

P-selectin as a biomarker in patients infected with *Giardia lamblia* parasite in Al-Najaf Governorate

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Abstract: The study was conducted on 450 out suspected patients and twenty two healthy persons, whom have meant the laboratory of AL-Hakeem hospital and AL-Zahra maternity and pediatrics in AL-Najaf province from October, 2015 till March 2016. This study was designed to determine the effects of *Giardia lamblia* infection on some biomarkers such as P-selectin, IL-8, IgE, iron and ferritin levels.

Whereas the numbers and percentage of infected patient were 44 (9.77%) vary with different sex 28 (6.22%) male and 16 (3.55%).

The results showed a significant decrease ($P < 0.05$) in serum concentration of IL-8, ferritin and iron in male and female infected with *G. lamblia* parasite in compared to control group, (44.30 ± 1.21 pg./ml), (751.7 ± 32.012) respectively in compared to the control group (13.56 ± 0.721 pg./ml), (1721 ± 89.153 pg./ml) respectively, ferritin was (14.91 ± 1.997) and (20.55 ± 3.6) respectively compared with control group (185.7 ± 52.25) and (180.6 ± 43.09) respectively, iron was (42.18 ± 4.802) and (44.19 ± 8.352) respectively compared with control group (206.5 ± 8.918) and (164.8 ± 38.58) respectively, whereas serum concentration of p-selectin and IgE was significantly increased ($P < 0.05$) in *G. lamblia* infection patients in compared to control group which the level of p-selectin in male and female were (60.65 ± 3.42) and (77.52 ± 3.86) respectively compared with control group (42.3 ± 4.63) and (57.04 ± 4.79) respectively, whereas the level of IgE in male and female were (382.2 ± 55.77) and (519.2 ± 40.45) respectively compared with control group (173 ± 51.2) and (164.8 ± 38.58) respectively. The current study has concluded that the infection with *G. lamblia* effect on some immunological and physiological biomarkers of human represent by IL-8, IgE, iron, ferritin and p-selectin. Also the p-selectin as a good biomarker may be used in detection of *G. lamblia* parasite.

Key word: giardiasis, Najaf, P-selectin, ferritin, IL-8.

Introduction

Giardia lamblia is a pathogenic protozoan that colonizes in the small intestine of humans which attachment strongly to the host intestine and caused severe gastrointestinal disease^{1, 2}. This microorganism a worldwide parasite may be leads to chronic diarrhea and malabsorption of human^{1, 3}. 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 symptoms in host⁴.

Giardiasis is a disease caused by *G. lamblia* parasite and may be acute or chronic infections, several symptoms associated with chronic infections such as vitamin deficiencies, lactase deficiency and fatty diarrhea

as well as cramping intestine irritable bowel syndrome and fatigue⁵. 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^{6,7,8,9} recorded that several pathological changes occur in the small intestine of human resulting in the malabsorption of nutrients similar to different non-infectious intestinal sicknesses like irritable bowel syndrome, celiac Crohn's and disease. Also chronic inflammatory may be lead to caused celiac disease and caused distraction in the mucosa of small intestinal¹⁰. TNF- α play essential role in inflammatory responses and pathogenesis.¹¹

Iron status, vitamin A status and growth cognitive development were influences by this disease^{12,13}. Clinical symptoms of giardiasis depend on different factors such as virulence of the *Giardia* strain, number of mature cysts swallowed, age of the human, and host's immune system¹⁴. The several studies revealed the role of human's giardiasis in nutrient malabsorption and micronutrient deficiencies such as zinc, vitamin B-12, vitamin A and iron^{15,16,17}.

P-selectin is essential protein of the selectin family of cell adhesion receptors expressed by platelets belong to selectin family which aid in initial attachment between the leukocytes and the activated endothelium^{18,19,20} shows that immune response against the infection depend on the recruitment of leukocytes from the bloodstream to sites of injury.

