



## Obestatin (OB), Calprotectin (FCP) and (SIgA) in Patients Infected with *Entamoeba Histolytica* in Al-Najaf Governorate, Iraq

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### Abstract

The study included the identification of changes in biomarkers to determine the effect of *E. histolytica* on the level of calprotectin, obestatin and SIgA in patients parasites, 250 patients were collected from AL-Hakeem Hospital in Najaf from (May 2018) to (October 2018), The number of patients with amoeba was 115(46%), 65(26%) males and 50 (20%). females and compared with a control group of 50 healthy subjects The results showed sig significant increase( $p \leq 0.05$ ) in the level of obestatin, calprotectin, SIgA control group.

### Introduction

*E. histolytica* is an intestinal protozoan that causes amoebiasis disease to human, it cause approximately of 100000 deaths every years [1, 2]. Infection by *E. histolytica* may be symptoms or without symptoms [3]. In developed world, protozoan parasites more commonly cause gastrointestinal disease compared to worms. The most spread protozoan is *E. histolytica* caused amoebiasis [4] *E. histolytica* is parasite that causes amoebiasis and liver abscess.

The disease is still one of the major health problems and most affects persons of decrease hygiene who live in developing countries [5-17]. This parasite is transmitted to humans by contaminated feeds and drinks; the diagnosis of *E. histolytica* is often not easy. The main aim of detection and differentiation of *E. histolytica* species in faecal specimen is the limitation of the causative factor of amoebic dysentery. Amoebiasis in facts approximately 500 million person in the world today [6], the prevalence of parasite infections and amoebic dysentery increase.

In Mediterranean region [18-25]. *E. histolytica* has a simple life cycle in which the transition is cross the faecal-oral route, infection occurs through swallow infective cyst size (8-20 $\mu$ m) or invade of mobile trophozoites size (20-40  $\mu$ m) [26].

Most infections are asymptomatic, but *E. histolytica* can invasion the intestinal barrier, causing severe ulceration and diarrhea contains blood [27-30]. If *E. histolytica* enables access to blood vessels, they may be carried extra intestinal sites anywhere in the body, the most important of which is the liver where cause liver abscess [18, 31]. Serbian [32] Explains that immune response against the infection depend on the recruiting of white blood from blood vessel to sites of infection and essential mucosal immunoglobulin is secretory (IgA) in the individual [33-35].

Calprotectin is calcium and zinc binding protein; it is biomarker in inflammatory gut disease and activates the recruitment of leukocytes as well as production the cytokine and chemokine [36]. Obestatin is a hormone that is found in humans, which is produced by stomach and small intestinal [19]. Obestatin has been reported to decrease feeds intake, body weight increase, gastric emptying, jejunal and duodenum mobility [17, 22].

### Aim

This study aimed to count and known of immune response in person infected with amoebiasis disease due to the determination of three biomarkers level obestatin, calprotectin and SIgA.

## Materials and Methods

### Sample Collection

The study included (115) diarrheal patients who were selected from (250) diarrheal patients at AL-Hakeem hospital during (May) - (October) in Najaf governorate. The stool was collected from patients in a broad vial with lid as 5ml of intravenous blood was drawn from patients and control group and blood samples were placed in sterile tubes at room temperature for 30 minutes and then placed in a centrifuge at 3000 rpm for 5 minutes to isolate and distributed to 3 parts at 20 C° and then placed in ELISA device for measuring calprotectin (CALP), (SIgA) and obestatin (OB).

### Diagnosis *Entamoeba histolytica*

#### Direct Stool Examination

Bring stool sample from patients suspected them in AL-Hakeem Hospital were processed and examined microscopically for intestinal

patients using X40 objective lens of the stool smears were examined as diarrhea after than X10 objective lens.

### Serum Biomarker Detection

In this study used three biomarkers: obestatin, calprotectin and (SIgA), the concentrations of biomarkers in serum were determined by using ELISA instrument.

### Statistical Analysis:

T-test was used in this study for comparison between samples by using a photograph. P-value in crease significant 0.05 [3].

