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Biomarkers evaluation for patients infected with *Entamoeba histolytica* parasite.

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ABSTRACT

The study was conducted on 380 out suspected patients and twenty healthy persons, whom have visited the laboratory of AL-Hakeem hospital and AL-Zahra maternity and pediatrics in AL-Najaf province from August, 2015 till January 2016. This study was designed to determine hemopexin, Lactoferrin as biomarkers of detection and effect of *Entamoeba histolytica* on levels of iron and ferritin. Yet the blood parameters gave a clear understanding to physicians to cure patients. Whereas the numbers and percentage of infected patient vary with different groups male 14 (3.68%), female 10 (2.68%) and 20 (5.26%) children, also the highest infected number and percentage of infected patient was 20 (5.26 %) respectively at the age (10-19) years and that the lowest at the age (40-49) years was 3 (0.78%) respectively. In infected male, female and children the results have also revealed that the hematological parameters of red blood corpuscular, Hemoglobin, Platelets count and red cell indices: mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin and packed cell volume were significant decreased ($P<0.05$) in patients infected with *E. histolytica* in compared to control group. The results showed a significant decrease ($P<0.05$) in serum concentration of hemopexin , Lactoferrin, ferritin and iron in male, female and children(32.86±1.687 ng/ml),(18.84± 2.275 ng/ml) and (62.04± 11.04 ng/ml) respectively in *E. histolytica* infection patients in compared to control group (46.99± 2.245 ng/ml),(26.96± 1.553ng/ml) and (79.73± 21.89 ng/ml) respectively. Lactoferrin (11.43± 0.5924ng/ml), (11.98±.8105ng/ml) and (14.04±3.713ng/ml) respectively in compared to control group (18.85±0.4908ng/ml), (20.72± 0.5944 ng/ml) and (24.07± 2.794 ng/ml) respectively. Ferritin (57.81± 5.31 ng/ml), (41.84 ng/ml) and (59.45± 1.61 ng/ml) respectively in compared to control group (163.6± 2.53 ng/ml), (89.44± 2.14 ng/ml) and (184± 1.33 ng/ml) respectively. Iron (128.5± 16.44 µg/dl), (98.61± 11.11 µg/dl) and (46.27± 6.592 µg/dl) respectively in compared to control group (179.9± 36.18 µg/dl), (224.4± 21.18 µg/dl) and (131.6± 27.3 µg/dl) respectively. There were positive correlations between hemopexin with ferritin and iron levels. The current study has also revealed positive correlation between Lactoferrin with ferritin and iron. The results have also exhibited a positive correlation between ferritin and iron. The current study has concluded that the infection with *E. histolytica* effect on blood parameter and iron status. Whereas the hemopexin and Lactoferrin as biomarker for infection with *E. histolytica*.

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INTRODUCTION

Entamoeba histolytica is a gastrointestinal protozoon caused amoebiasis disease for persons by contaminated food and water with cyst or trophozoite. The introduction of the parasite does not necessarily develop into symptoms, a bigger percentage of infected persons are asymptomatic and they can be in this state for as long as a year but after signs seem, the disease can be dangerous because it may present diarrhea which may lead to acute dehydration [1, 2].

Phagocytosis starts with the ligation of the Galactose/ N-Acetylgalactosamine (Gal/GalNAc lectin) lectin [3] and in order to promote degradation, amoebapores and cysteine proteinases are secreted to the phagosome [2].

There are evidences of the main role of cysteine proteinases as a virulence factor for *E. histolytica* being involved in the breach of the mucus barrier, which is crucial in the pathogenesis of amoebiasis [4].

E. histolytica is needs a high level of iron to continue, this protozoan pathogen is capable to get iron from host proteins, example hemoglobin, ferritin, Lactoferrin and transferrin .That *E. histolytica* trophozoites endocytose ferritin by clathrin-coated pits and degrade this protein by means of specific cysteine proteases in the endosome/lysosome pathway [5]. Ferritin is a heteropolymer composed of 24 subunits of two types, H and L, and the proportion of each subunit depends on the main function of the protein. For example, in the liver and spleen. [6]. The molecular mechanisms of iron acquisition in protozoa are still poorly understood, iron storage is extremely important to all forms of life, thus ferritin is found in the three domains of living beings, *Bacteria, Archaea,* and *Eukarya*. However, some eukaryotic cells like yeasts and protozoa apparently do not have a ferritin molecule, but have evolved other ways to store and maintain their own iron homeostasis [7, 8, 6]

Hemopexin is plasma protein bound of heme released from Hb and another plasma protein extracellularly, iron homeostasis depend on Hb heme removal which limited the toxicity and growth of pathogen. Many parasitic pathogens, including Trypanosoma, Leishmania and Entamoeba have advanced convergent techniques of (heme-iron) gaining from this molecule of host. Heme-iron is released by digested a protein portion of hemoglobin after capturing it by pathogen protozoa through the specific surface receptors or phagocytosis [9, 10, 11].

MATERIALS AND METHODS

The subjects

The study conducted on 380 suspected patient and 20 healthy peoples as control groups. All these cases were defined as suspected with *Entamoeba histolytica* and examined by wet mount microscopic method 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 by [12]. Before a slide was considered negative, ten X40 objective fields of the stool smears were examined.

