

Effect of *giardia* on some biochemical variables in human blood serum

Al-jebory K.Abdul-Hussien*

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الخلاصة

لقد تمت دراسة التغيرات في مستوى الإنزيمات الكبدية الأساسية ونسبة البروتين الكلي للأطفال المصابين بالإسهال نتيجة إصابتهم بطفيلي الجارديا لامبيليا , أجريت هذه الدراسة على 53 مصابا بالجارديا إضافة لمجموعة السيطرة المكونة من 22 طفلا غير مصابين بالمرض .

لقد تمت مقارنة النتائج لكل مجموعة عمرية من المرضى مع نتائج نفس المجموعة العمرية من السيطرة وأوضحت النتائج إن مستوى الإنزيمات الثلاثة قد كانت أعلى معنويا مما هي عليه في مجموعة السيطرة لجميع الفئات العمرية , كما أوضحت النتائج أن مستوى البروتين الكلي قد تغير هو الآخر للمصابين ولكن بصورة أقل من الإنزيمات ولجميع الفئات العمرية وهذا يشير إلى تأثير وظائف الكبد والأمعاء نتيجة الإصابة بالإسهال مما أدى إلى حدوث بعض التغيرات الكيميائية الحيوية في محتويات مصل الدم للأطفال تحت الدراسة .

ABSTRACT

The alteration in serum glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphotase (ALP) and total protein during giardiasis were studied.

This study has been performed on 53 patients with giardiasis; the control consists of 22 children without giardiasis. The results of each group of patients were compared with the same age of the control, the results indicated that the level of serum GOT, GPT and ALP was significantly higher than that of control for each age , while the level of protein was significantly lower than that for control for each age group.

The results indicate that the giardiasis affected the function of liver and the intestine, which lead to some biochemical changes in serum.

INTRODUCTION:

Diarrhoeal disease is among the most common infections through out the world⁽¹⁾, diarrhea in early child hood remains a major cause of morbidity and death in developing countries⁽²⁾. according to WHO and Unicef, childhood diarrhea kills between three and five million children under age five each year, 80% of these deaths involve children under two 90% of deaths caused by diarrhea diseases in young children are avoidable, provided they are treated sufficiently early and effectively.

The great killer has not completely disappeared, some, such as malaria and diarrhea, continue to weaker revenges⁽³⁾. In spite of infections and parasitic diseases decreased from 5% to 1% of total deaths in the developed world and from 45% to 43% of total deaths in developing world ,of more than 50 million deaths worldwide in 1997, about one-third were due to infections and parasitic diseases such as lower respiratory diseases, tuberculosis, diarrhea, and malaria⁽⁴⁾.

Diarrhoeal diseases present a major health problem in developing countries. It is reported that 750-1000 million cases of diarrhea occur in children below 5 years of age in Asia, Africa and Latin America every year. In addition, repeated attacks of diarrhea lead to malnutrition and more death⁽⁵⁾.

*Basic Science Department, college of Pharmacy, Almustansiriya University, Baghdad – Iraq.

Giardiasis is one of the most common parasitic diseases worldwide and causes both endemic and epidemic intestinal disease and diarrhea⁽⁶⁾ and one of the common causes of acute and persisting diarrhea in children in developing countries .there is some evidence from population studies that giardiasis interferes with intestinal absorption of nutrients and growth rate of children⁽⁷⁾. The organism has two stages, trophozoite and cyst⁽⁸⁾, the trophozoite divides by longitudinal binary fission⁽⁹⁾, the mature cyst is thick walled ,oval,8-12µm long ,7-10µm wide⁽¹⁰⁾ and contains four nuclei, it passed in the stool of infected individuals and may remain viable in water for as long as 2 months. The traditional method of diagnosis is a microscopic exam of stool trophozoite or cysts and is the assay with which other tests are compared⁽¹¹⁾. additional diagnostic tests include microscopic examination of duodenal fluid or duodenal biopsies.

EXPERIMENTAL:

The samples were obtained from children who presented to the Al-Mansur teaching hospital for children, Baghdad, the study include 53 patients with giardiasis, the patients were divided into three groups:

Group 1: included patients <6 years old.

Group 2: included patients from 6 to 10 years old.

Group 3: included patients from 11 to 15 years old.

