

# Trefoil factor3 (TFF3), Calprotectin (CALP) and (SIgA) as immunological markers in patients infected with *Giardia lamblia* Parasite

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## Abstract

The study was conducted on 400 out suspected patients and twenty four healthy persons, whom have visited the laboratory of AL-Hakeem hospital and AL-Zahra maternity and pediatrics in AL-Najaf province from October till May 2018. This study was designed to determine the effects of *Giardia lamblia* infection on some biomarkers such as Trefoil factor3 (TFF3), Calprotectin (CALP) and (SIgA) whereas the numbers and percentage of infected patient were 48 (12%) vary with different sex 28 (7%) male and 20 (5%). The result of study revealed that concentration of (TFF3), (CALP) and (SIgA) in male and female patients with *G. lamblia* were significant increase ( $p < 0.05$ ) in compared to the control group. Also it revealed the significant increase ( $p < 0.05$ ) in concentration in total patients infected with *G. lamblia* and total control group.

The current study has concluded that the infection with *G. lamblia* effect on some immunological markers of human represent Trefoil factor3 (TFF3), Calprotectin (CALP) and (SIgA) as a good biomarkers may be used in detection of *G. lamblia* parasite.

**Keywords:** Giardiasis, diarrhea, TFF3, CALP, SIgA, Iraq.

## INTRODUCTION

Giardiasis is a gastrointestinal disease caused by a microscopic parasite called *Giardia lamblia*, proliferating in the small intestine of humans which attachment strongly to the host intestine and caused severe gastrointestinal disease (Macpherson, 2005) This microorganism a worldwide parasite may be leads to chronic diarrhea and malabsorption of human, although many of the patients with *G. lamblia* parasite asymptomatic, the main acute symptoms appeared sudden diarrhea for several days, excessive gases (swelling and puff and tasting nasty taste), abdominal pain around the navel, nausea, diuretic diarrhea, and rarely there is vomiting or warming (Kim H. & Ravinder, 2010). The cyst of this parasite has ability to resistance the unsuitable condition and adaptations with external environment to survival, whereas trophozoite responsible on virulence properties and clinical symptomates in host (Robertson *et al.*, 2014).

Serbina (2008) shows that immune response against the infection depend on the recruitment of leukocytes from the bloodstream to sites of injury and the essential mucosal immunoglobulin is secretory IgA (SIgA) in the human and the other mammals. Calprotectin is calcium and zinc binding protein; it is essential marker in inflammatory bowel disease and stimulates the recruitment of leukocytes as well as produced the cytokine and chemokine (Yui *et al.*, 2003; Van *et al.*, 2010; Van *et al.*, 2012).

Also this infection may be lead to malnutrition, weight loss, growth impairment and even poor cognitive development due to persistent diarrhea but not in acute diarrhea (Fraser *et al.*, 2000; Berkman *et al.*, 2002; Muhsen *et al.*, 2012).

**Aim:** This study aimed to estimation of immune response in patients infected with Giardiasis disease due to the determination of three biomarkers level; Trefoil factor3 (TFF3), Calprotectin (CALP) and (SIgA).

## MATERIAL AND METHODS

### Study design and patients

From October till May 2018, 48 samples were collected from patients and 24 healthy as control (vary on 14 male and 10 female) who attended the clinics in AL-Hakeem hospital and AL-Zahra maternity and pediatrics in AL-Najaf province, the samples

of stool were collected into clean, wide-mouth specimen bottles, from patients and blood samples were also drawn from the same patients by vein puncture into specimen tubes to prepare the serum for estimation the markers of study.

### Diagnosis *Giardia lamblia* parasite

Freshly voided stool specimens were processed and examined microscopically for intestinal parasites using X40 objective lens as described by Paniker (1989). Before a slide was considered negative, then X40 objective fields of the stool smears were examined.

### Serum collection

Five ml of blood were collected from healthy and infected patients. Blood samples were drawn in sterile plain tubes and left at room temperature for 30 min. Centrifugation was done at 3000 rpm for 5 min (Mettler, Germany). Serum was collected and kept in sterile tubes at deep freeze at -20 until use.