Blood Specimens collection

Five ml was the total blood collected from each clinical suspected woman with *Entamoeba histolytica* infection and non-suspected person (as control group) by disposable syringe, 4 ml of blood kept at room temperature for 30 minutes. The blood samples have been centrifuged at 3000 rpm for 5 minutes to isolated of serum and have been collected in other sterile tubes, each sample of serum was distributed into four parts; each of them was kept in deep freeze at -20C ºuntil used for serological test and other part of blood 1 ml from each of blood samples were drawn in EDTA tubes were for Haematological Assessments, ''number of red blood cell (RBC), Haemoglobin concentration, packet cell volume (PCV). Mean corpuscular volume (MCV), mean

corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) and platelets (PLT)''.

The Kits

The biomarker s in the current Study were estimated by Eliza Kits such as Human Lactoferrin (LTF/LF) ELISA/ Kono Biotech/ Bulgaria(catalogue number KN0888Hu), Human Haemopexin ELISA Kit/ Kono Biotech/ Bulgaria (catalogue number KN1962Hu), 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

***Entamoeba histolytica* Detection**

The parasite was recognized after examinations of the stool sample by using general stool examination test, forty four out of 380 (11.57 %) 20 (5.26%) child, 14 (3.68%) male and 10 (2.68%) female as seen in table (1), infected is detection by recognizing cyst and trophozoite of *E. histolytica* as shown in figures (1) and (2).

Table 1: Incidence of *Entamoeba histolytica* among suspected patients.

Patient population	No. Exam.	Positive cases		Negative cases	
		No.	%	No.	%
male	200	14	3.68	186	48.94
female	100	10	2.68	90	23.68
child	80	20	5.26	60	15.78
Total	380	44	11.57	336	88.42

Table 2: Age Groups and Infection with *Entamoeba histolytica.*

Age group	Total No. Examined	No. of infected	%
10 – 19*	80	20	*5.26
20 – 29	190	13	3.42
30 – 39	90	8	2.10
40 – 49	20	3	0.78
Total	380	44	11.57

The highest infection with *Entamoeba histolytica

Hematological criteria

The statistical analysis of the results has shown a significant decrease (P < 0.05) in a count of red blood corpuscular, platelets count , hemoglobin concentration, packed cell volume, mean corpuscular volume ,mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin in patients with *E. histolytica* infection compared to the healthy control group, as seen in Table (1).

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Table 1: Blood Parameters in Healthy Group and Patients Suffering from *Entamoeba histolytica* Infection.

Blood parameters	Control male (n=7)	Male patient (n=14)	Control female (n=5)	Female patient (n=10)	Control Child (n=8)	Child patient (n=20)
RBCs $X10^6$/ mm3	**5.064± 0.041**	**4.771± 0.067***	**4.893± 0.055**	**4.201± 0.033***	**5.112± 1.047**	**3.253± 0.932***
Hb g/dL of blood	**13.016±0.671**	**9.013± 0.821***	**12.951±0.172**	**8.071±0.056***	**16.531±0.092**	**9.655±1.333***
PCV (%)	**39.341 ± 0.310**	**35.508±0.510***	**36.694 ± 0.418**	**30.562±0.721***	**39.598 ± 0.020**	**17.444±2.354***
MCV (mm^3)	**86.107 ± 0.901**	**80.076±0.912***	**82.931 ± 0.531**	**79.195±0.408***	**86.107 ± 0.901**	**62.453±5.655***
MCH(pg) /cell	**30.913 ± 0.331**	**21.621±0.853***	**28.910 ± 0.361**	**23.957±0.861***	**30.024 ± 0.821**	**25.009±1.222***
MCHC (g/dL of RBCs)	**30.745± 4.120**	**24.093±0.612***	**28.945± 3.780**	**21.702±0.912***	**32.610± 2.442**	**28.432±0.988***
PLT $X\ 10^3$ / mm3	**344.941 ± 6.210**	**314.710±0.061***	**310.530 ± 7.210**	**300.321±1.231***	**367.529 ± 4.810**	**310.656±33.327***

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

Hemopexin

The current study revealed that concentration of hemopexin in child, male and female infection with *Entamoeba histolytica* were significant decrease ($P< 0.05$) (62. 04 ±11.04 ng/ml), (32.86± 1.687 ng/ml), (18.48± 2. 275 ng/ml) respectively in compared to the control group (79.73 ± 21.89 ng/ml), (46.99± 2.245 ng/ml), (26.96± 1.553 ng/ml) respectively, as seen in figure (1).

