Effect of Giardia *lamblia* on some biochemical changes of the human

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Summary

The study was carried out in Kirkuk province from period 15th of October 2005 to 1st of June 2006. The main aim of the study was to measure the adverse effects of giardiasis on some biochemical tests of the host. For this purpose a total of 997 stool samples were collected from children aging from one 1---- 12 years, giardia was diagnosed by using double wet preparation of 0.85 % of normal saline to detect trophozoites & lugols iodine 1% to detect cystic stages, all positive cases were confirmed by using concentration method(flotation technique Zinc sulphate ZnSo4 solution). After detecting positive cases, venous blood samples were drawn from infected children (study group) and healthy children (not infected) they consider as a control group, to assess the effect of giardiasis on some biochemical tests of the host (random blood sugar, serum (S.) Cholesterol, serum total protein, serum albumin, serum copper & serum magnesium)were chosen . Biochemical tests were determined using special enzymatic kits purchased from local companies in the city. The overall rate of giardiasis was 13.23 % which was distributed in 132 stool samples. Stool examination by using concentration method (Flotation technique using ZnSo4 solution) shows high efficacy in demonstrating giardiasis (13.23 %) more than using wet preparations (9.62 %) the relation ship was significant (P<0.05).

Sera of infected children with Giardia *lamblia* Shows decreasing in mean value of blood sugar (91.3 mg/100 ml), S. cholesterol (143 mg/100ml), S. total protein (6.43gm/ml), S. albumin(3.51 gm/100ml) and S. magnesium, (1.55 mg/100 ml) P<0.05 compared to sera of non infected (control group), which shows (99.7 mg/100ml), (184.4mg/100ml), (7.47gm/100ml),(4.39gm/100ml) and(2.12 mg/100ml) for blood sugar, S. cholesterol, S. total protein, S. albumin and S. magnesium respectively P>0.05. While S. globulin & S. copper mean values were within normal P>0.05.

Introduction

Giardia *lamblia* is a flagellated waterborne protozoan parasite, that can infecthuman and other mammals including cats, cattle and dogs[1,&2] .Infants & young children may have increased susceptibility to giardiasis because of behavioral & immunological factors [3], it is easily transmitted by direct feco-oral contact, by drinking contaminated water (waterborne disease)[4] ,food [5]or by abnormal sexual practices[6].There is no evidence that this parasite causes both acute persistent diarrhea[7]. Illness can last several months if untreated & can be characterized by continued exacerbation of diarrheal symptoms, with chronic illness. Malabsorption of fat, lactose, vitamin A & vitamin B12 [8] & failure of children to thrive have been noted[5].

Although abundant number of studies were done in Iraq in regard of prevalence of giardiasis[9&10] , but the adverse effect of giardiasis in more studies are not investigated [11,12,13 & 14] .more studies refer to the demonstrating of Giardia stages in diarrheal stool, & intensive care was directed to management & blocking diarrhea that was caused by Giardia parasite either by monitoring oral fluid to compensate the fluid loss due to diarrhea or by application of anti-protozoan drugs[15]. The major health problems are the low sanitation level of patient & bad medical follow up to cases, within a period which may progress to chronic cases [16]. In addition to chance of mutation or Giardia challenges, this may have role in surveillance on the brush border (villi) which leads to changes in microvillus function especially the absorption of essential dissolved materials during the digestion process in the duodenum [17]. In order to shed light on these events that will occur, the aim of this study was directed to assess the adverse effect of Giardia *lamblia* on some biochemical tests.

Materials & methods

Time, location, patient selection & stool sampling:-

From 15th October 2005 to 1st June the study was carried on daily except holidays in Kirkuk pediatric general Hospital & centers of primary care In different location in the same province. A total of 997 stool samples were Collected from children's in primary schools & from whom they attends to Out patient clinic of Kirkuk pediatric general Hospital, their age was ranged From 1 year to 12 years old. Complete information including (name, sex, age, address, water supply, source of food & others) were put in special questionnaire form prepared for this purpose.

Initially stool sample from each patients was collected in clean, dry, tight fit cover (to prevent specimen loss & to avoid contamination). Regarding sampling from children in schools, stool containers were given to children in the school to collect stool in the morning of the next day (using 2.5 % of potassium dichromate as preservative solution), the collected samples were taken to pediatric Hospital laboratory for examination.