Diagnosis was confirmed on the basis of microscopic examination of fresh stool specimens. The amount of fresh stool specimen in normal saline was used for finding of either trophozoites or cysts. Twenty two children (1 month to 15 years old) were used as control; they were divided into three groups as in the patients groups.

Serum separation :

Blood samples were drawn from the groups of patients and controls, after that each blood sample was left in a tube for 10 min, then the blood samples was centrifuged, 3000rpm/min. for 5 min, then the serum was kept frozen at – 20°C in marked tube until analyzed.

Serum GOT test :

GOT transforms alpha-ketoglutarate into glutamate, and the aspartate into oxaloacetate. Oxaloacetate reacts with 2; 4-dinitrophenylhydrazine to produce colored solution which is measured by the spectrophotometer⁽¹²⁾ normal value is 2-20IU/L.

	Test tube	Control tube	Standard tube	Blank tube
GOT substrate	0.5 ml	0.5 ml	0.4 ml	0.5 ml
Serum	0.1 ml	—	—	—
Distilled water	—	—	0.1 ml	0.1 ml
Working pyruvate standard	—	—	0.1 ml	—

All tubes were incubated in water bath at 37°C for exactly 60 minutes, then:

2,4- DINITROPHENYLHYDRAZINE	0.5 ML	0.5 ML	0.5 ML	0.5 ML
Serum	—	0.5 ml	—	—

After 20 min, 5ml of 0,4N-NaOH was added to each tube .after 10 min, the absorbance of each tube was measured at 510 nm. The pyruvate formed per min per liter of serum is:

$$\frac{\text{Test} - \text{control}}{\text{Standard} - \text{blank}} \times 67 \text{ m mole}$$

The value opposite to the calculated value of pyruvate was found from the appendix⁽¹³⁾.

Serum GPT tests:

The pyruvate produced by transamination by GPT reacts with 2, 4-dinitrophenylhydrazine to give a brown-colored hydrazine, which was measured by spectrophotometer. Normal value is 2-15 IU/L⁽¹⁴⁾.

	Test tube	Control tube	Standard tube	Blank tube
GPT substrate	0.5 ml	0.5 ml	0.4 ml	0.5 ml
Serum	0.1 ml	—	—	—
Distilled water	—	—	0.1 ml	0.1 ml
Working pyruvate standard	—	—	0.1 ml	—

All tubes were incubated in water bath at 37°c for exactly 30 minutes, then:

	Test tube	Control tube	Standard tube	Blank tube
2,4- INITROPHENYLHYDRAZINE	0.5 ML	0.5 ML	0.5 ML	0.5 ML
Serum	—	0.1 ml	—	—

After 20 min, 5ml of 0.4 N-NaOH was added to each tube, after 10 min the absorbance of each tube was measured at 510nm. The pyruvate formed per min per liter of serum is:

$$\frac{\text{Test} - \text{control}}{\text{Standard} - \text{blank}} \times 133 \text{ m mole}$$

The value opposite to the calculated value of pyruvate was found from the appendix⁽¹³⁾.

Serum ALP tests:

It depends on the liberated quantity of phenol as a result of disodium phenyl phosphate lyses. King and Armstrong unit (KAU) is used for phenol quantity measurement. The normal value is 3–26 KAU/100 ml.

	Test tube	Control tube	Standard tube	Blank
Buffer (pH 10)	1.0 ml	1.0 ml	1.1 ml	1.1 ml
Disodium phenyl phosphate	1.0 ml	1.0 ml	—	—
Serum	0.1 ml	—	—	—
Working phenol standard	—	—	1.0 ml	—
Distilled water	—	—	—	1.0 ml

All tubes were incubated in water bath at 37 0 C for exactly 15 minutes, then:

	Test tube	Control tube	Standard tube	Blank
NAOH (0.5 N)	0.8 ML	0.8 ML	0.8 ML	0.8 ML
Serum	—	1.0 ml	—	—
Sodium bicarbonate (0.5 N)	1.2 ml	1.2 ml	1.2 ml	1.2 ml
4- aminoantipyrine	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Potassium ferricyanide	1.0 ml	1.0 ml	1.0 ml	1.0 ml

The absorbance of each tube was measured immediately at 510nm. The activity was calculated from the equation:

$$\text{Serum ALP (KAU /100 ml)} = \frac{\text{Test} - \text{control}}{\text{Standard} - \text{blank}} \times 10$$

Serum total protein:

Serum total protein is measured by atomic absorption normal value for serum protein is 6.4 – 8.3g/100 ml.