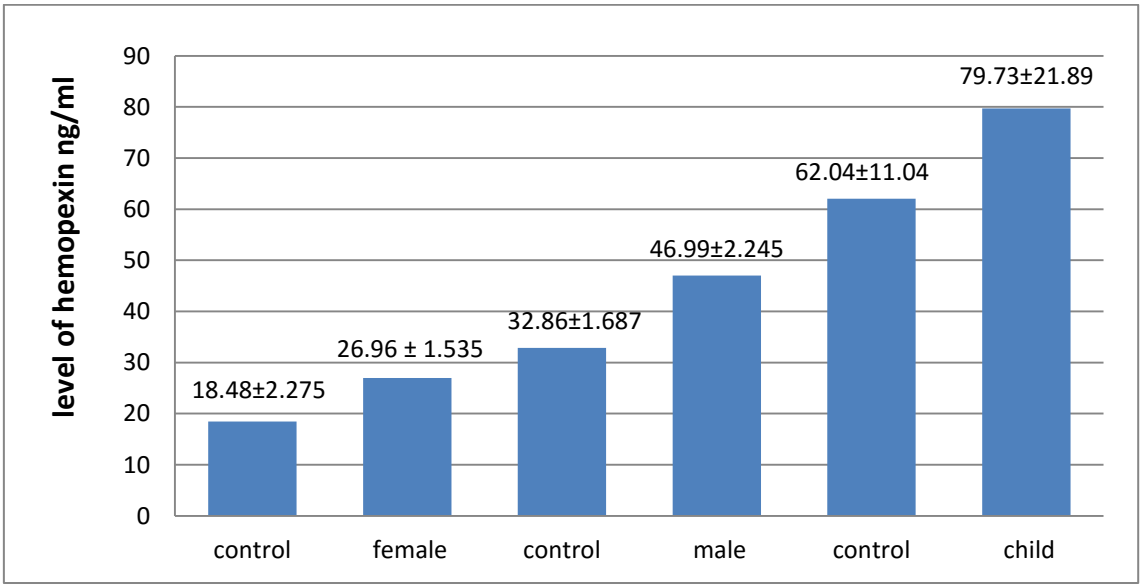

Figure 1: Concentration of Hemopexin (ng/ml) Comparison between Patients Suffering from *Entamoeba histolytica* Infection and Control Group.

* Significant difference P<0.05 between control group and patients

Lactoferrin

The current study revealed that concentration of Lactoferrin in child, male and female infection with *E. histolytica* were significant decrease ($P< 0.05$) (14.06 ±3.713 ng/ml), (11.43± 0.5924 ng/ml), (11. 98 ±0.8105 ng/ml) respectively in compared to the control group (24.07 ± 2.794 ng/ml), (18.85± 0.4908 ng/ml), (20. 72 ±0.5944 ng/ml)

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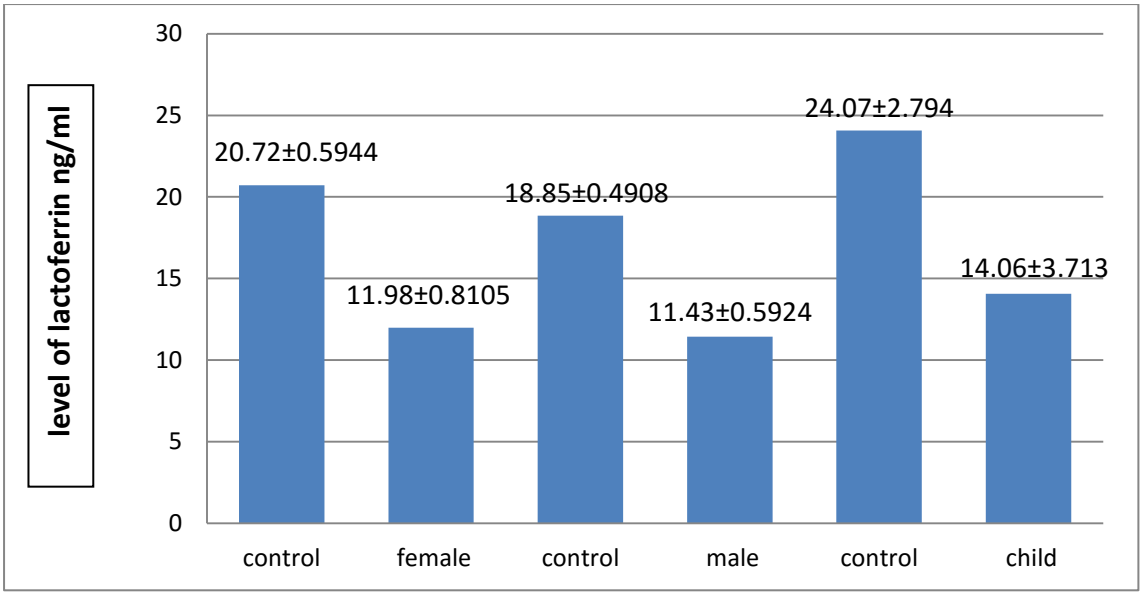

Figure 2: Concentration of Lactoferrin (ng/ml) Comparison between Patients Suffering from *Entamoeba histolytica* Infection and Control Group.

* Significant difference P<0.05 between control group and patients

Iron

The result of study revealed that concentration of iron in child, male and female infection with *E. histolytica* were significant decrease (P< 0.05) (46.27 ±6.592 µg/dl), (128.5± 16.44 µg/dl), (98. 61 ±11.11 µg/dl) respectively in compared to the control group (131.6± 27.3 µg/dl), (179.9± 36.18 µg/dl), (224. 4 ±21.18 µg/dl) respectively, as seen in figure (3).