## Results and Discussion

### Calprotectin

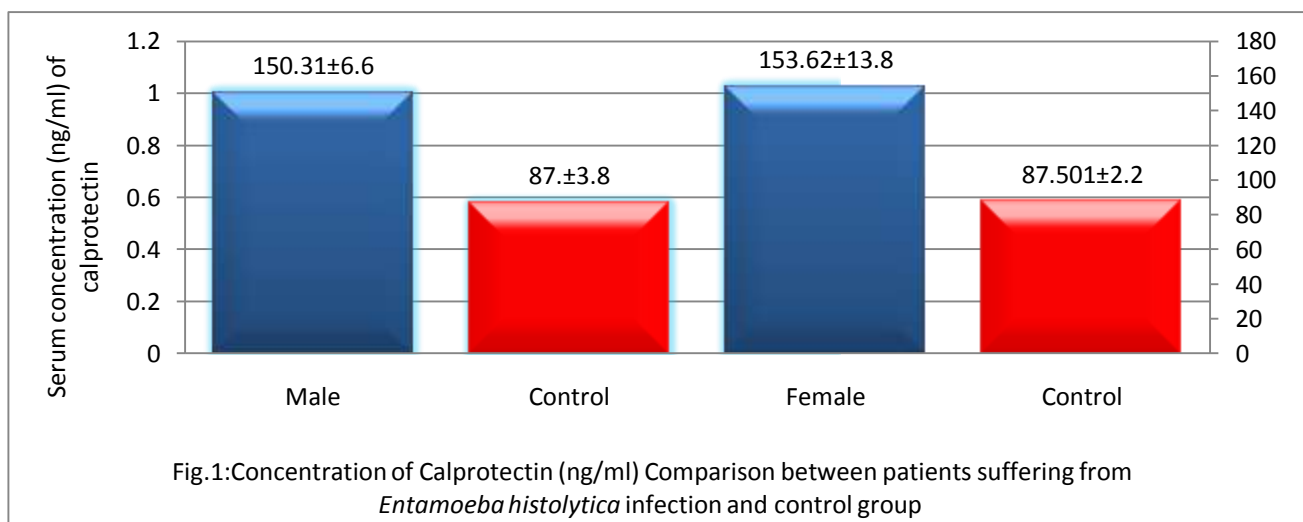
Figure (1) of this study revealed a significant increase  $p \leq 0.05$  of calprotectin level in patients with *E. histolytica* in male and female (150.31±6.6ng/ml), (153.62±13.8ng/ml) in compared with control group (86.991±2.5ng/ml), (87.501±2.2ng/ml).

Table 1: Compare between male patient and control group calprotectin

	M ± SD	Tc	Tt P< 0.05	
Male patient	150.3 ± 6.6	9.7	1.96	Significant increase
Control	87 ± 3.8			

Table 2: Compare between female patient and control group calprotectin

	M ± SD	Tc	Tt P< 0.05	
Female patient	153.6 ± 13.8	10.1	1.96	Significant increase
Control	87.5 ± 2.2			



The high of serum level of calprotectin may be due to role of calprotectin which plays in epithelial tissue ability to stimulate immunity. And body production of cellular motins [34]. Calprotectin antibody is produced from the white blood when they were resistant with the infection causing intestinal disease [30].

Calprotectin is considerable trial in gastroenterology for differentiating inflammatory gut infection from other non-inflammatory, in addition it can be used as a validated marker for disease stimulate and response to treatment [21,33], particularly in infectious diarrhea of both viral and bacterial etiologies [12].

*Entamoeba* infection may modify human immune responses and have protect against risk diarrheal disease [5], and cause accumulate and migrate large numbers of leucocyte in lumen in the bowel inflammation, so calprotectin synthesis occurs in activated neutrophils, macrophage, epithelial cells and monocytes. Calprotectin produced from mucosal neutrophils and bowel cells will mainly be released in the mucosa giving increase immune response [37].the recent study agree with the study of Bodil *et al.* [10]that showed increase of fecal

and serum calprotectin back to increase white blood, also Mansour *et al*[20] and Mustafa [27].