### Serum biomarkers detection

Three human biomarkers were used in this study: Trefoil factor3 (TFF3), Calprotectin (CALP) and (SIgA). All these biomarkers kits were provided from Elabscience Company, Bulgaria and the level of biomarkers in serum were determined by using ELISA device (Human reader, Germany) according to Manufacturer Company.

### Statistical analysis

T-test was used in this study for comparison between samples by using Graph-pad prism version 10 computer software.  $P$ -value less than 0.05 considered statistically significant (Aljanaby AAJ & Alhasnawi HMRJ, 2017).

## RESULTS

Result of study revealed that concentration of (TFF3), (CALP) and (SIgA) in male and female patients infected with *G. lamblia* were significant increase ( $P < 0.05$ ) (0.13907  $\pm$  0.006 ng./ml), (0.20530  $\pm$  0.03), (152.35  $\pm$  5.4 ng./ml), (157.15  $\pm$  14.5 ng./ml), (4.2490  $\pm$  0.5 ng./ml), (5.0586  $\pm$  0.7 ng./ml) respectively in compared to the control group (0.078864  $\pm$  0.005 ng./ml), (0.082629  $\pm$  0.008 ng./ml), (86.981  $\pm$  2.9 ng./ml), (87.701  $\pm$  2.3 ng./ml), (2.2107  $\pm$  0.5 ng./ml), (2.4531  $\pm$  0.6 ng./ml) respectively as seen in fig.1, fig.2 and fig.3 respectively.

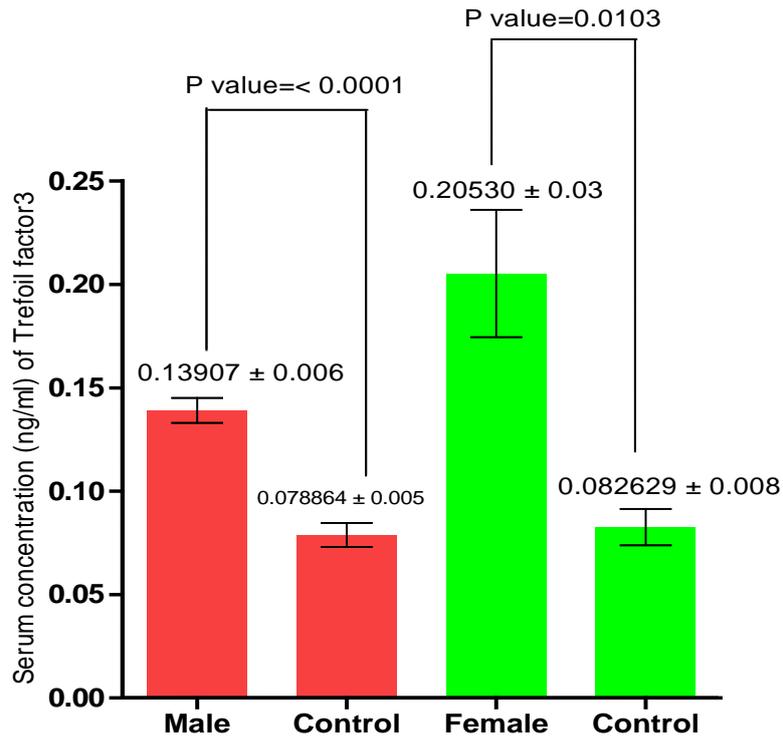


Fig. 1: Concentration of TFF3 (ng/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group.