Interleukin-8 as a cytokine belong to chemokine Family has been suggested to contribute in chronic inflammation and development of cancer disease, when IL-8 stimulation normal neutrophil adhesion and motility by Paxillin²¹. The growth of extracellular parasite and disease progression may be assistance IL-10 and IL-4, the various host defense induced by several external stresses and environmental stimuli such as exposure to heavy metals, inhalation of foreign materials, infections and pathogens^{22,23}. The current study aimed to investigate the effect of *G. lamblia* infection on some immunological and physiological biomarkers such as p-selectin, IL-8, IgE Ferritin, and iron by ELISA technique in patient with giardiasis and healthy as control group. Iron absorption may be increased in presence of vitamin C but continuous increase unnecessary for absorption^{24,25}. Iron is the important metal for brain functions and loss caused neuronal injury²⁶ and it is the chief heavy metal found in environment and low cost removal from the synthetic metal solutions^{27,28} who recorded the relationship between the concentration of iron and ferritin and transferrin storage in the liver cells. Whereas²⁹ conducted iron metabolism influences by vanadium levels deficiency.

Material and Methods

The subjects

The study conducted on 450 suspected patients with *Giardia lamblia* parasite and 22 healthy peoples as control groups vary on 12 male and 10 female. The collection of samples was approved by the institutional ethics committee of the faculty of science at the University of Kufa and all participants signed informed consent forms. All suspected samples are examined by wet mount microscopic method these samples collected from suspected patients when attended to AL-Zahra maternity and pediatrics and AL-Hakeem hospital in AL-Najaf province from August to January 2015.

Wet mount Examination

Freshly voided stool specimens were processed and examined microscopically using X40 objective lens for intestinal parasites as described by³⁰. Before a slide was considered negative, ten X40 objective fields of the stool smears were examined.

Blood Specimens collection

From October, 2015 till March 2016, 44 samples were collected from patients and 22 healthy (vary on 12 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 and remains for 30 minutes at room temperature. After that the samples were centrifugation at 3000 rpm for 5 minutes (Backman/counter, Germany) to separate the serum and collected in other sterile tubes, each sample of

serum was divided into five parts; each of them was kept in deep freeze at -20C° till used for the determination of IL-8, iron, p-selectin, ferritin and IgE.

The Kits

The biomarkers in the current Study were estimated by Eliza Kits such as Human p-selectin (SELP) ELISA/ Kono Biotech/Bulgaria (catalogue number KN0432Hu), Human Interleukin8 (IL-8) ELISA Kit/ Kono Biotech/ Bulgaria (catalogue number KN0923Hu), Immunoglobulin E(IgE)ELISA Kit/ Kono Biotech/ Bulgaria (catalogue number T1244A(69 Tests), Ferritin ELISA/ Monobind/ USA(product Code:2825-300) and spectro Kits such as Iron/ Biolabo/ France (02160Maizy France).

Statistical analysis

Data were analyzed using the software packages Graph pad prism for Windows (5.04, Graph pad software Inc. USA), Data are presented as the mean ± standard error (SE). The comparison between the patients and control groups were analyzed by student t- test. As well as the correlations between parameters were performed by Pearson's correlation coefficients (r). A p-value < 0.05 was considered significant.

Results and Discussion

Interleukin – 8 (IL – 8)

Result of study revealed that concentration of (IL-8) in male and female patients infected with *G. lamblia* were significant decrease (P< 0.05) (44. 30 ±1.21 pg /ml), (751.7± 32.012) respectively in compared to the control group (13.56 ± 0.721 pg /ml), (1721± 89.153 pg/ml) respectively,as seen in figure 1.

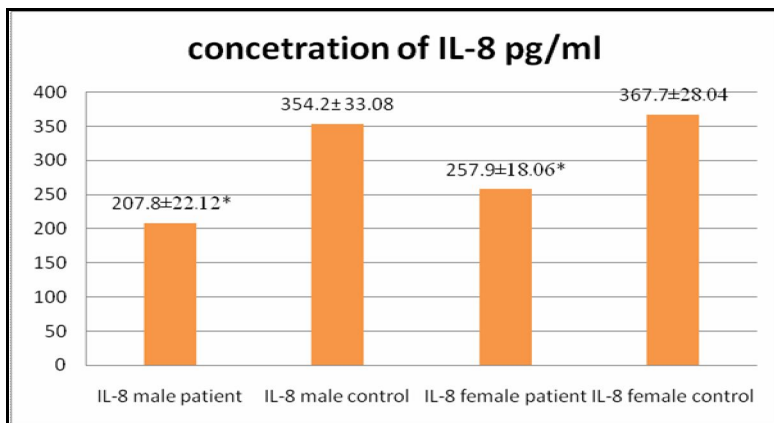


Figure.1. Concentration of IL-8 (pg/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group.

