears a significant increasing in the concentration of IL-6 for all patients with different age groups, compared to control and the highest concentration of them for the age group 40-49 years . Several studies have focused on the diagnostic value of IL-6 concentration in suspected appendicitis, comparing with patients of a normal control group (Erkasap *et al*, 2000; Yoon *et al*, 2002).

It has been shown that concentrations of serum IL-6 and IL-8 were elevated in adults with acute appendicitis, especially with perforation; and the serum IL-6 level is a valuable tool in diagnosing advanced appendicitis. But some findings had shown there was no correlation between IL-6 serum concentrations and the severity of appendiceal inflammation(Almagor *et al*, 2005; Groselj *et al*, 2007). Many studies have investigated the value of raised IL-1  $\alpha$  levels in improving the diagnosis of appendicitis which aggregate with present study (Paajanen *et al*, 2002; Dalal *et al*, 2005).

IL-6 have an important role in stimulating Th22 ,Th17 that secrete another set of cytokines that share in stimulating neutrophils and NK cells which provides extracellular microbes at the end by activity of macrophage (Kelso, 1995).

## References

- Almagor M, Mintz A, Sibirsky O, et al.(2005). *Preoperative and postoperative levels of interleukin-6 in patients with acute appendicitis. Surg Endosc.* ;19:331–333.
- Almusawy, M.M. (2004). Intestinal parasite in karbala city. Msc. Thesis . Colleg Science, Babylon university;56pp.
- Dalal, I.; Somekh, E.; Reich, A.B.; Boaz, M.; Gorenstein, A. and Serous, F. (2005). Serum and peritoneal inflammatory mediators in children with suspected acute appendicitis. *Arch Surg.*, 140:169- 173.
- Erkasap, S.; Ates, E.; Ustuner, Z.; Sahin, A.; Yilmaz, S.; Yasar, B. and Kiper, H. (2000). Diagnostic value of interleukin-6 and C-reactive protein in acute appendicitis. *Swiss Surg.*; 6:169-172.
- Fernando, A.; Rivera-Chaves ; Peters-Hybki, D.L.; Barber, R.C.; Lindberg, G.M.; Jialal, I.; Munford, R.S. and O'Keefe, G.E. (2004). Innate immunity genes influence the severity of acute appendicitis. *Ann. Surg.*, 240(2):269-271.
- Fisher, R. E. (2000). The primate appendix: a reassessment. *Anat Rec.*, 261: 228-236.
- Groselj-Grenc M, Repse S, Vidmar D, et al.(2007).Clinical and laboratory methods in diagnosis of acute appendicitis in children. *Croat Med J.* ;48:353–361.
- Gurleyik,G.; Gurleyik,E.;Cetinkaya,F. and Unalmiser,S.(2000). Serum interleukin-6 measurment in the diagnosis of acute appendicitis. *ANZJ sur. Sep*;72(9):665- 667.
- Hill , P.R. (2000). Principle and practical parasitology by John Wiley and Snos.LtB, Baffins Lane, Chichester , West Sussex po 191 UD. Engiland.
- Kelso, A. (1995). Th1 and Th2 subsets: paradigms lost?. *Immunol Today.*,16:374–379.

- Martin, D.L. & Gustafson, T.L.** (1985). A cluster of true appendicitis cases. *American Journal of Surgery* **150**: 554-7.
- Mowlavi, G., Massoud, J., Mobedi, I., Rezaian, M. & Mohammadi, S.S.** (2004). *Enterobius vermicularis*: a controversial cause of appendicitis. *Iranian Journal of Public Health* **33**: 27-31.
- Niazi, A.D.** (2004). Statistical analysis in Medical Research. 2<sup>nd</sup> ed. Coll. of Med., Nahrain Univ. Baghdad. P: 73-98.
- Oguntola, A.S.; Adeoti, M.L. and Oyemolade, T.A.** (2010). Appendicitis: Trends in incidence, age, sex, and seasonal variation in South-Western Nigeria. *Ann. Afr.*, **9**(4):213-217.
- Paajanen, H.; Mansikka, A.; Laaton, M.; Ristamaki, R.; Pulkki, K and Kostianen, S.** (2002). Novel serum inflammatory markers in acute appendicitis. *Scand. J. of Clin & Lab. Inves.*, **62**(8): 579-584.
- Pasupati, M.; Yothasamutr, K.; Wah, M.; Sherif, T and Palayan, K.** (2008). A study of parasitic infections in the luminal contents and tissue sections of appendix specimens. *Tropical Biomedicine* **25**(2):166-172.
- Pranesh, N.; Sathya, V. and Mainprize, K.S.** (2006). Serosal appendicitis: incidence, causes and clinical significance. *Postgrad Med J.*, **82**(974):830-832.
- Preidt, R.** (2010). Study Questions Need for Emergency Appendectomies Appendicitis may have viral origins, researchers suggest. University of Texas South-western Medical Center, news release, Jan. 18.
- Simpson J, Scholefield JH.** (2008). Acute appendicitis. *Surgery*. **26**:108–112.
- Terada, T.** (2009). Schistosomal appendicitis: Incidence in Japan and case report. *World J. Gastroenterol.*, **15**(13):1648-1649.
- Wangenstein OH, Dennis C.** (1985). Experimental proof of the obstructive origin of appendicitis in man. *Ann Surg*; **110**:629-47.
- Yoon, D.Y.; Chu, J.; Hiyama, S.; Thompson, J.E. and Hines, O.J.** (2002). Human cytokine levels in nonperforated versus perforated appendicitis: molecular serum markers for extent of disease?. *Am Surg.*, **68**(12):1033-1037.