ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Lactferrin as Biomarker in Patient Infected with Giardiasis.

Saleem Khteer Al-Hadraawy[1]*, Ali Hassan Abood[1], Shaymaa Rahim Hussein AL-saabar[2], and Kias Khteer Al-Hadraawy[3].

[1]Faculty of sciences / university of Kufa, Iraq.
[2]College of education for women / university of Kufa, Iraq.
[3]AL-Sadder city of medicine, Iraq.

ABSTRACT

This study amid to evaluated of some biomarker such as Lactferrin, ferritin and iron in male infected with *Giardia lamblia* in compared with control group. From 30 healthy male and 60 out patients stool samples were collected and diagnosis this parasite by using the wet amount microscope who have visited Al-Sadder teaching and Al-Hakeem Hospital in Al- Najaf Province during the period from August till march 2015. also serum sample collected from the same patients and control to estimated the Lactferrin, iron and ferritin. Results of study recorded significant decrease ($P<0.05$) in Lactferrin, iron and ferritin in *G. lamblia* infection patients in compared to control group.

Keywords: lactoferrin, Giardiasis, iron, Al-Najaf

Corresponding author

ISSN: 0975-8585

INTRODUCTION

In developed countries infection with *Giardia lamblia*is associated with type of water sources and nutrition, Giardiasis accounts for a high proportion of water-linkage and eruptions with disease of parasite[1],[2]. Giardiasis is endemic disease and have relalionship with contamented of water and food with infected stage (mature cyst) that found in feaces of infected human. Many Socio- factors example poor individual cleanliness and absence of right hygiene have been recognized as important danger aspects [3-5]. Classical identification by wet mont examination of stool specimens to detected and found of two stages of Giardia in the feces [6, 7]. There is another method that used for diagnosis of *Giardia lamblia* as small intestinal biopsy which is high sensitive but it is risky method [8].

Lactoferrin is classified as iron-binding protein from the transferrin family has been registered to have several affects. Insipite of this biomarker was first diagnosis in milk, Lactoferrin also present in granules of neutrophils and in exocrine secretions, Lactoferrin identified as anti-inflammatory activity and antimicrobial factor in host defense against infection [9].[10]reported that kidneys are chiefly source of production of human Lactoferrin. Also[11],[12] described that Lactoferrin may be saturated with iron therefore iron from external environment fully saturated this biomarker.

A. Specimens

From August till march, 2015, 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 ,Stool samples collected in clean wide-mouth specimen bottles from 30 control male and 60 male patients "who attended the clinics in AL-Sadder teaching and AL-Hakeem Hospital in AL-Najaf province" and blood samples from patients has been drawn into serum tubes and stayed for 30 min. at 25C º. and centrifugation at 3000 rpm for 5 min. (Backman/counter, Germany**).** The serum preserved at -20Cºtill used for the determination of iron, ferritin and Lactferrin.

B. Specimen processing

Stool examined by light microscopic using X40 objective lens for intestinal parasites as described by (Paniker, 1989)[13].

C. Serum Iron (Colorimetric Test)

The colorimetric test method was used to estimate the serum of iron via RANDOX reagents, code HB012. (RANDOX Kit, U.K) by cypress diagnostics biochemistry analyser.

D. Ferritin ELISA

This test was intended to quantify the serum levels of ferritin through the immunoezymatic technique Enzyme-Linked Immunosorbent Assay (ELISA) using bio Elisa reader ELx 800 (bio kit, U.S.A.) .The human Accu Bind ferritin ELISA kit was achieved according to the manufacturing company (Monobind Inc , U.S.A.) .

Procedure:

1. The components of the kit were equilibrated at the room temperature before use.
2. 25µ l of standard, controls and sample was added per well.
3. 100 µ l of Biotinylated ferritin Antibody was added to each well. Wells were covered with a sealing tape and incubated for 30 minutes. The timer was started after the last sample addition.
4. The micro plate was washed six times with 300 ml of wash buffer using bioeliser washer ELx 50 (bio kit, U.S.A).
5.100 µ l of ferritin Enzyme Reagent was added per well and incubated for 30 minutes. The bio Elisa reader ELx 800 (bio kit, U.S.A.) was turned on and set up the program in advance.
6. The micro plate was washed as described above.
7. 100 µ l of working substrate solution was added per well and incubated for about 15 minutes or until the optimal blue colour density develops.

ISSN: 0975-8585

8. 50 µ l of stop solution was added to each well. The colour will change from blue to yellow.
9. The absorbance on bio Elisa reader EL x 800 was read at a wave length of 450 nm immediately. Results were provided within 1 minute on the LCD display and printed out.