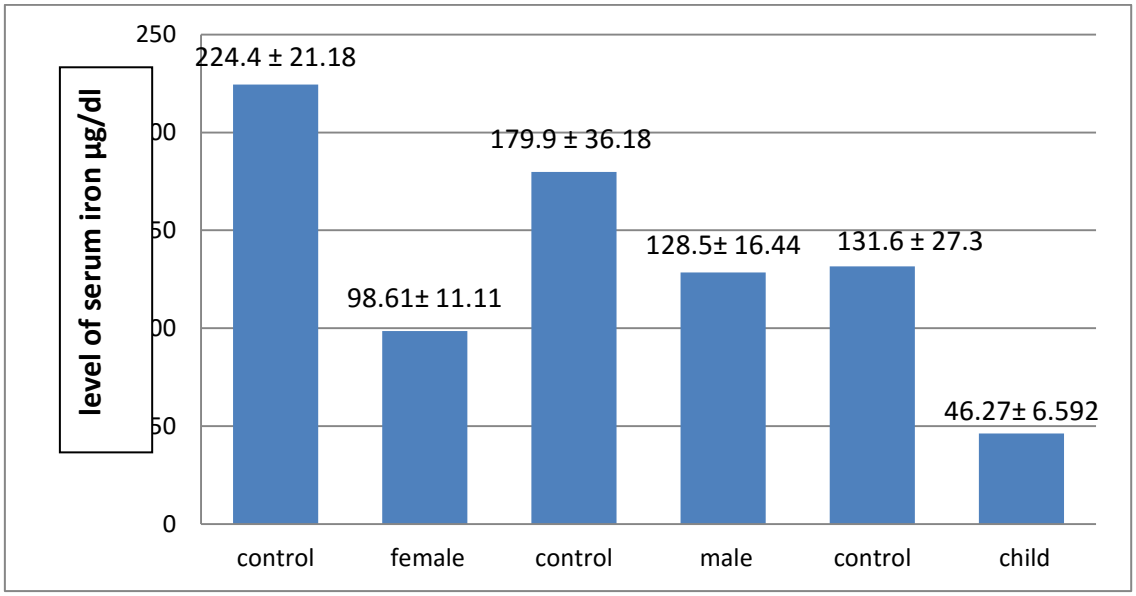

Figure 3: Concentration of iron (µg/dl) Comparison between Patients Suffering from *Entamoeba histolytica* Infection and Control Group.

* Significant difference P<0.05 between control group and patients

Ferritin

The current study revealed that concentration of ferritin in child, male and female infection with E. histolytica were significant decrease (P< 0.05) (59.45 ±1.61 ng/ml), (57.81± 5.31 ng/ml), (41.84± 5. 12 ng/ml)

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respectively in compared to the control group (184± 1.33 ng/ml), (163.6± 2. 53 ng/ml), (89.44± 2. 14 ng/ml) respectively, as seen in figure (4).

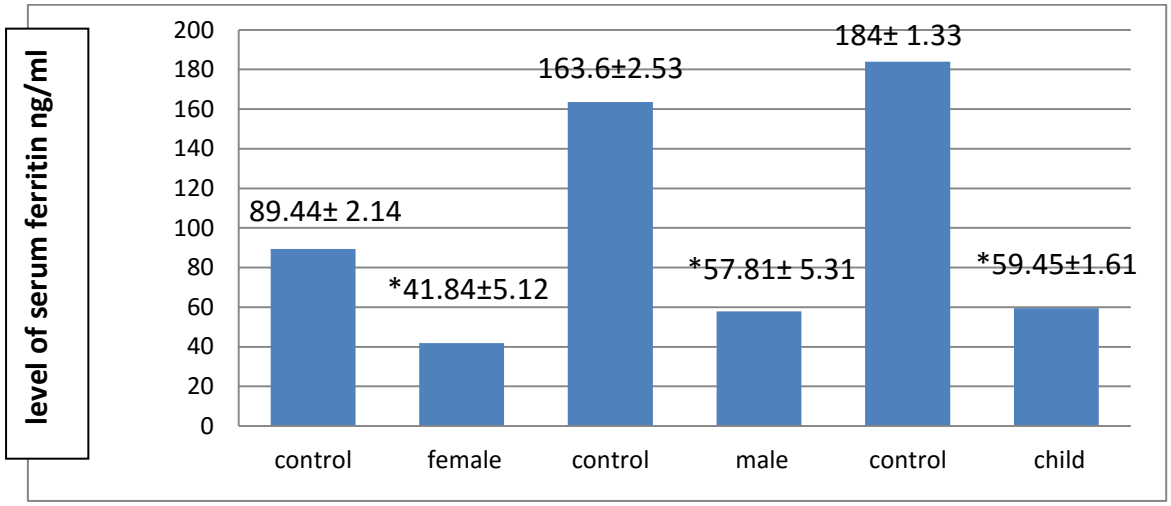

Figure 4: Concentration of ferritin (ng/ml) Comparison between Patients Suffering from *Entamoeba histolytica* Infection and Control Group.

* Significant difference P<0.05 between control group and patients

Correlation Between criteria

Serum concentration of hemopexin, Lactoferrin and Ferritin correlated positively and significantly with levels of Iron in serum of patients suffering from *E. histolytica* infection (r = 0.6179) as seen in Figure (5), (6), (7), (8), (9) and (10).

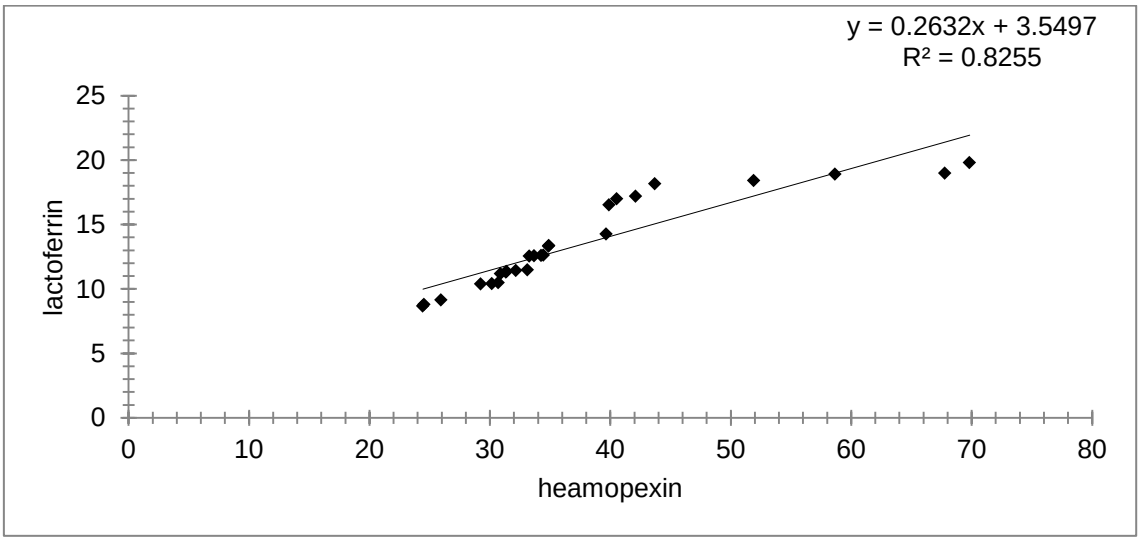

Figure 5: The Correlation between Serum Concentrations of hemopexin and Level of Lactoferrin in patients suffering from *Entamoeba histolytica* infection.