Stool examination

Double wet preparation for each stool sample was done using 0.85% of NaCl % for detecting trophozoites of giardia[18], &using of lugols iodine 5 % for detecting cystic stages [19&20].Each positive stool for giardiasis was tested by using concentration method using floatation technique by treating stool with 33% of zinc sulphate (ZnSo4) the solution was prepared & the experiment was done according to that used by [21].

Blood collection

Five ml of venous blood sample was collected from each infected patient with Giardia *lamblia*(total number examined =132) , also 104 venous blood samples were collected from non infected children (healthy negative for giardiasis),sera were separated as soon as possible in the same day(to avoid glycolysis of sugar, & decomposition of other chemicals in the serum).

Biochemical tests

Each serum was tested for blood sugar using enzymatic colorimetric kit On the basis of Trinder reaction [22], S. cholesterol (enzymatic method) [23], S. total protein using Biruet method [24], S. albumin using bromocresol green Complex forming [25], S. copper using enzymatic kit [25] S. magnesium using blue ixidile reaction in alkaline medium to produce color water soluble chelate [26].All kits were provided by Bio-meureax company Spain.

Statistical analysis:- All data were tabularized by the aid of computer, statistical analysis was done using Chi-square & t- student test to determine source of variances between study & control groups [27].

Results

Examination of 997 stool samples shows Gardia *lamblia* infection in 132 samples with the rate 13.23 %, see table (1).

Table (1) positive & negative percentages of Giardia lamblia among children below 12 years in Kirkuk province.

Total numbe Examined	No. of Positive	% Positive	No. of Negative	% Negative
997	132	13.23	865	86.77

The present study takes in consideration two ways of Giardia diagnoseIn stool by using double wet preparations (o.85 % of NaCl,& 5% of Lugols Iodine solutions) & Concentration method(Zinc sulphate flotation technique).The result shows that from 997 stool sample Giardia was found in 96 sample with the rate 9.62 %, while it was 132 with the rate 13.23 % by using concentration method (P <0.05). See table (2).

Table (2) Comparison between direct wet preparation & concentration Method used in Giardia diagnosis.

Type of stoo Examinatior	Total No. Examined	No. of Positive	% Positive	No. of Negative	% Negative
Direct wet Preparation	997	96	9.62	901	90.38
Concentratio Method	997	132	13.23	865	86.77

The results of biochemical tests were shown in table (3).Blood sugar mean value in test group (infected with Giardia) 5.07 mmol/ liter was lower than in control group (uninfected) 5.54 mmol/liter. P<0.05.

The serum cholesterol mean value in test group 3.7 mmol/liter was scientifically different P<0.05 from that in control group 4.77 mmol/L. Considering the level of total protein in sera of infected patients it was 64.3gm/liter. This level when compared with that in control group 74.7 gm/liter was lower& significant P<0.05. Also slightly hypoalbuminemia was seen in children infected with Giardia, the mean value was 35.1 gm/liter, while it was with in normal value 43.9 gm/liter, P<0.05.

Serum globulin in test group 29.2 gm/liter & 30.8 gm/liter in control group P>0.05. Spectrometric absorbance of samples used for measuring serum copper shows no statistical significance between children infected with Giardia 18.29micro mol/liter& control group 19.4 micro mol/liter.

P >0.05. It seems from the result of the study, that serum magnesium levels were decreased during Giardiasis. Its mean value was 0.63 mmol /liter, while in control group it was 0.87 mmol/liter. P<0.05.

Table (3): Effect of Giardia *lamblia* on some biochemical tests in both study group (infected with Giardia) control group (uninfected).

Biochemical Tests	Study group		Control group		Normal values In mg/100ml & Mmol/liter	t-student values
	Mg/100 ml X + Sd	Mmol/liter X + Sd	Mg/ 100 Ml X + Sd	Mmol / Liter X + Sd		
Blood sugar	91.3 +15.3	5.07 + 0.85	99.7+16.6	5.54+0.92	< 135 mg/100 7.5mmol/liter	8.65**
S. cholesterol	143 + 27.9	3.7 + 0.72	184.4+34.8	4.77+0.9	<200 mg/100ml <5.18mmol/liter	3.13**
S. total protein	6.43 + 0.89 gm/100ml	gm/1iter 64.3 + 0.89	7.47+0.58 gm/100ml	gm/liter 74.7+0.5.83	(6.6—8.7 gm/100ml 66—87gm/liter	1.68*
S. Albumin	3.51 + 0.37 gm/100ml.	35.1 + 0.37 gml/1iter	gm/100ml 4.39+0.29	43.9+2.9 gml/liter	3.6—5.2 gm/100ml 36—52gm/liter	4.9**
S. globulin	2.92 + 0.29 gm/100ml	29.2 + 2.96 gml/liter	3.08+0.31 gm/100ml	30.8+3.1 gm/liter	2.4---3.7gm/100ml 24—37gm/liter	0.015
S. copper	114.3 +15.3	18.29+ 2.48	121.1+19.9	19.4+3.19	70—150 micro gm/100ml 11---24 micro mol/liter	٠,٢٥
S. magnesium	1.55 + 0.3	0.63 + 0.12	2.1+0.29	0.87+0.11	1.9—2.5 mg/100ml 0.78---1.03 mmol/liter	4.14**