C. Human Lactoferrin (ng/ml) ELISA kit

Procedure:

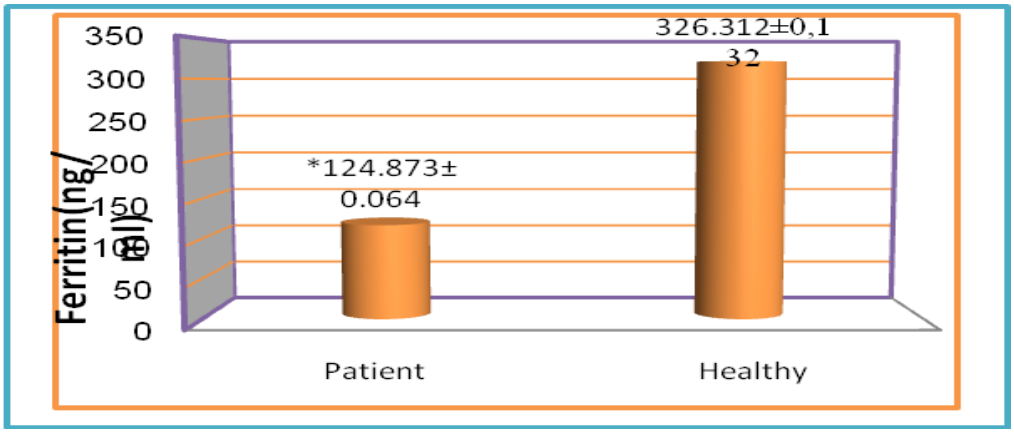
1- according to quantity to be tested sample and the standard, the quantity of the strips determined. You should be made every sample according to your required quantity, also attempt to use the duplicated wells for samples.
2- Set standard wells, test sample wells and blank wells
a-The Sample and Horseradish peroxide (HRP) do not add to blank wells.
b-50 µl of standard added to standard wells.
c-40µl of sample diluent added to test sample wells and then 10µl of sample also added (The final sample dilution is five times and the final result calculation should be multiplied by five times).
d-Except blank well. Add to each well 50µ of horseradish peroxide (HRP). Then the plate is sealed and gently shake, then incubate for 60 minute at 37°C.
3- Remove excess liquid, drying , each well is filled with diluted washing liquid , then mix up and shaking for thirty second , remove the washing liquid and then tap the plate into absorptive papers to dry .Repeat five time and then pat dry.
4- To each well added 50µl of chromogen solution A and then 50µl of chromogen solution B is also added, softly shake and incubated away from light for ten minutes at 37°C.
5-To stop the reaction 50µl of stop solution is added to each well (the blue changes into yellow).
6-Final measurement: set blank well zero. At 450 nm wavelength, the optical density is measure after 15 minute from adding the stop solution.
7- Calculate out the standard curve linear regression equation, according to the corresponding OD values and standards concentration and then apply the OD values of the sample on the regression equation to calculate the corresponding samples concentration. It is suitable to use a diversity of software to make calculations.

F. Statistical analysis

The software(Graph pad prism 5.04,USA) used to analyzed data of research, whereas (SE) as the mean ± standard error and one-way ANOVA used to appear the different between the patients and healthy groups. So significant difference as p-value < 0.05.

RESULTS

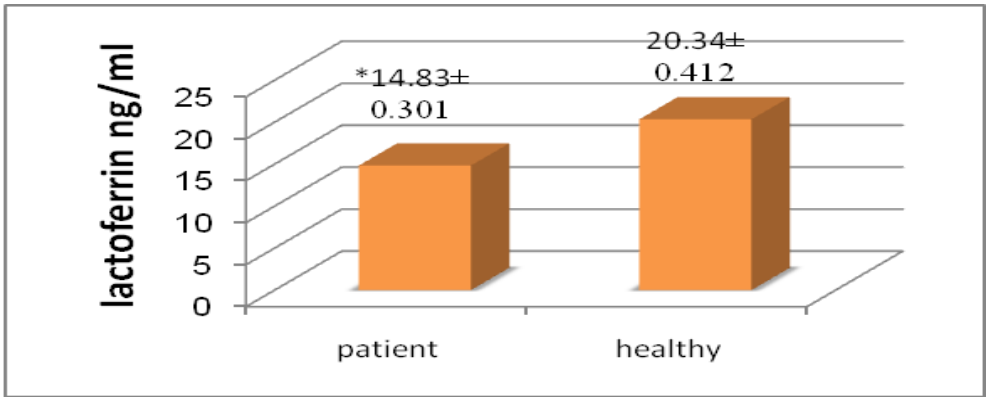
Figure 1: Comparison between Serum Iron in healthy Group and Patients Suffering from *Giardia lamblia* Infection.