* Significant difference P<0.05 between control group and patients

Serum iron

The statistical analysis of the current study showed a significant decrease (P< 0.05) in serum iron concentration in male and female patients with *G. lamblia* infection (42.18± 4.802) and (44.19± 8.352) respectively compared with control group (206.5 ± 8.918) and (164.8± 38.58) respectively; as seen in Figure 2.

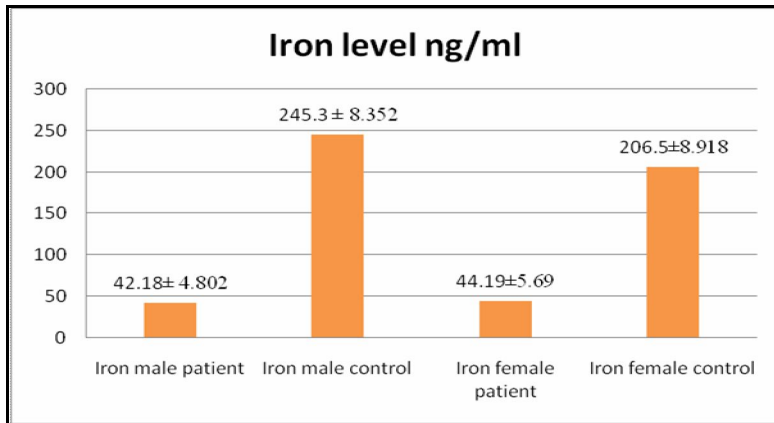


Figure.2. Concentration of iron (ng/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group.

* Significant difference $P < 0.05$ between control group and patients

Serum ferritin

The statistical analysis exhibited significant decrease ($P < 0.05$) in serum levels of ferritin in in male and female patients (14.91 ± 1.997) and (20.55 ± 3.6) respectively compared with control group (185.7 ± 52.25) and (180.6 ± 43.09) respectively, infected with *G.lamblia* parasite, as seen in Figure 3.

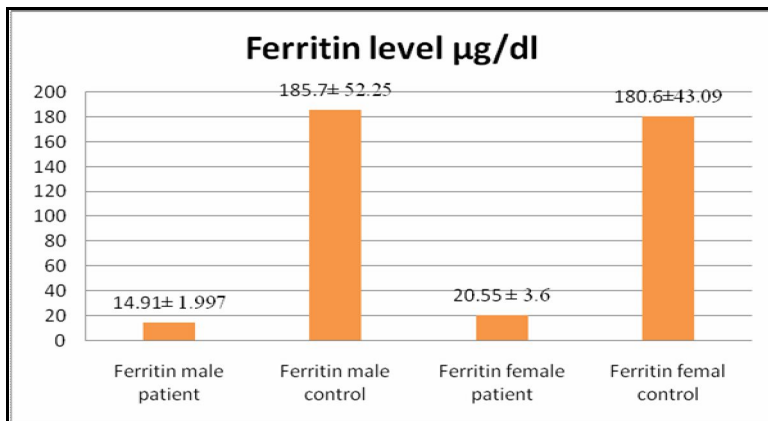


Figure.3. Serum Ferritin (µg/dl) in Control Group and Patients Suffering from *Giardia lamblia* Infection.

* Significant difference $P < 0.05$ between control group and patients

P-selectin

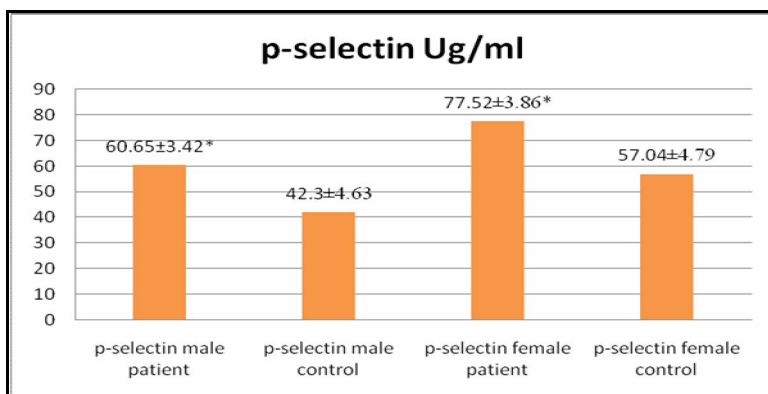


Figure.4. Concentration of p-selectin (Ug/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group

* Significant difference $P < 0.05$ between control group and patients

The current study revealed that concentration of p-selectin in patients infection with *G. lamblia* were significant increase ($P < 0.05$) in male and female patients (60.65 ± 3.42) and (77.52 ± 3.86) respectively compared with control group (42.3 ± 4.63) and (57.04 ± 4.79) respectively, as seen in figure 4.