### Obestatin

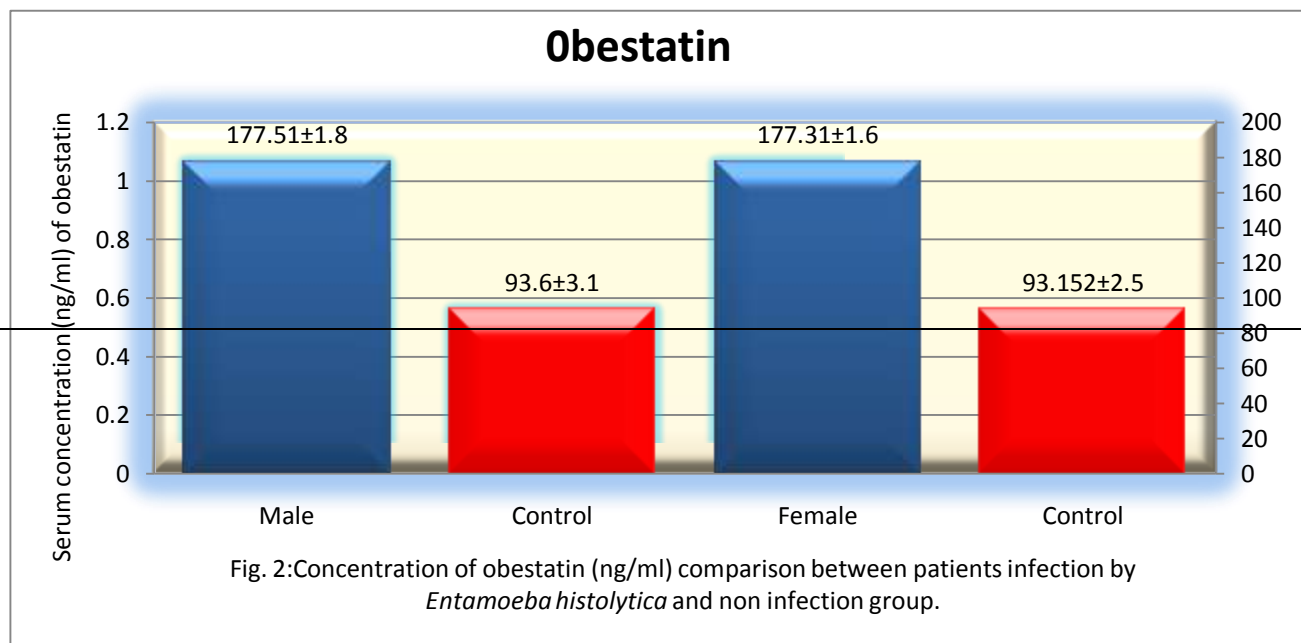
Figure (2) the study results showed that concentration obestatin in patients infected with *E. histolytica* were significant increase ( $p < 0.05$ ) in each male and female respectively ( $177.51 \pm 1.8$  ng/ml), ( $177.31 \pm 1.6$  ng/ml) with compared to non-infected group ( $93.6 \pm 3.1$  ng/ml), ( $93.152 \pm 2.5$  ng/ml) respectively.

**Table 3: Compare between male patient and control group obestatin**

	M ± SD	Tc	Tt P< 0.05	
Male patient	177.5 ± 1.8	8.6	1.96	Significant increase
Control	93.6 ± 3.1			

**Table4: Compare between female patient and control group obestatin**

	M ± SD	Tc	Tt P< 0.05	
Female patient	177.3 ± 1.6	8.2	1.96	Significant increase
Control	93.15 ± 2.5			



The increase of serum level of obestatin may be due to isease pathogenesis, *E. histolytica* cysteine enzyme necrosis of MUC to in the non-glycosylated c-domin, weakening its structure and helping *E. histolytica* contact with moscal layer in lumen intestinal [23].Obestatin hormone played role important of predominantly produced in the stomach, and inhibition of food mount body weight gain, gastric emptying and balance of jejuna mobility [24, 35-38]. The obestatin hormone released from epithelial cells of stomach and small intestine in the reduce of intestinal motility, appetite inhibition, weight loss, and increased memory function in mammals.

These roles rare different with the roles of ghrelin [28].

