

Study of lipid profile alteration in the patients infected with *Giardia lamblia* and compare the results with healthy individuals

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الخلاصة

تضمنت الدراسة جمع 40 عينة براز لاشخاص يعانون من الاسهال الدهني وبعد استخدام طريقة المسحة الرطبة لتلك العينات تم تشخيص وجود طفيلي *Giardia lamblia* الذي يسبب داء الجيارديات، بعد ذلك اخذت عينات الدم لنفس الاشخاص مع جمع 20 عينة دم لافراد اصحاء تم استخدامهم كعامل سيطرة وبعد استحصال المصل من عينات الدم انفة الذكر تم اجراء تقدير الدهون (الكوليستيرول،الدهون الثلاثية،البروتينات الدهنية عالية الكثافة،البروتينات الدهنية الواطئة والواطئة جدا). وقد اظهرت النتائج ان الاشخاص المصابين بداء الجيارديات يعانون نقصا ملحوظا في مستوى الكوليستيرول في الدم بينما كانت انواع الدهون مثل (الدهون الثلاثية والبروتينات الدهنية عالية الكثافة)، طبيعيه لدى نفس الافراد وذلك لان طفيلي الجيارديا يستهلك الكوليستيرول الموجود في دم المضيف لاغراض البناء الحيوي داخل الخلية حيث انه غير قادر على تخليق الكوليستيرول بمفرده.

Abstract

This study was include collection of 40 stool samples from people were suffer from steatorrhea and the genral stool examination for these stool samples showed the presence of the intestinal parasite *Giardia lamblia* the etiologic agent of giardiasis .

Blood samples were collected from 60 individual 40 of them were the infected with *Giardia lamblia* patients and the other 20 were healthy and used as control ,then the serum was obtained from all blood samples in the field of the study and the estimation of total (Cholesterol ,Triglyceride ,High density lipoproteins, Low density lipoproteins and Very low density lipoproteins)was done .

the result showed that the cholesterol levels were decreased in giardiasis patients while the other types of lipids were normal in the same patients because *Giardia lamblia* consumed the cholesterol of the host in the bio synthesis of the cell . because the parasite is unable to synthesize cholesterol by itself .

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Introduction

Giardiasis is a protozoan disease caused by *Giardia* genus. This disorder is the most parasitic infection in the world especially in the developing countries [1, 2, 3]. Iran is an endemic area for the infection and the prevalence is 10.9% [4]. *Giardia* protozoan is seen in two cyst and trophozoite forms.

Trophozoites attach to upper portion of small intestine by its sucker plate and may present with severe damage affecting nutrient absorption [5]. *Giardia lamblia* is unable to synthesize lipids *de novo* and must obtain them from the dietary products of the host small intestine [6].

Detailed pathogenesis mechanisms of *Giardia* in host are not clear, however, colonization of the microorganism appears cause microvillus shortening, villous flattening or atrophy [7]. Infected children revealed malnutrition and growth retardation compare to health group [8]. One of the main complications of giardiasis is lipid malabsorption so that steatorrhea (foul smelling, greasy stool) is a clinical sign of giardiasis [9]. Therefore the infected people

especially children, may lack the important caloric source and lipid soluble vitamins (K, A, D, E) [10, 11, 12]. Lower level of hemoglobin concentration and, iron-deficiency anemia associated with giardiasis were observed [13, 14]. Giardiasis is one of the protozoan infections associated with peripheral blood eosinophilia [15]. Information on the effect of *G. lamblia* on lipid parameters. Therefore this study aimed to

assay total cholesterol, triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), in peripheral blood in *Giardia* cyst passers.

Materials and Methods

• Fecal Samples Examining

Stool samples containing *Giardia lamblia* cysts were collected from 60 infected individual. The samples were from sporadic cases and food born outbreaks.

Giardia infection was diagnosed by microscopy of wet amount and concentration method

- **Blood sample collection**

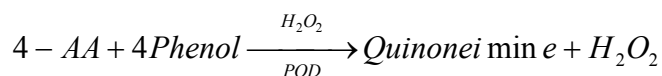
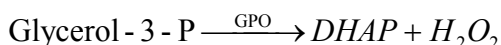
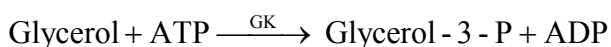
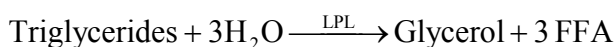
Blood samples were collected from 60 individual at different ages (2-60) years 40 of them were suffer from giardiasis, 20 were healthy and used as a control , 3 ml of venous blood was collected from each individual transferred immediately into plain plastic tubes and the serum was obtained by centrifugation at(3000 rpm for 10 minutes) . the serum was dispensed in plastic tubes and used immediately in Lipid profile process .