RESULTS & DISCUSSION:

1-The effect of Giardiasis on Serum GOT & GPT:

The results showed that the level of serum GOT was 10.62 ± 1.38 IU/L in control group 1 compared with 25.2 ± 2.29 IU/L in group 1 of patients with giardiasis. The level of sGOT was 7.71 ± 1.2 IU/L in control group 2 compared with 19.46 ± 2.79 IU/L in group 2 of patients with giardiasis. The level of sGOT was 7.42 ± 1.64 IU/L in control group 3 compared with 14.37 ± 1.13 IU/L in group 3 of patients with giardiasis as shown in (fig-1).

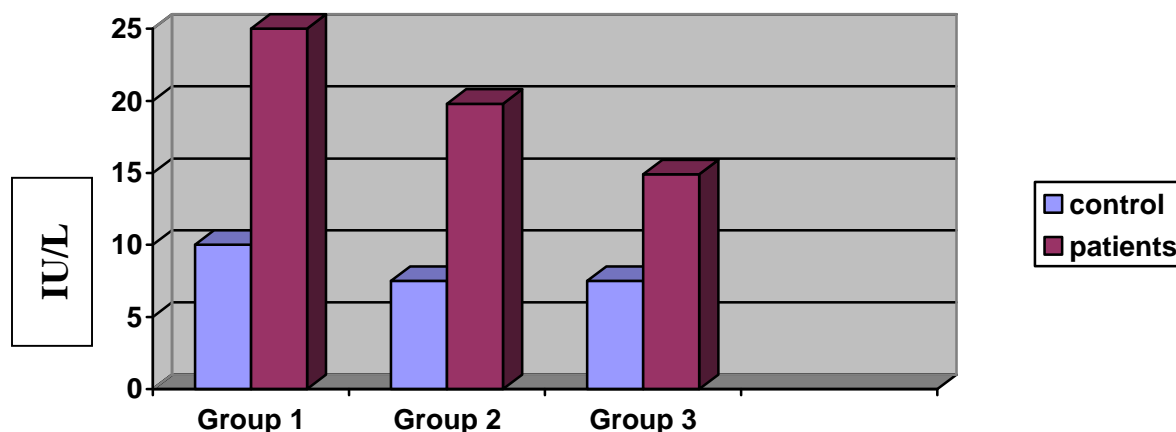


FIG -1: EFFECT OF GIARDIASIS ON SERUM GOT

By one way ANOVA, the level of GOT in group 1 of patients was significantly higher than that for control group 1 ($p < 0.01$), the level of GOT in group 2 of the patients was significantly higher than that for control group 2 ($p < 0.05$), the level of GOT in group 3 of patients was significantly higher than that for control group 3 ($p < 0.01$). By one-way ANOVA, the level of GOT in group 1 of patients was significantly higher than that for group 2 of the patients which was significantly higher than that for group 3 of patients ($p < 0.05$). The level of GPT was 6.87 ± 0.91 IU/L in control group 1 compared with 14.4 ± 1.16 IU/L in group 1 of patients. The level of GPT was 6 ± 1.09 IU/L in control group 2 compared with 12.8 ± 1.2 IU/L in group 2 of patients. The level of GPT was 6.14 ± 0.79 IU/L in control group 3 compared with 12.87 ± 0.89 IU/L in group 3 of the patients (fig -2).