The current study was agree with study of Burhan Hakan *et al*[11] that revealed an increase in obestatin level with acute pancreatitis with high in ghrelin level in this infection . also the current study was agree with study of thikra A. [35] that showed and elevated in obestatin level with increase HDL-C in diabetic patients.

### SIgA

Figure (3) the study results showed that concentration SIgA in patients infected with

*E. histolytica* were significant increase ( $p \leq 0.05$ ) in each male and female respectively ( $4.552 \pm 0.51$  ng/ml), ( $5.351 \pm 0.61$  ng/ml) with

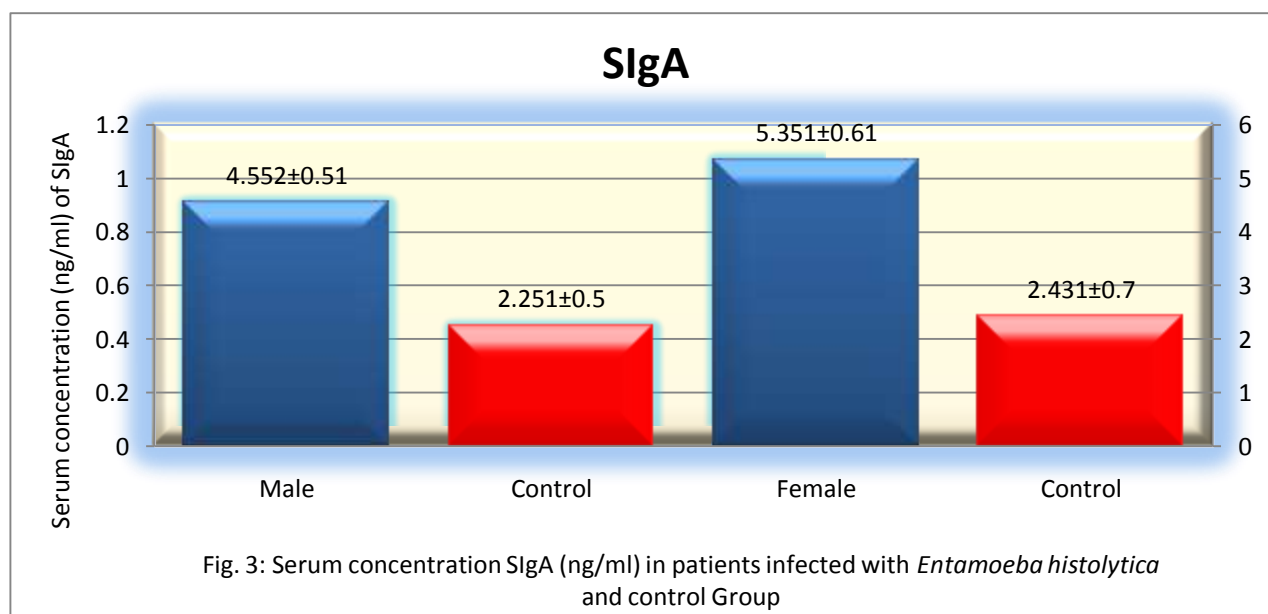
compared to non-infected group ( $2.251 \pm 0.5$  ng/ml), ( $2.431 \pm 0.7$  ng/ml) respectively.

**Table 5: Compare between male patient and control group SIgA**

	M $\pm$ SD	Tc	Tt P < 0.05	فروقات معنوية
Male patient	4.6 $\pm$ 0.5	4.2	1.96	
Control	2.3 $\pm$ 0.5			

**Table 6: Compare between female patient and control group SIgA**

	M $\pm$ SD	Tc	Tt P < 0.05	فروقات معنوية
Female patient	5.4 $\pm$ 0.6	5.5	1.96	
Control	2.4 $\pm$ 0.7			



The elevated of serum level of (SIgA) may be due to that mucosal IgA antibody response is linked with immune protection against *E. histolytica*. The primary role of SIgA is referred to use immune exclusion, is inventor process microorgens and mucosal antigens to

mucosal layer [7]. SIgA play role important in the remove of the parasite [13] This study agree with study AL Mahdawy [4] where showed high SIgA level in patients with *E. histolytica*, also agree with study Sehgul *et al*[31].

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