

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.501.076>

Prevalence of *Giardia lamblia* among Iraqi Displaced Peoples in Kirkuk Province

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ABSTRACT

Keywords

Giardia lamblia,
Formal-ether,
*Blastocyst
homonis*,
*Hymenolepis
nana*

Article Info

Accepted:
December 2015
Available Online:
January 2016

Iraq in 4 decades ago was undergo several wars in particular that started from 1980 till to recent time has had potential effects on economic outcome, subsequently impact of public health of Iraqi peoples living in areas where the war was continuous. So, that the current study was conducted to estimate prevalence of *Giardia lamblia* and other intestinal Parasites among Iraqi displaced people (IDPs) using two different laboratory techniques. For this purpose, 417 stool samples from both gender and ages from below one year to 60 years were collected from peoples in 12 districts in Kirkuk Province, whom they live under poor hygienic condition and low level of sanitation. Two laboratory methods where performed on each collected stool sample; namely direct double wet preparation of 0.85% of NaCl solution and 1% Lugol s iodine and formalin-ether concentration method. The overall rate of intestinal parasitic infection was 19.66 %, this rate was divided in to 10.31 % for *Giardia lamblia* and 9.35 % for other 9 intestinal parasites. They involve: *Blastocyst homonis* 4.17 %, *Entamoeba histolytica* 1.67 %, *Cryptosporidium parvum* 1.43 %, *Entamoeba coli* 0.71 %, *Cyclospora cayetanensis*, 0.49 %, and 0.23 % for each of *Entamoeba hartmani*, *Iodomoeba butschili*, *Hymenolepis nana* and *Ancylostoma duodenale*. Statistically formalin-ether technique show high efficacy and significance than wet preparation technique in demonstrating other intestinal parasites. Also *Giardia lamblia* was highly found among peoples aging from 1 year to 10 years than in other age groups. While relationship between giardiasis and types of applied laboratory methods and patients gender was not significant. The rate of giardiasis among IDPs was high specially particularly among young aged peoples. Formalin-ether concentration technique had value in detecting other intestinal parasites rather than detecting giardiasis.

Introduction

Giardiasis is caused by a flagellate protozoan parasite, *Giardia lamblia* (order

Diplomonadida, family Hexamitidae) and affects people worldwide including people

living in developed countries, but is more prevalent in areas with inadequate sanitary conditions (El-Safi, et al 2013). It is one of the most important non-viral infections causing diarrheal illness in humans (Meyer 1990; Dib et al. 2008). *Giardia lamblia* is recognized as the most common intestinal protozoan parasite infecting humans in Iraq (Abd-Alzahra et al, 2012) particularly in Kirkuk Province (Salman and Mustafa, 2013). These diseases are often overlooked during routine parasitological or serological testing of intestinal parasites (Salman and Salih, 2013). Depending on availability of equipment, reagents, technical experience, considerations of time, and cost, there are several methods for the detection of giardiasis (Ndao 2010). Microscopic examination of stool samples, either direct or concentrated, for the recovery of *G. lamblia* both stages trophozoites and cysts (CLSI, 2005).

The recent crisis and violence lead to massive population movement, and presence of over 1,500,000 internal displaced in Iraq (Xiao et al., 1999). The displacement of populations from different communities often brings people into proximity either due to increased concentrations of displaced populations and also increased density in terms of the living environment (Salman et al, 2015 a). If one group is a carrier of illness, disease outbreaks reflecting endemic pathogens circulating within the community may occur (Watson et al., 2007). Because of the internally displaced persons are at high risk for emerging parasitic infections, since in most cases they have a history of poor utilization of medical care and vaccination, living conditions of low socioeconomic status and a high possibility to be carrying symptomless diseases, so it is important to carry out an assessment for diarrheal risk factors particularly intestinal parasites with an emphasis on *Giardia lamblia* and to

avoid any health risks. Therefore this study was conducted to investigate giardiasis rate in this particular category.