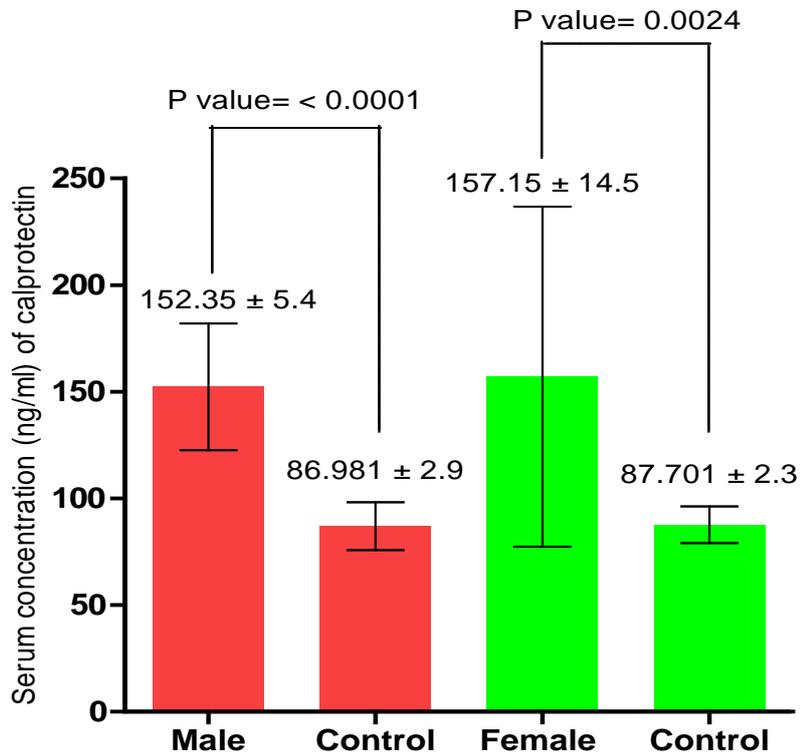


Fig. 2: Concentration of calprotectin (ng/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group.

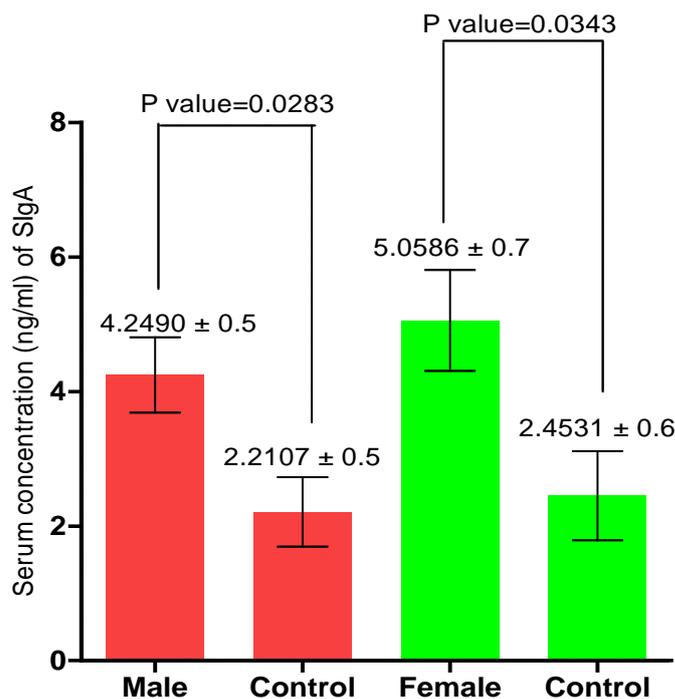


Fig. 3: Serum SIgA (ng/ml) in Control Group and Patients Suffering from *Giardia lamblia* Infection.

#### DISCUSSION

The results of study showed a significant increase in the serum level of Trefoil factors3 (TFF3), Calprotectin (CALP) and secretory IgA (SIgA) in male and female patients infected with *G. lamblia* parasite compared to control group, also in total patients with *G. lamblia* compared to the total control group, the increase of serum levels of TFF3 may be due to essential role of TFF3 in mucosal protection, cell proliferation, and cell migration, the aberrant expression of TFF3 is correlated with gastrointestinal inflammation, clinical diseases and tumor (Van . *et al.*, 2012)

The results of study agrees with study of Susumu *et al.*, (2011) that showed the serum concentration of TFF3 was confirmed to be higher in the gastric cancer patient group and serum levels of TFF3 are a better marker of gastric cancer than pepsinogen.

