

# Role of Cytokines in Giardiasis

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## Abstract

**Objectives:** The purpose of this study was to determine the relationship of interleukins with *G. lamblia* infection.

**Methods:** Serum interleukins were estimated in 42 patients suffering from giardiasis and 42 apparently healthy controls. Tumor Necrosis Factor (TNF) was also studied in 14 patients and 14 controls.

Interleukins (IL-2, IL-4, IL-10) and TNF alpha were determined by ELISA.

**Results:** IL-4 was present in 14 (33.3%) patients (mean value 220 pg/ml) and 8 (19%) controls (mean value 93 pg/ml). IL-10 was present in 5 (12%) patients (mean value 57 pg/ml) and 6 (14%) controls (mean value 79 pg/ml). IL-2 was present in one patient only but absent in controls. TNF alpha was not detected in patients but was present in 2 (14.2%) controls (mean value 75 pg/ml).

**Conclusion:** Results indicate that IL-4 being an inflammatory regulator appears to have some relationship with probably because *G. lamblia* is a non invasive parasite giardiasis, while TNF alpha was not detected in patients (JPMA 50:113, 2000).

## Introduction

As in other parasitic diseases<sup>1-4</sup> Cytokines may have a role in *G. lamblia* infection. Cytokines or Interleukins are secreted by lymphocytes, monocytes or macrophages. They act on other cells of the immune system to regulate their function. Interleukins cause inflammatory response in parasitic diseases<sup>3</sup> TNF, a mediator of inflammatory response also plays an important role in parasitic diseases<sup>2-4</sup>. This study was done to determine the relationship of cytokines in *G. lamblia* infection.

## Materials and Methods

Forty two patients with abdominal pain, persistent diarrhoea and stool positive *G* for *glamblia*, were selected for this study. Forty two apparently healthy age and sex matched adults from the same socioeconomic group with no diarrhoea were selected as controls. For TNF alpha, 14 patients diagnosed positive for *G. lamblia* and 14 apparently healthy controls were selected. Faecal samples from both groups were screened for *G. lamblia* by direct microscopy and concentration method.

Direct microscopic examination of faecal samples was done in saline and iodine. A drop of saline was placed on one end of the slide and another drop of iodine on the other end. A small amount of faecal sample was emulsified with a toothpick or wire loop to make a smooth thin preparation which was covered with a cover slip and examined under low and high power objectives.

### Concentration method

The faecal parasite concentrator (FPC Evergreen Scientific USA) was used. The methodology was the modification of the accepted formal ether concentration method. The FPC Concentrator consists of a polypropylene tube with an interconnecting precision molded EPC strainer. FPC strainer and the conical tube were assembled and the vent straw pulled out about 1 inch. The function of the straw is to equilibrate pressure in both tubes to help straining. The FPC strainer assembly was screwed firmly and tightened on to the tubes and shaken vigorously for 30 seconds. After putting the conical tube section

downwards the specimen was shaken through the strainer into the conical centrifuge tube. The flat bottomed tube was unscrewed and discarded. The conical tube was spun at 2000 rpm for 2-3 minutes to layer the specimen. With the applicator stick the double layer was removed and the debris and supernatant was poured off leaving the sediment. Few drops of saline were added to mix sediment which was examined under low and high power objectives.

Five milliliters of blood sample was collected from patients positive for *G. lamblia* on direct microscopy of faecal samples and equal number of controls. Serum samples were stored at -20°C for further analysis. Serum Interleukins (IL-2, IL-4, IL-10) and TNF alpha were determined by ELISA (Cytokines Combi Kit DRG Germany).

#### **Detection of Interleukin (IL-2, IL-4 and IL-10) by ELISA method**

Frozen serum samples were brought to room temperature and mixed thoroughly without foaming. Leaving substrate blank well, empty 100ul of standard or sample were added into the antibody precoated well. Plates were gently agitated for 15 seconds to thoroughly mix the contents of each well. The wells were covered with a fresh plastic sealer and incubated at 37°C for 60 minutes in the incubator. Care was taken not to place the plate very close to the side of the incubator to prevent uneven heating.