- **Serum Triglycerides assay**

Principle

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL) .

The glycerol is phosphorylated by adenosin to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP) . G-3-P is oxidized by glycerphosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide . red chromogen is produced by the peroxidase (POD) catalyzed coupling of 4-aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H₂O₂) , proportional to the concentration of triglyceride in the sample .[16]



Procedure

The reagents and samples were brought to room temperature and then. pipetted into labeled tubes .

Table (1) Serum Triglycerides assay Procedure

TUBES	Blank	Sample	CAL. Standard
R1. Monoreagent	1.0 µl	1.0 µl	1.0 µl
Sample	-	10 L	-
CAL. Standard	-	-	10 L

The tubes were mixed and let the tubes stand 15 minutes at room temperature (16-25)° C or 5 minutes at 37° C.

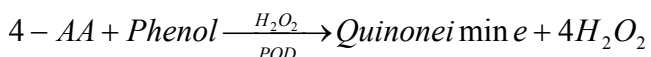
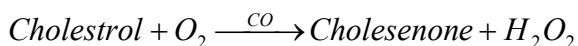
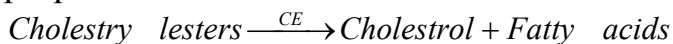
The absorbance (A) of the sample and the standard was read at 500 nm against the reagent blank .

The color stable for at least 1 hour protected from light.

- **Serum Cholesterol Assay .**

Principle

This method for the measurement of total cholesterol in serum involves the use of three enzymes : cholesterol esterase (CE) , cholesterol oxidase (CO) and peroxidase (POD) . In the presence of the former the mixture of phenol and 4-aminoantipryne (4-AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample [16].



Procedure

reagents and samples were brought to room temperature and pipetted into labeled tubes .

Table (2) Serum Cholesterol Assay Procedure

Tubes	Blank	Sample	Cal. Standard
R1. Monoreagent	1.0 µl	1.0 µl	1.0 µl
Sample	-	10 L	-
CAL. Standard	-	-	10 L

The tubes were mixed and incubated stand 10 minutes at room temperature or 5 minutes at 37 °C.



The absorbance (A) of the sample and the standard was read at 500 nm against the reagent blank .

The color stable for at least 30 minutes protected from light.

- **Serum High density lipoprotein assay**

Principle

The chylomicrons and lipoproteins of very low density (VLDL) and low density (LDL) contained in the sample are precipitated by the addition of phosphotugstic acid in the presence of magnesium ions .

The supernatant obtained after centrifugation contain high density lipoproteins (HDL) from which the cholesterol and phospholipids can be determined using the 'Cholesterol Enzymatique' and 'Phospholipids Enzymatique ' reagents [16].

Procedure

Precipitation

sera containing > 3.5 mmol / l of triglycerides was diluted in 9 g /NaCl

- Serum.....500 µl
- Reagent 1 (precipitant) 50 µl

Then mixed and let stand for 10 min .

The tube was centrifuged for 15 min at 5000 rpm.

Determination of HDL cholesterol .

The absorbance (A) of the sample and the standard was read at 500 nm against the reagent blank .

Table (3) Serum High density Lipoprotein assay Procedure

	Reagent blank	Standard	sample
Distilled Reagent (HDL cholesterol Calibrating solution)	50 µl	-	-
Supernatant " Cholesterol Enzymatique"	-	50 µl	-
Working solution	-	-	50 µl
	1 µl	1µl	1µl

- **Serum very Low density lipoprotein assay**

Very low density lipoproteins were estimated by Friedewald equation as the following :

Very low density lipoproteins = Serum Triglycerides – 5 [17].

- **Serum Low density lipoprotein assay**

The low density lipoproteins were estimated according to Friedewald equation as the following :

Low density lipoprotein = Serum cholesterol - (VLDL+HDL) . [17].