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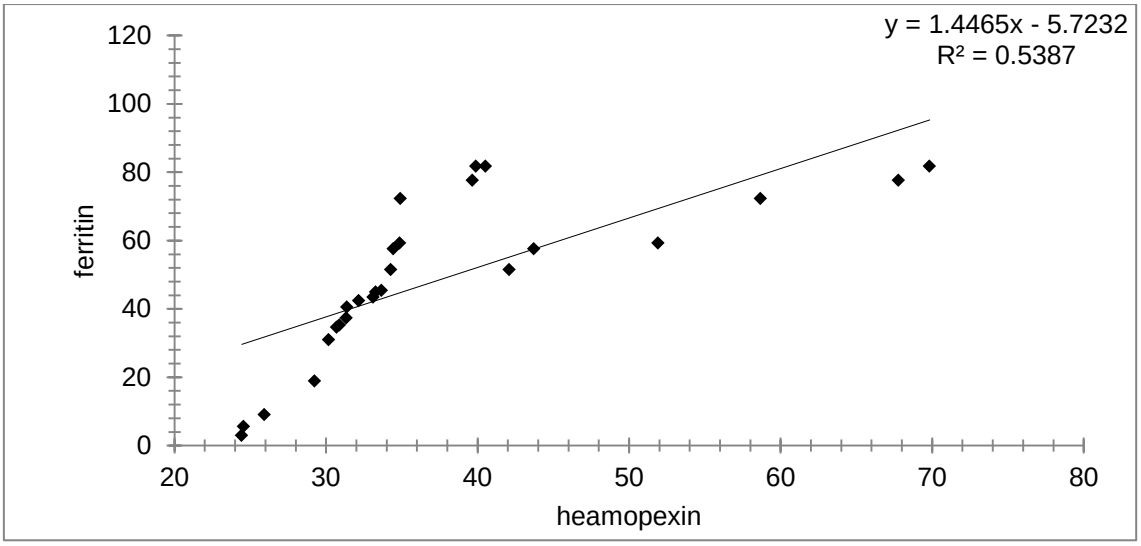

Figure 6: The Correlation between Serum Concentrations of hemopexin and ferritin in patients suffering from *Entamoeba histolytica* infection.

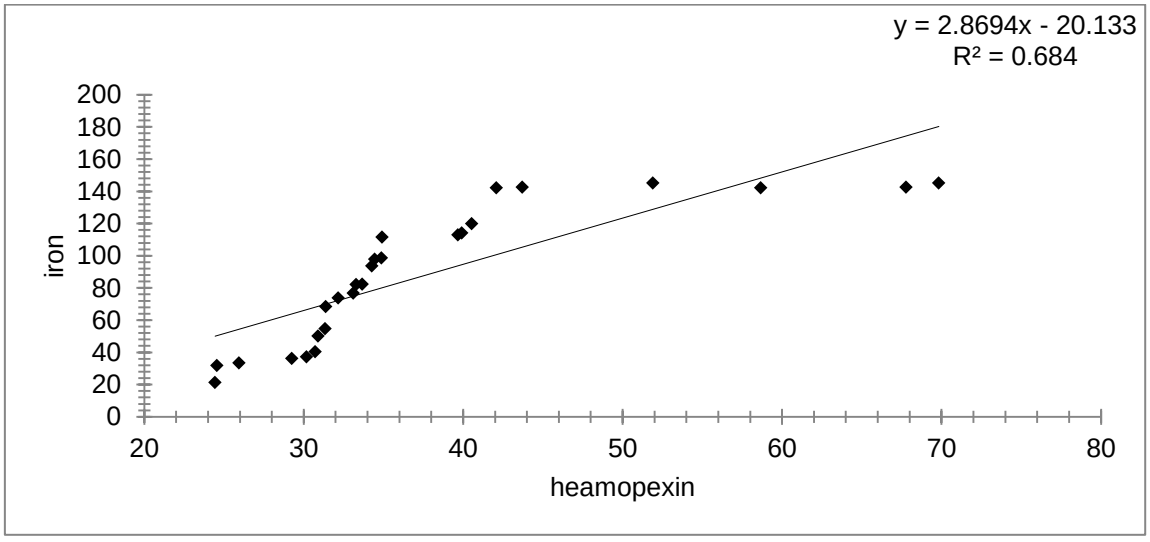

Figure 7: The Correlation between Serum Concentrations of hemopexin and Level of Iron in Patients suffering from *Entamoeba histolytica* infection.