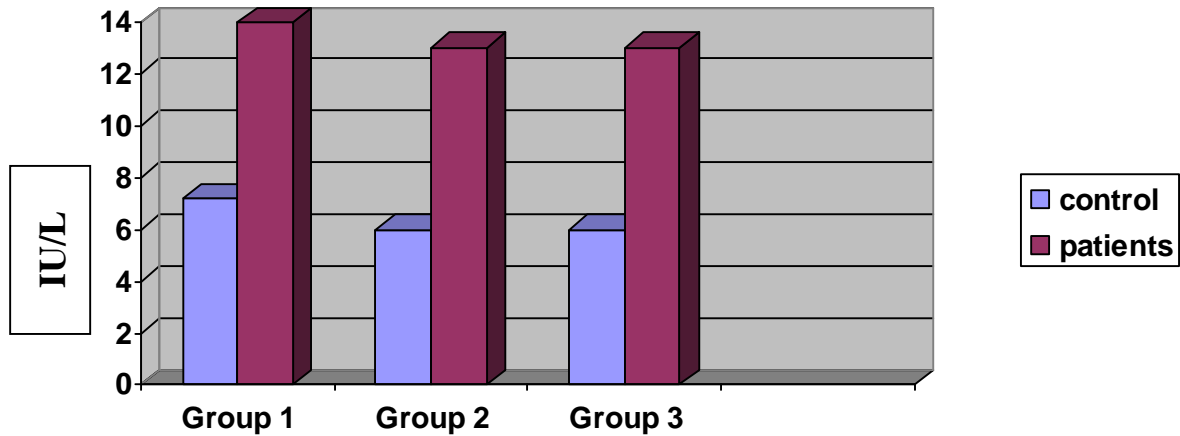


FIG -2: EFFECT OF GIARDIASIS ON SERUM GPT

By one-way ANOVA, the level of GPT in group of patients was significantly higher than that for group for each age group ($p < 0.01$). By one-way ANOVA, the difference in the level of GPT between the groups of patients was not significant. The available references did not refer to the effect of giardiasis on GOT and GPT in serum. The elevation of serum GOT and GPT during giardiasis is because of that, since giardiasis may produce diarrhea and malabsorption⁽¹⁵⁾, the diarrhea causes malnutrition, the malabsorption and malnutrition cause disturbance of liver function⁽¹⁶⁾, this lead to increase the level of GOT and GPT in serum. When the adequate energy for the child from the food is not obtained during diarrhea, or when the child is not obtain basal metabolic rate, the body will return to the energy storage in the liver and the skeletal muscles to supply its requirement of energy, this lead to increase the level of GOT and GPT because the skeletal muscle is considered from the region, which GOT and GPT is concentrated in⁽¹⁷⁾.

2- Effect of giardiasis on Serum ALP:

The results showed that the serum ALP was elevated in all groups of patients compared with that of controls of each group. Its level was 15.25 ± 0.35 KAU/100 ml for control groups 1, 2 and 3 respectively while the sALP level was elevated to 41 ± 2.67 , 37.46 ± 3.82 and 31 ± 2.13 KAU/100ml for patients of groups 1, 2 and 3 respectively (Fig-3).

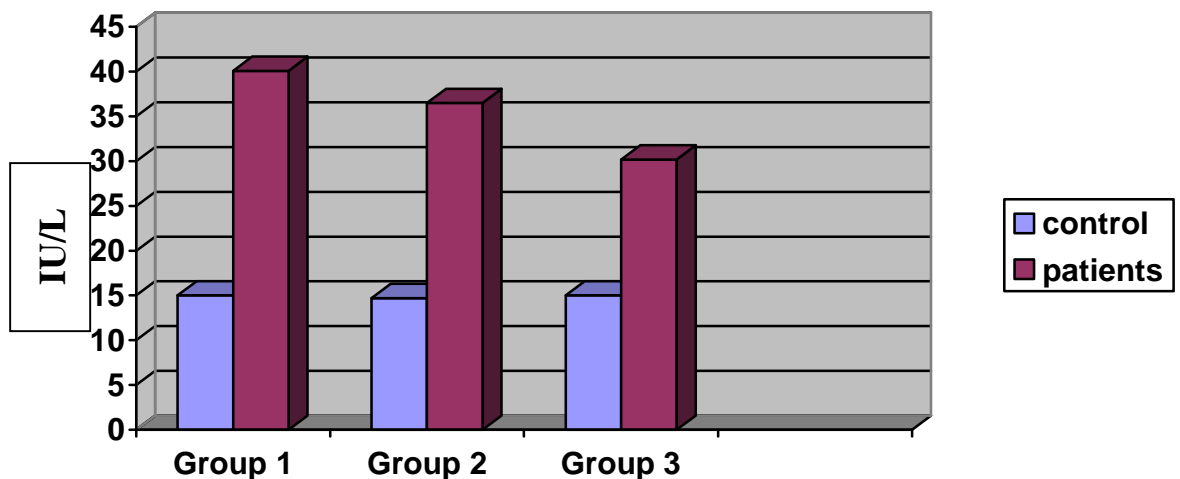


FIG- 3: EFFECT OF GIARDIASIS ON SERUM ALP

By ANOVA, the level of ALP in group of patient was significantly higher than that for control group for each age group ($p < 0.01$). By one-way ANOVA, the difference in the level of ALP between the groups of patients with giardiasis was not significant.

