Comparison of diagnostic microscopy with direct fluorescent assay and flotation methods for detecting Giardia lambliain stool samples

Yahya Jirjees Salman[1] , Maha Ismail Mustafa[2] , Wazera Ghaieb Mustafa[3]

[1]*College of Medicine , Kirkuk University , Iraq*
[2]*College of Science Kirkuk University , Iraq*
[3]*Kirkuk General Hospital*

Abstract

There is an increasing demand for diagnostic testing for Giardia intestinalis (G. lamblia) ,with a priority being placed on obtaining diagnostic results in an efficient and timely manner. Several commercial companies have developed rapid diagnostic tests that are simple to perform and can be completed in less time than traditional methods for detecting Giardia lamblia. We compared one of these rapid tests, MERIFLUOR direct fluorescent-antibody (DFA) test, and zinc sulphate flotation technique with microscopy of direct double wet preparationson 310 stool samples, taken from children below 12 years in Kirkuk city from 1st December 2012 to 30th of June 2013. The all rate of intestinal parasitic infections was 51.93 % that divided into 42.58 % and 9.35 % for protozoan and helminthic infections respectively. Giardia lamblia rate 20.32 % was the highest rate among protozoan infections P<0.05.According to gender the rate of giardiasis among males 27.92 % was higher than 16.08%in females P<0.05.While according to age of the patients, statistically the differences among the ages were not significant P>0.05, but correlation age with the gender high rate of giardiasis14.41 % and 7.2 % were recorded in males aging from 7 to8 years and from 11 to 12 years respectively , P<0.05. For detection of Giardia lamblia, the differences in sensitivities, specificities and accuracy of three methods were not significant P>0.05, but when comparing these elements alone, direct microscopy have had significant differences on other two methods. Statistical analyses reveal significant differences in Positive Predictive Values (PPV) among three employed methods P<0.05, While Negative Predictive Values (NPV) analyses were not significant P>0.05.

Keywords: Giardia, DFA, flotation, sensitivity, specificity, accuracy.

Introduction

Giardia lamblia and Cryptosporidium parvum are common protozoan parasites in humans, causing intestinal infections with watery diarrhea, abdominal pain and mal-absorption that may last for weeks to months [1,2] . In travelers, Giardia lamblia and Cryptosporidium spp. are, together with Entamoeba histolytica, the most frequent causes of intestinal protozoan infection [3] . Conventional microscopical diagnosis of Giardia is time-consuming, and relies crucially on the microscopist's skills and experience [4,5]. Furthermore, microscopical examination must be performed on three stool samples to increase sensitivity [6], which leads to problems concerning patient compliance and delays in the final diagnosis. In an attempt to establish sensitive and cost-effective methods to diagnose intestinal infections with Giardia, a number of copro-antigen tests have been developed [4,7and 8] . The aim of the present study was to compare direct double wet preparations with direct fluorescent-antibody assay (DFA) and flotation technique using Zinc sulphate solution in clinical samples (stool) from patients attending private clinics and medical laboratory in Kirkuk city.

Materials and methods

From 1st of December 2012 to 30th of June 2013 a total of 310 fresh stool samples were collected from children below 12 years in Kirkuk city. The study was carried out at the Medical Research laboratory, Kirkuk College of Medicine. The samples were obtained from patients attending the private clinics and medical labs in Kirkuk city, who presented with abdominal symptoms, mainly diarrhea. Prior to processing complete informations were reported in a special questionnaire prepared for this purpose. One aliquot of each sample was immediately examined using direct wet preparations of lugols iodine 1% and 0.85 % of NaCl for detecting motility of Giardia trophozoites and other intestinal protozoan parasites. The residue of each specimen was preserved by adding sufficient amount of 2.5 % of potassium dichromate and concentrated by floatation method using ZnSO4 technique [9] . Concentrated samples were examined by light microscopy for ova and parasites (O & P),[10] and by a direct fluorescent-antibody assay (DFA) (MeriFluor Crypto and Giardia; cell labs company).For DFA, small amount of stool sample approximately 200 µl was placed on special microscopic slide supported with the kit. The specimen was completely air dried. Some drops of acetone were applied on the smear for five minutes. Fixation of the smear was done by adding 25µl of RR2 to smear and, control positive and covering well area. The slides were kept in a humid chamber and incubated at 37 C^0 for 30 minutes, with avoiding the dryness of the slides. The slides were gently rinsed with Phosphate Buffer Saline (PBS) for one minute. Excess moisture around the well was gently drained by a piece of soft tissue. A drop of RMG was applied on the slide, and then covered with cover slip. Scanning the entire specimen using a fluorescence microscope initially at x200magnification, then at x400, and x1000 for confirmation .The slide either read immediately or stored at 2-8C^0 in dark place for 24 hours. Demonstration of Giardia parasite was assessed by using Nikon fluorescent microscope by using wave length 480nm and 550 nm .The following terms and equations were used for detecting the