The Immunoglobulin IgE in Patient and Control Group:

The results of present study as shown in figure 5 revealed that the concentration of immunoglobulin E was significant increase ($P < 0.05$) in male and female patients (382.2 ± 55.77) and (519.2 ± 40.45) respectively compared with control group (173 ± 51.2) and (164.8 ± 38.58) respectively.

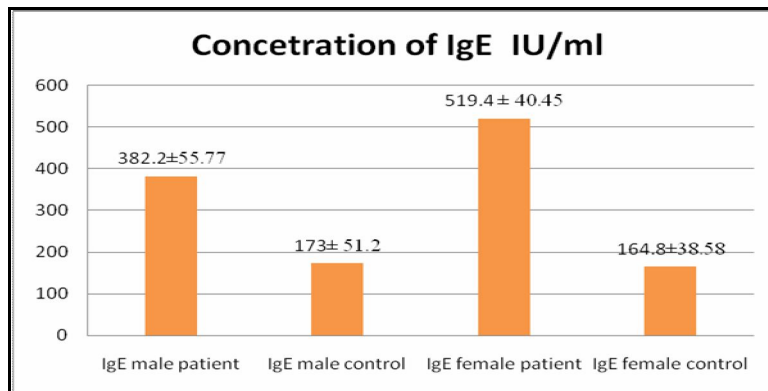


Figure.5. Concentration of IgE (IU/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group.

* Significant difference $P < 0.05$ between control group and patients

The results showed a significant decrease ($P < 0.05$) in serum concentration of IL-8, ferritin and iron in male and female infected with *G. lamblia* parasite in compared to control group, whereas serum concentration of p-selectin and IgE was significantly increased ($P < 0.05$) in *G. lamblia* infection patients in compared to control group.

This may be due to the impairment of cell-mediated immune response leading to decrease cytokine production by immunologically effector cells which is characterized produce cytokines leading to further damage of the host defense against infection this in turn badly affects all the biological processes in with IL-8 is involved in particular, activation of neutrophils and chemotaxis of different leukocytes.

This result agree with³¹ which was showed that IL-8 decreased in patients with giardiasis compared to control group, also indicate that *G. lamblia* trophozoites produce some material which act as immunomodulatory factors that may inhibit proinflammatory intestine response in *G. lamblia* infected individuals.

Study achieved by³² showed that the patients with cryptosporidium have significant decrease in the concentration of IL-8 compared to control group. But³³ indicate that no increase in IL-8 concentration which indicates the minor role of Th2 cytokines in immune response to giardiasis, IL-8 is the chief cytokine immune response to giardiasis which is accountable for the call neutrophil to the place of inflammation. Trophozoites of *G. lamblia* parasite stimulated neutrophils and monocytes to produce of Interleukin-8³⁴.

Also^{35,36} showed that these chemokines may have no influence on immunity to giardiasis and explain the chronic nature of this disease as *G. lamblia* usually extracellular parasite does not able to penetrate the epithelial layer therefore removal this parasite depends on the immune response of the host. Decrease in the serum level of iron in patients infected with *G. lamblia* may be due to significant effect of giardiasis on iron malabsorption as it infects the duodenum the main site of iron absorption another possible reason for this significant change may be due to the possible high load of parasites in the intestine³⁷. The result of study agree with study of^{38,39} whom showed reduced iron absorption and reduced iron levels in children with symptomatic giardia in Turkey and Egypt respectively showed iron level were decreased during giardiasis due to malabsorption these conclusion were also suggested in a rat model⁴⁰.

⁴¹shows over on quarter 26.4% of the children were identified as having iron deficiency anemia also⁴² showed through his study among children from endemic areas of intestinal parasitic infection that the population was found to have iron deficiency and appeared to be the dominant cause of anemia.

In the ⁴³study, parasite infections were insignificantly associated with anemia which was found in only 12.3% of girls infected with *G. lamblia* and in the ⁴⁴study malabsorption of iron was reported in the children with symptomatic giardiasis, however asymptomatic giardiasis did not affect the intestinal absorption of iron but⁴⁵ showed in endemic setting there was no evidence that giardia infection impair iron status.