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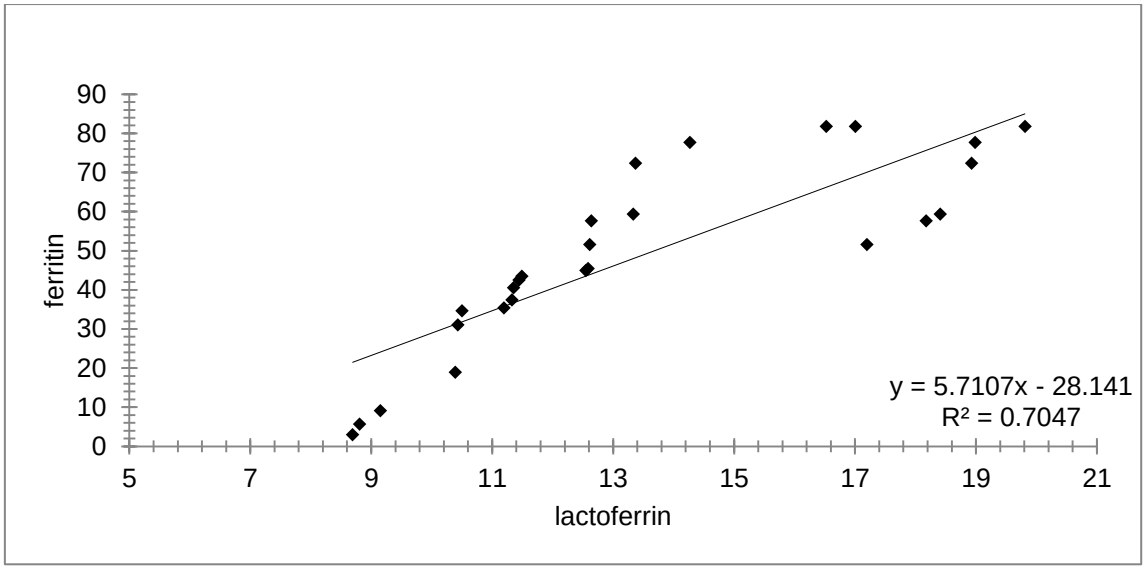

Figure 8: The Correlation between Serum Concentrations of Lactoferrin and Ferritin in Patients suffering from *Entamoeba histolytica* infection.

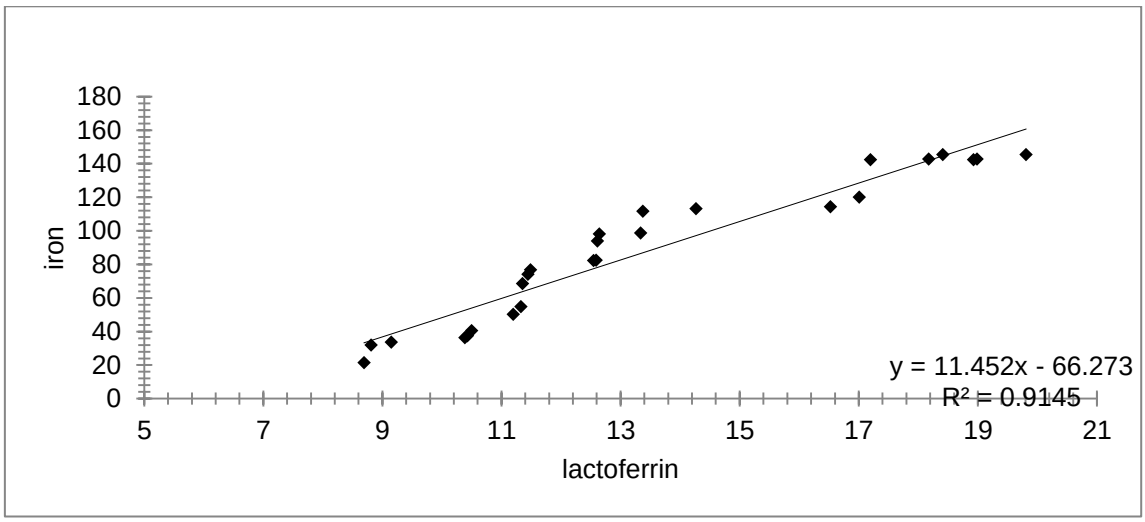

Figure 9: The Correlation between Serum Concentrations of Lactoferrin and Level of Iron in patients suffering from *Entamoeba histolytica* infection.

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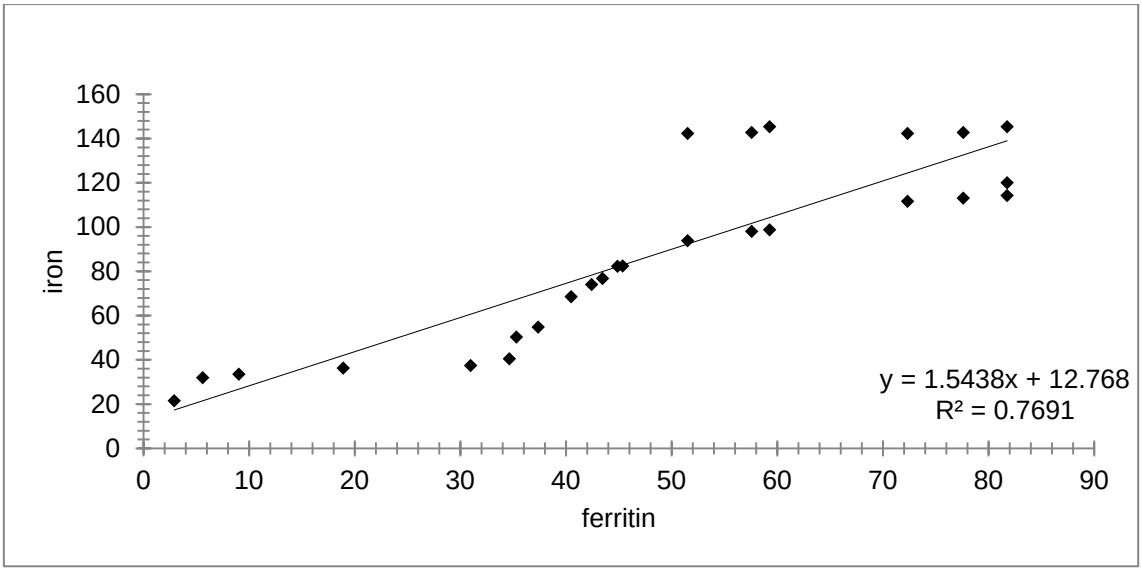