efficacy of laboratory methods in detecting Giardia lamblia; TP=True positive, NP=True negative, FP=false positive,FN=false negative , PPV=positive predictive value and NPV=negative predictive value . Sensitivity=TP/(TP+FN).Specificity=TN/(TN+FP),Accuracy=(TN+TP)/(TN+TP+FN+FP),PPV=TP(TP+FP)and NPV=TN/(TN+FN).[11] .

Statistical analysis

all data in the present study were stored in Microsoft Excel program and arranged in tables. Variances between methods were adjust at P<0.05 and <0.01.

Results

From a total of 310 stool samples examination the all rate of intestinal parasitic infection was 51.93 % distributed in 161 stool samples .

This rate involve high frequency of protozoan infection in 132 (42.58 %) compare to 29(9.35 %) for helminthes ,P<0.05.

The more common intestinal protozoan parasite was Giardia lamblia63(20.32 %) followed by Blastocyst hominis, Cryptosporidium parvum, Entamoeba coli, Entamoeba histolytica, Iodomoeba butschili, Endolimax nana and Balantidium coli with the rates : 6.77%,6.45%,4.18 %,2.58 %,1.29 %,0.64 % and 0.32 % respectively.

Concerning intestinal helminthic infection, high number 23 (7.41%) was with Hymenolepis nana compare to 1(0.32 %) record for Ancylostoma duodenali. Table -1.

Table -1-Distribution of Giardia lamblia and other intestinal parasites among children in Kirkuk province

Name of parasites	Number positive	Percentages Positive	T-value and P
Protozoan parasites			
Giardia lamblia	63	20.32	Df=7 t-table value =2.36 P>0.05
Blastocyst homonis	21	6.77	
Cryptosporidium parvum	20	6.45	
Entamoeba.Coli	13	4.19	
Entamoeba.histolytica	8	2.58	
Iodomoeba butschili	4	1.29	
Endolimax nana	2	0.64	
Balantidium coil	1	0.32	
Total	**132**	**42.58 ***	
Helminthes			
Hymenolepis.nana	23	7.41	Df=3 t-table value =3.18 P>0.05
Enterobius virmicularis	3	0.96	
Ascaris lumbricoides	2	0.64	
Ancylostoma duodenali	1	0.32	
Total	**29**	**9.35**	
All Total	**161**	**51.93**	

Total No. Exam=310 *P<0.05

Table -2- showing relationship between Giardia lamblia distribution and age, sex of the patients, the rate of Giardiasis was 27.92 % in 31 samples of males, while in female was 16.08 % in 32 samples ,P<0.05.Concerning the age, high rate of Giardia lamblia 14.41 % was recorded among males aging from 7 to 8 years ,while in female the high rate of giardiasis 8.04% was among females aging from 9 to 10 years ,P<0.05.In other age groups the relationships were not significant P>0.05.