The decrease in serum ferritin level in both male and female infected with giardiasis may be due to depletion in iron stores in body as result of chronic giardia infection where the mean concentration of serum ferritin reflects the iron body stores. This result corresponds with study of⁴⁶ that showed decrease in ferritin level in patient with *G. lamblia* compared with control group.

Also ⁴⁷ showed the level of iron and ferritin in both human and animals are significantly decreased in giardiasis infection. Study achieved by⁴⁸ showed lower level of iron and ferritin in patients with giardiasis as result of damage to the intestinal mucosa. In other intestinal parasitic infection showed decrease in ferritin level in children infected with *E. histolytica*⁴⁹. Also ⁵⁰ who showed that ferritin was in it is lower level in children infected with some intestinal parasites such as *Ascaris* and *Trichuris*.

⁵¹Who show that ferritin serum concentration was higher in infected children with *G. lamblia* than non-infected. Also⁴⁵ showed that the levels of ferritin in infected children with giardiasis are higher than non-infected children. The difference between this result and other result which showed increased level of ferritin may be due to age and sample size.

P-selectin may be the predominant and endothelial selectin involved in recruiting leukocytes into chronic inflammation lesions^{52,53}. The result of study revealed that the concentration of p-selectin is significantly increased in serum of patients infected with *G. lamblia* compared to control group this may be due to the host response to giardiasis infection that requires increased expression of cell adhesion molecules in order to achieve their role in recruitment of effector cells to the site of infection, several studies have underscored the importance of p-selectin in leukocyte homing to the inflammation tissues^{54,55,56}.

Blocking p-selectin in experimental infection by leisteria monocytogenes result in altered lymphocyte population in the gut⁵⁴. ⁵⁶Demonstrated that p-selectin facilitated T-cell migration to the site of infection blocking p-selectin had no effect on parasite replication or immunity to reinfection by *Leishmania major*.

Participation of p-selectin in transient neutrophil attachment to endothelium under conditions of flow was previously suggested by *in vitro* studies by⁵⁷ who recorded that neutrophils could roll on artificial lipid bilayers comprising p-selectin but not on bilayers having ICAM-1 a member of the Ig superfamily. In study achieved by⁵⁸ shows that inhibit human p-selectin expression suggest an important mechanism for down-regulation of the quantity of leukocytes entering the tissues during chronic immune mediated inflammation response. ⁵⁹Also showed in his study that the p-selectin is increased in patients with cryptosporidium infection compared to control group.

In some parasitic infection hypersensitivity reaction may occurs at response to antigens of enteropathogens and produce of IgE and lead to diarrhea production which help in parasite annihilation. The result of study show elevation serum level of IgE in *G. lamblia* patients in compared to control group, this result was agreed with^{60,61} whom showed that the patients with symptomatic giardia infection have elevated IgE levels in their serum.

⁶²Also showed in his study, elevated IgE levels are an appearance of amplified immunization of the host in giardiasis and elevation of IgE in patients in compared to healthy. The study of⁶³ was showed in children with giardiasis, increase in total serum immunoglobulin E level compared to control group.

⁶⁴Was showed in many parasitic infections the eosinophils and the antibody-dependent cell cytotoxicity may be the essential mechanisms against the parasite which may cause the obstruction the receptors on the outer surface the parasite induce direct destruction of the parasite via increase IgE or activation of the

complement system. The effect of excretory and secretory antigens of *G. lamblia* is observed an increase in the total IgE level as well as systemic and local stimulation of IgA secretion⁶⁵.

In study achieved by⁶⁶ indicated was IgE activates platelets and induces cytotoxic functions against parasites the excretory and secretory proteins released by *G. lamblia* are responsible for production of specific IgE and activation of eosinophil^{67,68} showed increase in IgE in patients with giardiasis and mechanism of parasite to cause starting out IgE synthesis was not yet been understood.

Conclusions

1. The infection with *Giardia lamblia* causes increased in concentration level of p-selectin, IL-8 and IgE
2. *Giardia lamblia* has an important role in change of iron and ferritin levels in patients infected with this parasite.
3. P-selectin is an important biomarker may be used for detection of *Giardia lamblia* in patients infected with this parasite.

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