Figure 10: The Correlation between Serum Concentrations of Ferritin and Level of Iron in patients suffering from *Entamoeba histolytica* infection.

DISCUSSION

The present study has revealed the number and percentage 44 (11.57%) out of 380 parted on 20 (5.26%) child, 14 (3.68%) male and 10 (2.68%) female ,examined and were found to be infected with *E. histolytica*.. The results of study showed that the two age groups (10-19) years and (20-29) years have higher incidence with this infection than the age group (30-39) years and (40-49) this result can be explain by the fact that incidence with this parasite maybe related with these age groups more activity and more contiguity with environment [13]. In Al- Najaf province the high morbidity rate by gender was 2.68% in females and 3.68% in males associated with the higher risks for infection in males due to daily activities carried out. This result agrees with explanation of [14].The present study has revealed a significant decrease in RBCs count, concentration of Hb and PCV in patients with *E. histolytica* infection compared to control group. This result may be due to hemolysis of RBCs and phagocytosis by *E. histolytica* parasite and the increased bleeding through ulceration and this leads to decrease in the number of RBCs and the hemolysis of RBCs may lead to decrease in the Hb concentration [15, 17, 18). One of the most frequent causes of anemia in *E. histolytica* infection is the disabsorption and inhibit the nutrient eating ,storage, absorption and use of many nutrients such as iron, vitamin A, vitamin B12, vitamin C and folic acid through small intestine and increase in CD4 production [19, 20]. These lead to iron deficiency anemia (IDA).

The results have shown a decrease in MCV and MCHC in male, female and child in infection with *E. histolytica* parasite compared to control group. A decrease in MCV may be due to a decrease in Hb inside RBCs caused by *E. histolytica* infection; decrease in MCHC is caused by iron deficiency anemia that lead to decrease in formation of Hb in RBCs. the results also provided a decrease in level of PCV in patient compared to control group; this result may be due to a decrease in RBCs counts or due to decrease in MCV caused by decreased level of Hb in RBCs [13]. Also, there is a decrease of hemopexin, Lactoferrin, serum iron and ferritin in *Entamoeba histolytica* infection patients compared to healthy group , Due to its ability to bind heme with high affinity Hpx represents the principal line of defense against heme toxicity and to function as a heme special transporter from the bloodstream to the liver [21].

E. histolytica contain cysteine proteases that cleave Lactoferrin [22]. Iron-binding human proteins, including hololactoferrin, hemoglobin, and holotransferrin, but not ferritin is degraded by the proteases which released from free-living amoebae. In the case of the parasitic protozoan *E. histolytica* have several mechanisms such as receptors and proteases to obtain iron [23].

Inside the host, both the host and the pathogens require iron for surviving. [24]. Which is significant for amoebic growth and enzymatic activities, [25] .furthermore many pathogens contain a huge number of cellular mechanisms, for iron acquisition or iron deprivation dedicated to both, microbial growth and host

defense [26].for obtaining iron these microorganisms elaborate elegant mechanisms and then transport into the iron-poor endophagosomal environment. Specifically iron-binding proteins bound to the amoeba surface, are up taken by endocytosis, traffic through the endosomal/ lysosomal route and are degraded by neutral and acidic cysteine-proteases [17, 27, 28, 29]. Serum concentration of ferritin and Lactoferrin correlated positively and significantly with levels of iron in serum of patients suffering from *E. histolytica* infection, the consuming of iron by *E. histolytica* 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 *E. histolytica* as an iron source [30], the consuming of iron by *E. histolytica* may cause a decrease in the iron levels. The decrease in Lactoferrin levels may be due to an increase in consuming iron by this parasite and this leads to decrease in the Lactoferrin or increased utilized by parasite whereas some studies describing *E. histolytica* as an iron source This parasite requires about 100 mM iron for growth; thus it has developed mechanisms to scavenge iron, for example through the removal of iron from Hb, holoTf, holoLf and ferritin [9, 31, 18, 32, 33].

In serum from patients with *E. histolytica* infection the concentration of hemopexin is correlated positively and significantly with iron and ferritin. Thus, different means of cellular iron uptake have evolved to accommodate different forms of iron and, probably, to allow differential regulation. Biochemical data suggest that additional iron uptake mechanisms may exist (e.g., through putative receptors for ferritin and hemopexin), but these have not yet been characterized at a molecular level or *in vivo*, some studies conducted about capable of some microorganism can be used hemopexin, Lactoferrin and transferrin as iron sources such as *Porphyromonas gingivalis*,. [33, 34].

REFERENCES

[1] Haque, R., C. D. Huston, M. Hughes, E. Houpt, and W. A. Petri, Jr.(2003). N. Engl. J. Med. 348:1565–1573.

[2] Botelho, F., Lopes, R., Franca, J. and Gomes, M. (2011). Research Journal of Parasitology, 6: 1-17.