Table -2-Frequency of Giardia lamblia in relation to ages and gender

Age groups /years	Male		Female		Total		T-value and P
	No. +ve	Prevalence +ve rates	No .+ve	Prevalence +ve rates	No . +ve	Prevalence +ve rates	
3 to 6	-----	----	1	0.50	1	0.32	P>0.05
7 to 8	16	14.41	12	5.52	28	9.03	P<0.05
9 to 10	7	6.30	16	8.04	23	7.41	P>0.05
11 to 12	8	7.2	3	1.50	11	3.54	P<0.05
Total	31	27.92 %	32	16.08 %	63	20.32 %	P<0.05 *

Total number of male = 111Total number of female= 199 *Chi-square value= 2.699 Regarding Giardia lamblia detecting according to laboratory methods high rate of giardiasis 20.32% was reported using direct wet preparation technique, followed by 19.03% and 17.74 % using DFA andflotation technique respectively. In spite of recording the following rates: 78.75 5,77.20 % and 76.19 5 for sensitivity,93.56 %,91.27 % and 91.07 % for specificity and 93.53%,91.71 % and 89.59 % for accuracy of three methods respectively ,but statistical analysis show no differences among methods in detecting giardiasis P>0.05.

Negative predictive values NPV in relation to type of laboratory methods exert the following rates:97.16 %,95.61 5 and 94.11 % for direct microscopy, DFA

and flotation respectively, but statistically were not significant ,controversy to positive predictive values PPV that showed significances ,P<0.05(Table-3) .

Table-3- Distribution of Giardia lamblia according to laboratory methods

Lab methods	No.+ve	% +ve	Sensitivity % *	Specificity %	Accuracy %	PPV	NPV	T-value and P
Direct wet preparation	63	20.32	78.75	93.56	92.53	78.75 **A**	97.16	A*T=42.51 P<0.05
DFAT	59	19.03	77.20	91.27	91.71	73.75 **B**	95.61	B* T=68.86 P<0.05
Flotation	55	17.74	76.19	91.07	89.59	68.75 C	94.11	C*t=74.38 P<0.05

PPV=Positive predictive value NPV=Negative predictive value

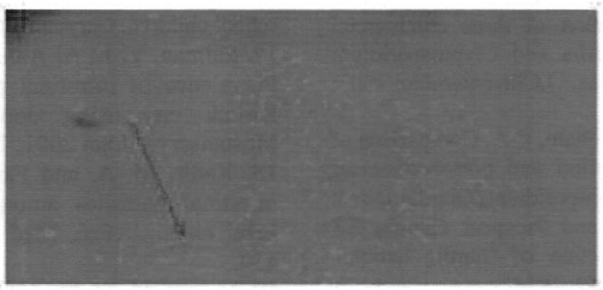

Figure-1- Giardia lamblia cyst using DFA visualizing by fluorescentmicroscope 550 nm

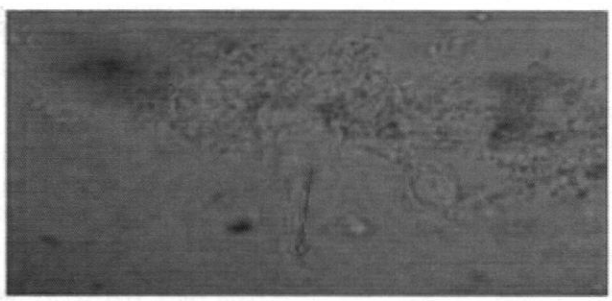

Figure-2- Giardia lamblia cyst using DFA visualizing by fluorescentmicroscope 480 nm

Discussion

The all rate of intestinal infections 51.93 % and Giardia lamblia rate 20.32 % in the present study were high, this reflects: lower educational level to health hygiene among children in general, poor experience in toilet use, overcrowded families, water contamination with Giardia parasite, and lack of insecticides that had role in mechanical transmission of the infective stages of intestinal parasites. High prevalence of parasitic infection was not agreed with that 13.23 %, 13.3% and15.9 % recorded in Iraq by Juomaa, Saliem and Hawiadi [12,13and14]. Also it was not agree with those 11.4 % and 17.1 % recorded in Libya and Brazil respectively by [15 and 16].The rate of Giardia lamblia 20.32 % was lower than those 44.59%, 35.89 %, and 62.2 % recorded in Kirkuk, Erbil in Iraq and in Egypt by [17, 18 and 19].The variance of Giardia rates from one region to another might be due to nature of residence survey, level of personal hygiene and sanitation, safety of water consumption from water supplies. In addition to type of diagnostic techniques, size of samples. The rate of infection in males was higher than in females. This might be due to that males are mostly outside their houses and are mostly exposed to faecally transmitted parasites. This finding was not agreed with those reported in two studies done among different localities of Al-Tameem governorate by Salman and Hayder[20 and 21] and with that recorded by Kadir and al-Barzanji in Erbil [22] and also with that recorded by Al-Hanoon in Mosul [23] whom they did not found significant difference in the rate of infection between males and females. These differences were probably due to the differences in technique used, or could be due to socioeconomic status [24].For diagnosis of Giardia infections, direct wet preparation microscopy detection of Giardia parasite provided the best results, with sensitivities ranging from 78.75%. In contrast, the Giardia-DFA and flotation techniques had a sensitivity of only 77.20 % and 76.19 %, this might be due to fact that in direct preparation, the sample was not contain any preserved solution that had role in missing some giardia stages in stool samples when it was preserved, while in flotation low sensitivity, specificity and accuracy might be due to centrifugation that destroy