The available references did not refer to the effect of giardiasis on ALP .the elevation of sALP during giardiasis is because of that, giardiasis may produce diarrhea and malabsorption⁽¹⁵⁾, and because of the important role of ALP in the process of transportation of phosphate, calcium, sodium and potassium, in addition to its important role in the metabolic process, the level of ALP is increased during diarrhea because of the loss of these minerals during this period⁽¹⁸⁾.

Effect of Giardiasis on Serum total Protein:

The results showed that, the level of serum total protein was 6.75 ± 0.26 g/100 ml in control group 1 compared with 4.52 ± 0.06 g/100 ml in group 1 of patient with giardiasis. The level of serum total protein was 7.02 ± 0.23 g/100 ml in control group 2 compared with 4.40 ± 0.08 g/100ml in group 2 of patients with giardiasis. The level of serum total protein was 7.58 ± 0.11 g/100 ml in control group 3 compared with 4.72 ± 0.04 g/100 ml in group 3 of patients with giardiasis (fig-4).

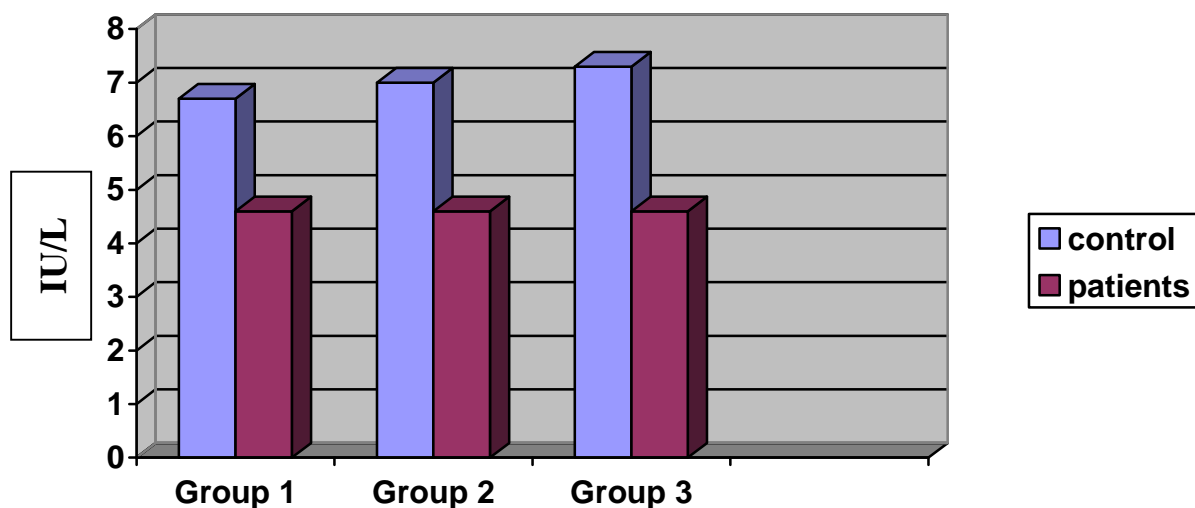


FIG-4 . EFFECT OF GIARDIASIS ON SERUM TOTAL PROTEIN:

By one-way ANOVA, the level of total protein in group of patients with giardiasis was significantly lower than for control group for each age group ($p < 0.01$). The difference was not significant by one-way ANOVA in the level of serum total protein between the groups of patients with giardiasis.

These results which indicate that the level of serum total protein is decreased during giardiasis are agreed with the results reported by Gillon⁽¹⁹⁾ in a study of adults presenting with giardiasis .the decrease of serum total protein during giardiasis is because of that giardiasis can cause acute and chronic diarrhea with intestinal malabsorption⁽²⁰⁾, the malabsorption leads to disturbance in the metabolic functions of the liver⁽¹⁶⁾. This decrease the formation of albumin, the alpha and beta globulins ,this decrease their concentrations in serum and this lead to decrease serum total protein .

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