.Study group =children infected with Giardia lamblia , N=132 Control group= children not infected , N= 104. * P<0.05 significant. ** means P<0.05 highly significant.

Discussion

The all rate (13.23%)of giardiasis in the present study is high, the causes can be attributed to several factors, such as lower education level to health hygiene among children, poor experience in toilet training , over crowded family in small house, especially after the return of departures to Kirkuk , building the undesigned camps in different area around Kirkuk city and lack of insecticides or even of most imported insecticides had poor action on

insects ,which enhance the development & proliferation of more insect specially flies, which had role in transmitting the infective stage of Giardia mechanically. The result of this study is similler with that recorded in Brazil [28], Lybia[29] & with that in Iraq[30,31,&32]Whom they recorded 13.3%,13.75% & 12 % respectively. While it was disagree with that recorded in Saudi Arabia, Tunisia & Egypt 62 %, 42% & 62.2% [33,34&35], and disagree with That in Baghdad, Nassaria , Kirkuk,Arbil & Duhok 33%, 26.1%, 44.59% 35.39% & 38.5 % recorded by [36,37,38,39 & 40] respectively. Variation is probably due to the nature of residence survey, level of personal hygiene& sanitation& the safety of water consumption from water supplies. It is evident that Giardia cyst can resist chlorination of drinking water [41&42].In addition diagnostic techniques, number of stool examination& type of population in the study; all can affect the incidence rate of this parasite [43].

The high efficacy of flotation method (ZnS04 solution) in detecting Giardia than using direct wet preparation, may be explained by the fact ,that Giardia cyst had a light weight which can float easily on ZnS04 solution(which had heavy specific gravity)[44]. Further more the little amount of stool sample taken in wet preparation did not represent the whole sample, especially in case of low parasitemia. [38].

Regarding biochemical tests: - Blood sugar mean value in children infected with giardia5.07 mmol/liter than in uninfected children 5.54 mmol/liter with significant differences can be due that glucose is an essential substance for growth of Giardia trophozopites. It was found experimentally that when glucose concentration is reduced the replication rate is reduced [45].

About the relationship between giardiasis &Serum cholesterol which shows decreases during giardiasis 3.7 mmol/liter comparing to 4.77 mmol/liter in control group, this finding can be explained by In-Vitro study[46]who shows that [H] palmitic acid& [H] arachdonic acid are incorporated into various phospholipids ,giardia has developed the ability to take up or transport free fatty acids across the plasma membrane & incorporate them into cellular lipids. serum total protein& serum albumin mean value are slightly lower than their normal limit in study group, but when they compared to that in control group, the relation ship is significant statistically this means that, although Giardia lacks synthesis of amino acids & depends on scavenging them from the intestinal milieu in which trophozoite replicates[47],Also it has been found that kinetics of amino sugar phosphate synthesis in encysting Giardia favors the direction that supports cyst wall synthesis[48&49].

The relationship between Giardia *lamblia* & magnesium mean levels was significance; the levels were o.63 mmol/L during giardiasis & 0.87 mmol/L in control group. Clinically significant magnesium deficiency is most commonly associated with malabsorption syndrome, vomiting & diarrhea, in which elevated fecal magnesium is probably related to the level of steatorrhea rather than to deficient bowel absorptive sites [49].