Giardiatrophozoites as well as technical aspects, might explain why the DFA and flotation techniques did not achieve satisfactory sensitivity values. The DFA copro-antigen and flotation assays were less time-consuming and easier to perform, but were less sensitive and specific than conventional microscopicalmethods[25]. Thus, these tests might be a useful addition to stool examination for parasites including Giardia lamblia, but not a substitute for microscopical methods in the diagnosis of giardiasis .

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مقارنة التشخيص المجهري مع طريقة الومضان المباشر والتطويف في تحديد الجياردیا لامبليا في نماذج البراز

يحيى جرجيس سلمان[1] ، مها إسماعيل مصطفى[2] ، وزيرة غائب مصطفى[3]

[1] كلية الطب ، جامعة كركوك ، كركوك ، العراق

[2] كلية العلوم ، جامعة كركوك ، كركوك ، العراق

[3] مستشفى كركوك العام

الملخص

الحاجة المتزايدة الى أيجاد طرق تشخيصية لطفيلي الجياردیا لامبليا مع الأسبقية لتلك الطرق التي تعطي كفاءة عالية و تستهلك وقتا اقصر للحصول على النتيجة دعت الكثير من الشركات التجارية لتطور طرق تشخيصية سريعة بسيطة لكي تطبق و تنجز تحديد طفيلي الجياردیا لامبليا في نصف الوقت المستخدم مع الطريقة التقليدية . تم في هذه الدراسة مقارنة طريقة الومضان المباشر وطريقة التطويف مع طريقة تحضير المسحة الرطبة المزدوجة على 310 أنموذج براز جمعت من الأطفال دون 12 سنة في مدينة كركوك من الأول من كانون الأول 2012 الى 30 من حزيران 2013. كانت النسبة المئوية الكلية للطفيليات المعوية 51.93 % قسمت الى 42.58 % و9.35 % للابتدائيات المعوية والخمج بالديدان على التوالي. نسبة الجياردیا لامبليا 20.32 % كانت من بين أعلى النسب المسجلة في الدراسة . حسب جنس المريض نسبة الجياردیا لامبليا 27.92 % في الذكور كانت أعلى من 16.08 % في الأناث ، الفرق معنوي. أحصائيا الفروقات بين الأعمار والخمج بالطفيلي كانت غير معنوي . ولكن عند ربط علاقة العمر وجنس المريض مع توزيع الخمج فأن نسب الخمج في الذكور كانت 14.41 % و 7.2 % للمرحلة العمرية بين 7 الى 8 سنوات وبين 11 الى 12 سنة على التوالي الفرق معنوي. لم تظهر أستخدام الطرق الثلاثة المستخدمة لتحديد الخمج بالطفيلي فروقات بين حساسية ، خصوصية وضبط الطرق الثلاث، ولكن لدى مقارنة هذه العناصر الثلاثة مع كل طريقة فأنها كانت معنوية وبخاصة طريقة تحضير المسحة الرطبة المزدوجة على الطريقتين الأخريتين . أظهر التحليل الأحصائي فروقات معنوية لنسب التنبؤ بالحالات الموجبة بين الطرق الثلاثة المستخدمة لتحديد الخمج بالطفيلي ، بينما نسب التنبؤ للحالات السالبة للخمج كانت غير معنوي.