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Study of P-selectin and some markers in patients with *Giardia lamblia* parasite

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Abstracts

The study was conducted on 60 patients and 30 healthy as control group to determine the influences of infected with *Giardia lamblia* on levels of p-selectin, ferritin and IL-5, patient infected with *G. lamblia* in compared with healthy group. Who have visited Al-Sadder medical city and Al-Hakeem Hospital in Al- Najaf AL-Ashraf Province during the period from October, 2015 till March 2016. Diagnosis infection with this parasite by using the wet amount microscope for stool from patients. The results showed significant increase (P<0.05) in p-selectin and IL-5 in *G. lamblia* infection patients in compared to control group**.** Whereas the results showed a significant decrease (P<0.05) in serum concentration of ferritin in *G. lamblia* infection patients in compared to control group.

Key word: *Giardia lamblia*, P-selectin, ferritin, IL-5

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 symptomates in host[4] .

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 fatigue [5]. 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 iron status, vitamin A status and growth cognitive development were influences by this disease.[10], [11]

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 system [12]. 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 [13-15] .

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 [16],[17] [18] 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 Paxillin [19]. The current study aimed to investigates 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.

MATERIAL AND METHODS

Wet mount Examination

Freshly voided stool specimens were processed and examined microscopically using X40 objective lens for intestinal parasites as described by [20]. 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, 60 samples were collected from patients and 30 healthy 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 three parts; each of them was kept in deep freeze at -20C° till used for the determination of IL-5, p-selectin and ferritin.

The Kits

The biomarker s in the current Study were estimated by Eliza Kits such as Human p-selectin (SELP) ELISA/ Kono Biotech/ Bulgaria (catalogue number KN0432Hu), Human Interleukin 5 (IL-5) ELISA Kit/ USBIO/ U.S.A (catalogue number L14103161), Ferritin ELISA/ Monobind/ USA(product Code:2825-300) .

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

P-selectin

The current study revealed that concentration of p-selectin in patients infection with *G. lamblia* were significant increase (P< 0.05) patients (62.923± 0.672) compared with control group (40.421 ± 0.538) as seen in figure (1).

Serum ferritin

The statistical analysis exhibited significant decrease (P< 0.05) in serum levels of ferritin in patients infected with G. *lamblia* parasite (12.312± 0.651) compared with control group (25.106 ± 0.832) as seen in Figure (3).

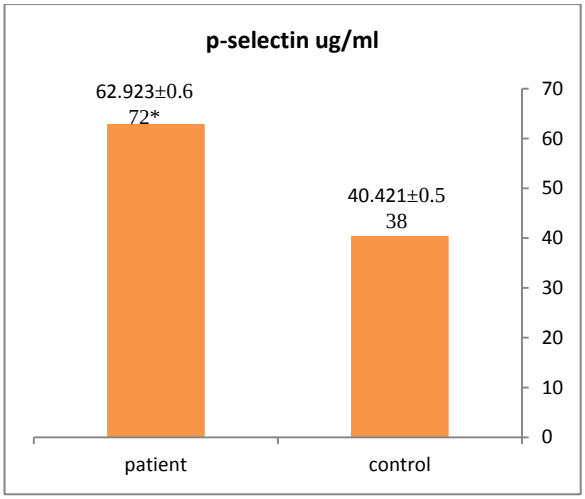

Figure 1: Concentration of p-selectin (Ug/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group

* Significant difference P<0.05 between control group and patients

Interleukin – 5 (IL – 5)

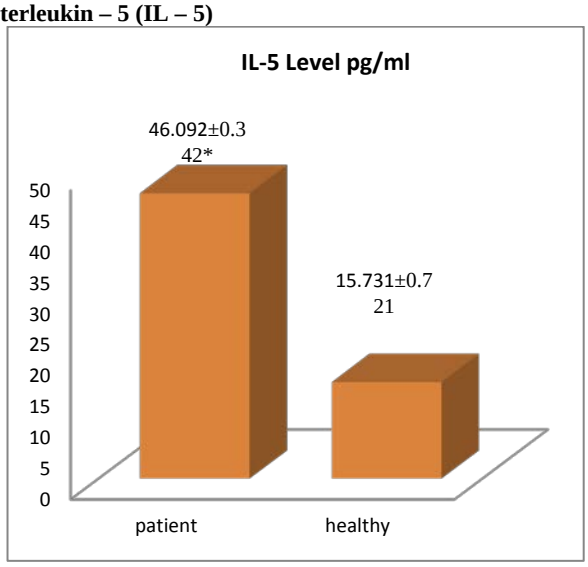

Figure 2: Concentration of IL-5 (pg/ml) Comparison between Patients Suffering from *Giardia lamblia* Infection and Control Group.