- **Statistical Analysis**

All results of lipid profile test were performed by Chi square test at the level of significant when P-value < 0.01. The specificity, sensitivity and diagnostic accuracy of the results were also calculated by applying the following equations: $(a / a + b) \times 100 =$ sensitivity, $(d / d + c) \times 100 =$ specificity. diagnostic accuracy = $(a+d / a+b+c+d) \times 100$. also calculated positive predictive value (PPV) = $(a / a + c) \times 100$ and negative predictive value (NPV) = $(d / b + d) \times 100$. (a = the total number of positive cases, b = false positive those bearing positive reading from negative samples, d =total number of true negatives, c=those with negative reading from positive cases [18] .

Results

The lipid profile results were as the following :

Serum cholesterol results

The levels of serum cholesterol were decreased for giardiasis patients and different for healthy individual who used as control these results are shown in table (4).

Table (4) the results of serum cholesterol

	number	Mean ± S.E.
Control	20	5.0145 0.31 A
Treated	40	2.6431 0.19 B

A,B the different letters refers to the significant differences under (P≤0.05)

• **Serum triglyceride results**

Triglyceride results were different for samples i.e control and giardiasis patients after reading in the spectronic at 500 nm. Table 5 clear this result.

Table (5) the results of serum Triglyceride

	number	mean	S.E.
control	20	1.571	0.12A
treated	40	1.736	0.2 A

A,A the similar letters refers to the non-significant differences under(P≤ 0.05).

• **Serum High Density Lipoproteins Results**

The results of High Density Lipoproteins were read in the spectronic at 500 nm and the samples showed different for the all (control ,and giardiasis patients).table (6).

Table (6)Serum High Density Lipoproteins results

	number	Mean ± S.E.
control	20	1.362A 0.02 A
treated	40	0.836B 0.02 B

A,B the different letters refers to the significant differences under (P≤0.05)

• **Serum Low Density Lipoproteins Results**

Serum Low Density Lipoproteins were calculated by the equation depends on cholesterol results therefore the results of LDL were different in control and decreased in giardiasis patients. table (7) clear the statistical analysis for this results .

Table (7) Serum Low Density Lipoproteins Results

	number	Mean	±	S.E.
control	19	2.5989		0.23A
treated	40	1.2790		0.14B
A,B the different letters refers to the significant differences under ($P \leq 0.05$)				

- **Serum Very Low Density Lipoproteins Results**

Serum Very Low Density Lipoproteins was also calculated by equation depends on cholesterol results so patients with giardiasis were showed clear decreasing in their serum VLDL levels while control samples which had been taken from healthy individuals showed different results as cleared in table (8) .

Table (8) Very Low Density Lipoproteins results

	number	Mean	±	S.E.
control	20	1.0025		0.06A
treated	40	0.5285		0.03B
A,B the different letters refers to the significant differences under ($P \leq 0.05$)				

Discussion

In the present study the lipid profile was used to understand the relationship between the infection with *Giardia lamblia* and steatorrhea (the most diagnostic symptom of giardiasis). and to determine whether that steatorrhea affect the levels of lipids in the blood of giardiasis patients and whether this impact is considered significant or not.

The association of steatorrhea with the infection of *Giardia* may be observed on the basis of damaging the intestinal mucosa , causing functional derangements , reducing brush border enzymes along with other factor such as synergism with agents like Salmonella and rotavirus [19].

Recent studies showed that Cysteine rich proteins(CRPs) which is produced by *Giardia lamblia* can bind to heavy metals like zinc in the small intestine and as this binding inhibit the enzymes of small intestine [20]. thus prevent lipids metabolism .

Giardia may consumed only cholesterol and neglect the other lipids [16] who showed that cholesterol starvation consider a trigger for trophozoite differentiation into cyst he was also proved that the affect of T.G. or HDL or another lipid on the growth was neglected . this result agree with the results obtained in the present study and it may explain why only serum cholesterol levels were decreased in patients with giardiasis patients while the values of triglyceride or high density lipoproteins were normal in the same patients considering that low density lipoproteins(LDL) and very low density lipoproteins (VLDL) levels depend primarily on cholesterol and triglyceride levels.

Or *Giardia lamblia* trophozoites may inhibit lipolysis (the process of lipids degredation) and the degree of inhibition increased with longer duration of lipase exposure to trophozoites [20], but this result do not agree with lipid profile results in the present study which showed only serum cholesterol in giardiasis patients was clearly decreased and the levels of triglyceride and high density lipoproteins were normal or sometimes rising more than normal rate

Giardia obtains cholesterol which consider necessary for membrane biogenesis from the serum of the host because giardiasis is unable to synthesize cholesterol it must therefore obtain this compound from the milieu of the upper small intestine , which particularly rich in biliary and dietary cholesterol [21]

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