[3] Ocádiz, R.; Orozco, E. and Carrillo, E., (2005). *Cell Microbiol.*7(2),221-232.

[4] Lejeune, M., Rybicka, J. and Chadee, K. (2009). *Future Microbiology*, 4: 105-110.

[5] Saleem k. A. Al-Hadrawy (2013). Iraq international jurnal of scientific engineering and technology research, 2: 1416-1419.

[6] Arosio P, Ingrassia R, Cavadini P. Ferritins (2009). Biochim Biophys Acta. Jul; 1790(7):589-99.

[7] Suchan, P.; Vyoral, D.; Petrak, J.; Sut'ak, R.; Rasoloson, D. and Nohynkova, E. (2003).. *Microbiology.* Jul;149 Pt 7:1911-21.

[8] Koorts, AM. and Viljoen, M. (2007). Arch Physiol Biochem. Feb;113 1):30-54.

[9] Serrano-Luna JJ, Negrete E, Reyes M, de la Garza M (1998). Exp Parasitol 89: 71–77.

[10] Carvalho S, Cruz T, Santarem N, Castro H, Costa V. (2009). Acta Tropica 109: 131–135.

[11] Vanhollebeke B, De Muylder G, Nielsen MJ, Pays A, Tebabi P. (2008). A. Science 320: 677–681.

[12] Paniker, C. K., (1989).. 2nd ed., Joypee Brothers, Daryaganj". *New Delhi , India.* pp. 224.

[13] Oguntibeju. O.O., (2003). ". *Journal, Indian Academy of Clinical Medicine*. Vol. 4, No. 3: 210-2. July-September.

[14] Al-Malki, J., (2014). *American-Eurasian Journal of Agricultural and Environmental Science*, 14 (1): 50-56.

[15] Devinder sehgal, alok bhattacharya and sudha bhattacharya., (1996). University, New Delhi 110067, India, *J. Biosci.,* 21 (3) . 423-432.

[16] Tolosano, E.; Fagoonee, S.; Morello, N.; Vinchi, F. and Fiorito, V., (2010). *Antioxid Redox Signal* 12, 305-320.

[17] Boczo, K.; Deryo, A. and Drewa G., (2002). *PWN Warszawa*: 104-117.

[18] Leon-Sicairos, N.; Reyes-L´opez, M.; Canizalez-Rom´an, A.; Berm´udez-Cruz, R. M.**;**Serrano-Luna, J.; Arroyo, R. and Garza, M., (2005). .*Microbiology* 151,3871-3859.

[19] Frederick, o.; akinbo1; Christopher, e.; okaka, and richard omoregie., (2011). *Tanzania Journal of Health Research* , Vol 13(1) .10-16.

[20] Lopez-Soto, F.; Gonzalez-Robles, A.; Salazar-Villatoro, L.; Leon-Sicairos, N.; Pina-Vazquez, C. and Salazar, EP. (2009). Int J Parasitol. Mar; 39(4):417-26.

[21] Petrat, F.; Paluch, S. and Dogru¨oz, E., (2003).*The Journal of Biological Chemistry*, vol. 278, no. 47, pp. 46403–46413.

[22] Frederick, o.; akinbo1; Christopher, e.; okaka, and richard omoregie., (2011). *Tanzania Journal of Health Research* , Vol 13(1) .10-16.
[23] World health organization/who., (2011). *SEA-CAH-02*. Pp 1-50.
[24] Espinosa, A.; Yan, L. and Zhang ZL., (2001) *J Biol Chem*; 276: 20136–43.
[25] Nevitt T., (2011). *Biometals.* 24(3):547-558.
[26] Tsutsumi, V.; Ram´ırez-Rosales,A. and Lanz-Mendoza, H., (1992). *Transactions of the Royal Society of Tropical Medicine and Hygiene,* vol. 86,no. 2, pp. 170–172.
[27] Ort´ız-Estrada, G.; Luna-Castro, S. and Pi˜na-V´azquez, C., (2012). *Future Microbiology,* vol. 7, no. 1, pp. 149–164.
[28] Talam´as-Lara, D.; Ch´avez-Mungu´ıa, B. and Gonz´alez-Robles, A., (2014). BioMed Research International, vol. 2014,Article ID 626259, 10 pages.
[29] Gkouvatsos, K.; Papanikolaou, G. and Pantopoulos, K., (2012). *Biochimica et Biophysica Acta (BBA)* - General Subjects.; 1820(3):188- 202.
[30] Reyes-López ,M.; Serrano-Luna, JJ.; Negrete-Abascal, E.; León-Sicairos, N.; Guerrero-Barrera, AL. and de la Garza M., (2001). *Exp Parasitol.* Nov;99(3):132-40.
[31] Lopez-Soto, F.; Gonzalez-Robles, A.; Salazar-Villatoro, L.; Leon-Sicairos, N.; Pina-Vazquez, C. and Salazar, EP. (2009a). Int J Parasitol. Mar; 39(4):417-26.
[32] López-Soto, F.; León-Sicairos, N.; Reyes-López, M.; Serrano-Luna, J.; Ordaz-Pichardo, C. and Piña-Vázquez C., (2009b *Infection, Genetics and Evolution*. vol. 9, no. 6, pp. 1038–1050.
[33] Bramanti, T. E., and S. C. Holt., (1993). *J. Bacteriol.* 175:7413–7420.
[34] Waltena simpson; teresa olczak, and caroline attardo genco., (2000). *Journal Of Bacteriology*, *American Society for Microbiology*;183(20): 5737–5748.