***Significant difference (P<0.05) between control group and patients.**

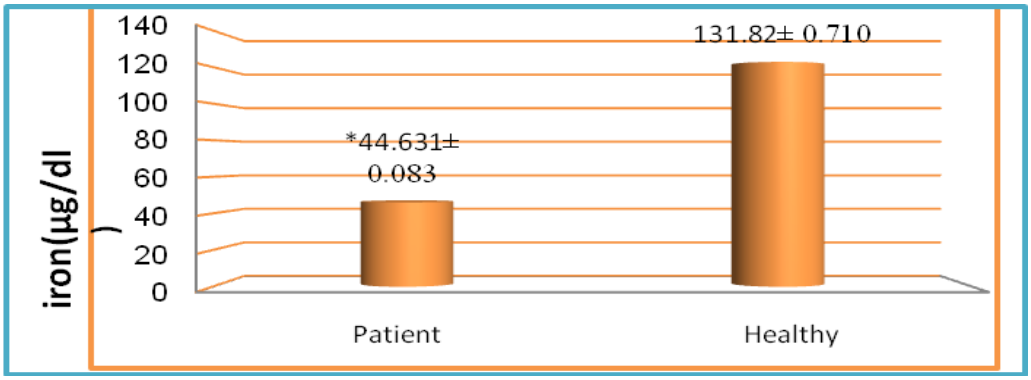
ISSN: 0975-8585

Figure 2: Serum Ferritin in Healthy Control Group and in Patients Suffering from *Giardia lamblia* Infection.



Significant difference (P<0.05) between control group and patients.

Figure 3: Serum Lactoferrin in Healthy Group and in Patients Suffering from *Giardia lamblia* Infection.



Significant difference (P<0.05) between control group and patients.

The statistical analysis of the results showed a significant decrease ($P < 0.05$) in serum Lactoferrin, ferritin and iron concentration of patients with *G. lamblia* infection (14.83± 0.301), (124.873± 0.064) and (44.631± 0,083) respectively compared to the control group (20.34± 0.412), (326.312± 0.132) and (131.82± 0.710) respectively, as seen in Figure (1), (2) and (3).

DISCUSSION

This study showed that there is a decrease of, iron, ferritin and Lactoferrin in *Giardia lamblia* infection patients compared to healthy group. The decrease in level of iron and ferritin may be due to blood loss in the gastrointestinal or from impaired absorption of iron in patients with *G. lamblia* [14],[15].

The result has revealed that the serum iron and ferritin significantly decrease in *Giardia lamblia* infection patients compared to control group. The decrease in iron level in patients with *G. lamblia* may be due to the pathogenicity of this parasite dependent on the relationship between iron concentration and adhesion of parasite on epithelial cell or due to malabsorption [16].

The consuming of iron by *G. lamblia* may cause a decrease in the iron levels. The decrease in ferritin levels may be due to an increase in consuming iron by this parasite and this leads to decrease in the storage of iron as ferritin or increased utilized by parasite whereas some studies describing *G. lamblia* as an iron source [17]. As confirmed by the data from[18] that *G. lamblia* use the ferritin as an iron source; therefore, ferritin decreased when an infectious process occur. The current study agrees with the study of [18] who recorded that the *G. lamblia* uses the ferritin as source of iron and these lead to decrease in the ferritin in serum of

patients infected with *G. lamblia* compared to control group. Several mechanisms may be used by pathogens to get iron from the host holo-lactoferrin [19].

During parasitic infection that lactoferricin caused penetrated parasitic membrane than, integrity causing subsequent changes in interactions between the host and the parasite [9].

Some protozoan parasites able are able to use Lactoferrin as source to ferric ions [20]. Iron is essential as a catalyst for the production of reactive oxygen species. Therefore, Lactoferrin can diminish the harmful influence of reactive oxygen species produced by leukocytes at the sites of inflammation [21].

Several factors influences on the absorption of nutrients such as intestinal acidity, intact mucosal surface and malnutrition [22],[23].Anemia caused by celiac disease reflecting reduced absorption of essential nutrients like iron and various vitamins [24].

This results agreement with study of [25] which recorded those levels of iron and ferritin were lower in patients with giardiasis in compared with healthy group.

The N-terminal cationic amino acids may also be responsible for some of the antiparasitic effects of Lactoferrin although not specifically demonstrated for major parasitic enteropathogens such as *Giardia* or *Entamoeba histolytica*.

REFERENCES

[1] Kourenti, C., Karanis, P., Smith, H.,.J. Water Health 2007 ;92: 361–365.
[2] Baldursson, S., Karanis, P., Water Res 2011, 45: 6603– 6614
[3] Prado, M.S., Strina, A., Barreto, M.L., Oliveira-Assis, A.M., Paz, L.M., Cairncross, S., Brazil. Epidemiol. Infect. 2003;131: 899–906.
[4] Faustini, A., Marinacci, C., Fabrizi, E., Marangi, M., Recchia, O., Pica, R., Giustini, F., LaMarca, A., Nacci, A., Panichi, G., Perucci, C.A., Epidemiol. Infect. 2006. 134: 649–658.
[5] Naelah, A.A., Mohammed, A.K.M., Rohela, M., Lim, Y.A.L., Yemen. PLoS One 2011;6: e22044.
[6] Wolfe MS Plenum, New York1984;pp 147–161