Our finding is similar to that recorded by [50].Although serum copper mean values in present study not reveals differences between children infected with Giardia & uninfected children. This finding can be explained by another finding in the study particularly serum albumin which shows decreased mean value. This means that great amount of copper was bound to albumin to form ceruloplasmin in children infected with Giardia, while serum albumin in control group not changed. The result of serum copper agrees with that recorded in Turkey by[51] and disagrees with that recorded in the same country by[52] who found that serum copper was increased during giardiasis. From the result of the present study the following can be concluded:-

Giardia *lamblia* infection was highly prevalent among children below 12 years in Kirkuk province, the rate was 13.23 %.Concentration method (flotation technique using ZnSo4 solution) shows high efficacy in detecting giasrdiasis than using double wet preparations technique.

Blood sugar, S. cholesterol, S. total protein, S. albumin & S. magnesium levels reveals significant statistical relationship between children infected with Giardia & uninfected children. Serum copper& serum globulin levels are not affected significantly during giardiasis.

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تأثير الخمج بطفيلي الجيارديا لامبليا على بعض المعايير الكيموحيوية للمضيف

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[3] قسم علوم الحياة، كلية العلوم، جامعة كركوك، كركوك، جمهورية العراق

الملخص

أجريت الدراسة في محافظة كركوك من الفترة ٢٠٠٥/١٠/١٠ إلى الأول من حزيران ٢٠٠٦. الهدف الرئيسي للدراسة هو معرفة تأثير الخمج بطفيلي الجيارديا على بعض فحوصات الكيموحيوية، لهذا الغرض تم جمع ٩٩٧ نموذج براز من الأطفال بعمر من سنة واحدة لغاية ١٢ سنة. شخصت الطفيلي باستخدام طريقة المسحة الرطبة المزدوجة (٠,٨٥% من المحلول الملحي للتحري عن الأطوار الخضرية و استخدام اللوكال ايودين ٥% لتحديد الأطوار الكيسية).تم استخدام طريقة التركيز – تقنية التطويف(استخدام محلول كبريتات الخارصين ٣٣%) كفحص توكيدي.بعد التأكد من الحالةالموجبة، ولغرض معرفة تأثيرالخمج بالجيارديا على بعض الفحوصات الكيموحيوية فقد تم سحب ٥ مل من الدم الوريدي من الأطفال المخمجة بالجيارديا (مجموعة الدراسة) وكرر سحب نفس الكمية من الدم من أطفال أصحاء كمجموعة ضابطة واختيرت أجراء الفحوصات التالية (فحص السكر بالدم، الكوليستيرول، أجمالي بروتين المصل،الأح ،ألكلوبيولين فحص النحاس والمغنيسيوم بالمصل (باستخدام عدد خاصة أنظمية تعتمد على امتصاص الشدة اللونية بوساطة جهاز الطيف الضوئي) وتم شراء موادالفحص من الأسواق المحلية. النسبة الكلية للخمج بالجيارديا ١٣,٢٣ %، أظهرت فحص البراز بطريقة التركيز(تقنية التطويفباستخدام محلول كبريتات الخارصين ٣٣%) كفاءة عالية في تشخيص مرض الجيارديا ١٣,٢٣%قياسا تحضير المسحة الرطبة المزدوجة ٩,٦٢ ., P<0.05 أظهرت مصول الأطفال الخمجة بالجيارديا معدل قيم واطئة بالنسبة لفحص السكر بالدم ٩١,٣ ملغم/١٠٠ مل، الكوليستيرول١٤٣ملغم/١٠٠مل،وأجمالي بروتين المصل ٦,٤٣ غم/١٠٠مل، الأح ٣,٥١غم/١٠٠مل ، المغنيسيوم بالمصل ١,٥٥ ملغم/١٠٠مل مقارنة لمعدل قيمها في المجموعة الضابطة حيث أظهرت المصول القيم التالية: ٩٩,٧ ملغم/١٠٠مل، ١٨٤ملغم/١٠٠مل،٧,٤٧ غم/١٠٠مل، 4.39غم/١٠٠مل و ٢,١٢ ملغم/١٠٠مل لفحص السكر بالدم و الكوليستيرول، أجمالي بروتين المصل،الأح والمغنيسيوم على التوالي P< 0.05. أما بالنسبة لفحص النحاس و الكلوبيولين بالمصل فلم تظهر الدراسة فرق معنوي في معدل قيم الفحصين في الأطفال الخمجة بالجيارديا والأطفال الأصحاء P>0.05.

الكلمات الدالة: الجيارديا ، طريقة التطويف، السكر، الكوليستيرول، ألأح ، النحاس ، المغنيسيوم