This increase may be due to goblet cell mucins and TFF3 play important roles in defending the intestinal mucosa against enteropathogen such as *G. lamblia* parasite (Bergstrom *et al.*, 2015). TFF3 can be found *in vitro* and *in vivo* studies indicate that TFF3 facilitates intestinal epithelial restitution, repair and mucosal protection, also TFF3 in the intestine showed increased resistance to intestinal damage and ulceration that results from intestinal infection (Taup, 2003). The intestinal pathogen have physiological effect on mucus layer which plays important physiological roles in the gut by simultaneously lubricating the intestinal surface, limiting passage of luminal molecules into the mucosa, functioning as a dynamic defensive barrier against enteric pathogens by secreting TFF3 and others markers (Deplancke B., & H. R., 2001).

The increase of serum levels of calprotectin may be due to important role of calprotectin which plays important role in the protection of intestinal tissue in addition to its ability to stimulate immunity and body production of cellular motions and the assembly and calling of white blood cells to the site of injury, which stimulates the immune response against infection of *Giardia* parasites, calprotectin is a heterodimer of two calcium-binding proteins present in the cytoplasm of neutrophils and expressed on the membrane of monocytes. Upon neutrophil activation or endothelial adhesion of monocytes, calprotectin is

released and may be detected in serum or body fluids as a potentially useful clinical inflammatory marker Taup (Taup, 2003).

*Giardia* infections may modify host immune responses and have been necessitate to protect against the development of diarrheal disease (James *et al.*, 2015) and due to accumulate and migrate large amount of leucocyte to the lumen in the intestinal inflammation, So Calprotectin synthesis occurs in activated neutrophils, macrophages, epithelial cells and monocytes. Its localization is mainly cytosolic (up to 60% is soluble) but it can also be located into the monocytes membranes. In the context of inflammatory bowel disease, calprotectin produced by mucosal neutrophils and intestinal epithelial cells will mainly be released in the mucosa and the intestinal lumen leucocyte giving arise to immune response (Voganatsi *et al.*, 2001).

This study coincided with the study of Bodil *et al.*, (2017) that dealt with the increase of fecal and serum calprotectin due to increase in neutrophils as are spend to infection, also Mustafa *et al.*, (2009); M.-A. Meuwis *et al.*, (2013); H. E. Mansour *et al.*, (2017) study agrees with the current study.

The increase of serum levels of SIgA may be due to important role of *Giardia* parasite infection in stimulate the immune response and inflammation or accumulation of white blood cells in infection area, also may be due to disorder of host immune response.

Pathophysiological response to *Giardia* trophozoite which disrupts the mucosal surface of intestine and due to the ability of *Giardia* to stimulate immune response to its antigen because of Secretory IgA (SIgA) is the predominant antibody in the intestinal lumen and probably the most important mechanism involved in defense mechanisms against this parasite, and the primary function of SIgA is referred to as immune exclusion, a process that limits the access of numerous microorganisms and mucosal antigens to these thin and vulnerable mucosal barriers. Where the Secretory IgA (SIgA) plays an important role in the protection and homeostatic regulation of intestinal mucosal epithelia, and due to the Clearance by peristalsis that one of the numerous mechanisms whereby SIgA fulfills its function at mucosal surfaces (Blaise,

2013) where the secretory immunoglobulin A (SIgA) antibodies play a central role in the clearance of the parasite (Eckmann, 2003) Even though *G. lamblia* has not often been considered an invasive organism (Katarina *et al.*, 2005).

In addition, the infections may stimulate the production of this immunoglobulin, and an inverse relationship between the levels of this antibody and gastrointestinal symptoms associated with this infection (Walterspiel *et al.*, 1994). In agreement with these findings (Orquídea *et al.*, 2004) and our results demonstrated elevated levels of serum and secretory anti-*Giardia* IgA.

*Giardia* is the most clinical protozoan can cause diarrhea that lead to significant morbidity and mortality worldwide. Giardiasis can be cause of disorder of host immune response Begaydorova *et al.*, (2014) which study reveals a significantly high total serum SIgA in the infected with giardiasis compared to control group that agree with the current study that showed a significant increase in the serum level of secretory IgA in patients infected with *G. lamblia* compared to control group.

### CONCLUSION

The pathogenic effect of *Giardia lamblia* in patients infected with recurrent diarrhea lead to activate of immune response and overproducing of Trefoil factor3 (TFF3), Calprotectin (CALP) and (SIgA) as a good signaling markers against infection.

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