* Significant difference P<0.05 between control group and patients

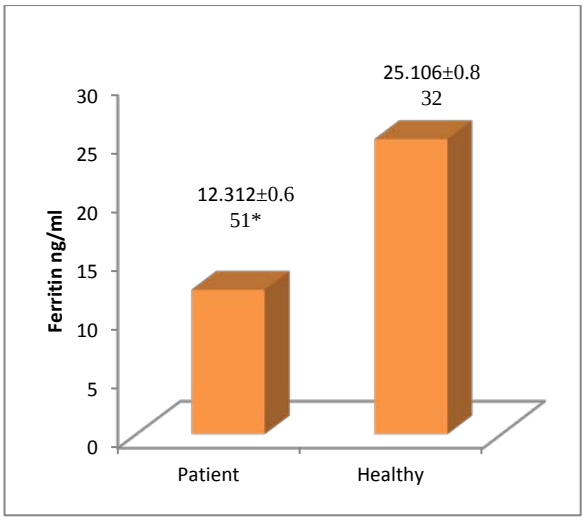

Figure (3): Serum Ferritin (ng/ml) in Control Group and Patients Suffering from *Giardia lamblia* Infection.

* Significant difference P<0.05 between control group and patients

DISCUSSION

The results showed a significant decrease (P<0.05) in serum concentration of ferritin and in infected with *G .lamblia* parasite in compared to control group, whereas serum concentration of p-selectin and IL-5 were 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.

In parasite infection Interleukin- 5 is produced by eosinophil and mast cell. It triggers the activation, differentiation, growth and chemotaxis of eosinophils. This may be due to a pathogenic role of parasite infection, also causes increase in intestinal necrosis (Nickdel, 2001). The presence of eosinophils in human congenital toxoplasmosisis probably related to the production of IL-5[21] (Nakazaki, 2000).

Nickdel (2001) reported that tumer necrosis factor-α and interleuken-5 produced by macrophages, mast cells and lymphocytes, and these cytokines increase the cytotoxic activity of eosinophils. Many specific cytokine synthesize by Th2 lymphocytes such as "(IL-4, IL-5, IL-6, IL-10, IL-13, and IL-14)" the major cytokine responsible for the increase in the eosinophil population in parasitoses is interleukin-5 and play an important role in the pathogenesis of parasitic diseases[22]

Also[23],[24] 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 intestine [25]. The result of study agree with study of [26],[27] 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 model [28] .

The results[29] show over on quarter 26.4% of the children were identified as having iron deficiency anemia also[32] 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 [30] study, parasite infections were insignificantly associated with anemia which was found in only 12.3% of girls infected with *G. lamblia* and in the [31] study malabsorption of iron was reported in the children with symptomatic giardiasis, however asymptomatic giardiasis did not affect the intestinal absorption of iron but [32] showed in endemic setting there was no evidence that giardia infection impair iron status.

The decrease in serum ferritin level in patients 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 of [33] that showed decrease in ferritin level in patient with *G. lamblia* compared with control group.

Also [34] showed the level of ferritin in both human and animals are significantly decreased in giardiasis infection. Study achieved by [35] showed lower level 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* [36]. Also [37] who showed that ferritin

was in it is lower level in children infected with some intestinal parasites such as Ascaris and Trichuris.

[38] Who show that ferritin serum concentration was higher in infected children with *G. lamblia* than non-infected. Also [33] 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 [39], [40]. 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 [41],[42],[43] Blocking p-selectin in experimental infection by leisteria monocytogenes result in altered lymphocyte population in the gut [44]. [45] Demonstrated 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 by[46] 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 by [47] 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. [48] Also showed in his study that the p-selectin is increased in patients with cryptosporidium infection compared to control group.

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