The sealer was removed and the solution was aspirated from all wells. The wells were washed 3 times with approximately 400 ul of wash buffer with thorough aspiration between washes. The plate was inverted and blotted on paper towel after final wash. Leaving the blank well empty 100 ul of interleukin antibody was pipetted to all other wells. The wells were covered with a fresh plate sealer and incubated at 37°C for 60 minutes. The sealer was removed and the solution was aspirated from the wells. Each well was washed 3 times with 400 ul of wash buffer per well with aspiration between washes. The plate was inverted and blotted on paper towel after final wash. Leaving the blank well empty 100 ul of Horse radish peroxidase (HRP) conjugate Streptavidin and goat anti-rabbit IgG was added to all wells. The plate was covered with the sealer and incubated for 30 minutes at room temperature. The sealer was removed and the sample aspirated from all the wells. The wells were washed 3 times with approximately 400 ul of wash buffer per well with thorough aspiration between washes. The plate was inverted and blotted on the filter paper after final wash. 100 ul of TMB substrate solution was pipetted into each well including blank well and the plate was covered with a plate sealer and incubated for 15 minutes at room temperature. 100 ul of stop solution was added to each well and tapped gently to mix. Absorbance of the wells was read at 450 nm versus substrate blank.

#### **Detection of Tumor Necrosis Factor by ELISA method**

The titre screen enzyme immunoassay was used for the qualitative determination of TNF levels in serum from patients diagnosed positive for *Giardia lamblia* in stool samples.

Leaving the substrate blank well empty 100 ul of standard I to III (0pg/ml, 500 pg/ml and 1000 pg/ml) and serum samples were pipetted onto antibody precoated wells. Serum diluent was used as standard (0pg/ml). After adding the standard and serum samples from patients and controls the plate was gently agitated for at least 15 seconds to thoroughly mix content of each well. The plate was covered with a fresh sealer and incubated for one hour at room temperature. After incubation the sealer was removed and the solution aspirated from all wells. The wells were washed 3 times with approximately 400 ul of wash buffer per well with thorough aspiration between washes. The plate was inverted and blotted on paper towel after final wash. Leaving the blank well empty 100 ul of TNF antibody was pipetted to all wells. The wells were covered with a fresh plate sealer and incubated at room temperature for one hour. The sealer was removed and the solution was aspirated. The wells were washed 3 times with approximately 400 ul of wash buffer per well with thorough aspiration between washes. The plate was inverted and blotted on tissue paper after final wash. Leaving the blank well empty 100 ul of HRP conjugated Streptavidin and goat anti-rabbit IgG was added to all the wells. The plate was covered with a plate sealer and incubated for 30 minutes at room temperature. The sealer was removed and the solution aspirated from all wells. The wells were washed 3 times with approximately

400 ul of wash buffer per well with through aspiration between washes. The plate was inverted and blotted on paper towel after final wash. 100 ul of TMB substrate was added to all wells including blank well. The plate was covered with plate sealer and incubated for 15 minutes at room temperature. After incubation 100 ul of stop solution was added to all wells including blank well and mixed by tapping the plate gently. Absorbance of the wells were read at 450 nm versus substrate blank.

## Results

Results indicate that serum IL-4 was elevated in 14 (33.3%) out of 42 patients (mean and 8 (19%) controls (mean value 93 not significant). IL-10 was estimated in 42 patients and 42 controls. It was detected in 5 (12%) patients (mean value 57 pg/ml) and 6 (14%) controls (mean value 79 pg/ml) as shown in the table.

**Table. Serum Interleukins (IL-4, IL-10) levels in giardiasis.**

		Patients (42)			Controls (42)		
		No (%)	Mean±SD (pg/ml)		No (%)	Mean±SD (pg/ml)	
Interleukin	4	14 (33.3)	220	366	8 (19)	93	57
Interleukin	10	5 (12)	57	14	6 (14.3)	79	28

IL-2 was present in one of 42 patients and absent in controls. TNF alpha being an inflammatory mediator, was absent in 14 patients but present in 2 (14.2%) controls (mean value 75 pg/ml).

## Discussion

Role of cytokines in G.lamblia infection is not clear. G.lamblia normally does not penetrate the epithelial barrier therefore the spontaneous elimination of the parasite depends largely on immune mechanism. Cytokines or other inflammatory mediators may play a role in G.lamblia infection. IL<sup>4</sup> appears to have some relationship in patients with G.lamblia infection as reported by us previously<sup>5</sup>. Chronic diarrhoea and malabsorption produces mucosal inflammation associated with T cell activation and cytokine release<sup>6</sup>. Cytokines were not altered after infection of colonic cell with G.lamblia<sup>7</sup>. As G.lamblia is a non invasive parasite, TNF alpha did not appear to have any role in giardiasis. This is in contrast to patients suffering from amoebiasis where TNF alpha was found because E. histolytica is an invasive parasite<sup>8</sup>. TNF appears to act synergistically with other cytokines<sup>9</sup> and may be of therapeutic benefit in G.lamblia infection<sup>10</sup>.

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