Materials and Methods

Time and Location

From 1st of September 2014 to 30th of June of 2015, cross sectional study was conducted in laboratory department of dentistry College Kirkuk University, and in Ibn-Nafies private medical laboratory.

Source of Samples

A total of 417 stool samples were chosen from internal displaced persons living in schools, houses under construction and rented accommodations and houses in over 12 residential districts in Kirkuk city with variant economical and hygienic levels and those people are originated from different cities and villages like Anbar, Mosul, Salah-Eldin, Diyala, Fallujah and other areas which severely affected from conflicts and crisis in Iraq. Sample size was validated by applying the equation of sample size determination in unknown population. Patients are segregated into age groups for both males and females subjects by applying Yule method (Daniels, 1985). Also sample chooses were involve two sources: 360 stool samples for peoples living under low level of sanitations, low income and poor hygienic condition. Whereas 57 stool samples for people living in normal good level of sanitation, high income and good level of hygiene were chosen.

Stool Samples Collection

Prior to sampling a special questionnaire was filled for each patients consisting of essential information. Disposable container with wide screw lid was given to each

patient to bring stool samples. The container label contain: name, date, address and number of container. Immediately about 3-5 ml of Potassium chromate solution was added to each container for preservation (Salman, 2015). Stool samples were kept in ice box and transferred directly to laboratory department for processing.

Sample Processing

After samples arrival to laboratory, each sample was examined for detecting *Giardia lamblia* stages and other intestinal parasites stages using direct double wet preparation of 0.85% of NaCl and 1% of lugols iodine (Salman, 2015). While formalin –ether technique was applied as follows: 10 ml of formalin was added in a clean test tube containing approximately 1gm of stool sample, the tube then agitated for two minutes till to obtaining cloudy suspension. A second centrifuge tube was fixed in a plastic rack and double layer of gauze was fitted in a funnel fixed in the top of centrifuge tube; carefully the content of first tube was converted into the tube on the rack. The funnel and gauze were removed and 3 ml of ethyl acetate or ether-ether was added quickly and the tube was plugged with rubber. The tube was converted several times then centrifuged for 3 minutes using 3000 rpm. The supernatant was discarded and few drops of malachite green 3 % stain or lugols iodine 3% was added to deposit. The tube was shaken for several times all content of the deposit were examined using 10 X and 40 X ,(WHO,1991).

Results and Discussion

From the examining of 417 stool samples using direct double wet preparations, the overall rate of the intestinal parasitic infections was 19.66 % distributed in 82 stool samples. This rate involve 10.31 %

(43) as pure *Giardia lamblia* infection and 9.35 % (39) for other intestinal parasitic infections. Statistically the differences between giardiasis and other intestinal parasitic infections in regard of direct microscopy was not significant, $P>0.05$. Table 1.

Nine species of other intestinal parasites were recorded in current study beside *Giardia lamblia*. The rates were: 4.176% for *Blastocyst homonis* followed by 1.678%, 1.438 %, 0.719 %, and 0.479 % for *Entamoeba histolytica*, *Cryptosporidium parvum*, *Entamoeba coli*, *Cyclospora cayetanensis*. While low rate 0.239 % was recorded for each of *Entamoeba hartmani*, *Iodamoeba butschili*, *Hymenolepis nana* and *Ancylostoma duodenale*, $P<0.05$; Table -2.

To get accurate and more precise rate of giardiasis in addition to assess the efficacy of suitable laboratory methods in detecting *Giardia lamblia* and other intestinal parasites, the all 417 stool samples were examined using direct double wet preparations in parallel with formalin-ether sedimentation technique. The results were obvious in table-3; which exert 5.51 % of *Giardia lamblia* stages using ether formalin compare to 4.79 % using double preparations, $P>0.05$. While other intestinal parasitic rate 6.23 % by using ether-formalin technique was higher than 3.12 % using double preparations, $P<0.05$. Whereas collectively *Giardia lamblia* and other intestinal parasites statistically show significant differences between to laboratory methods; through which ether-formalin technique contribute 11.75 % from total of 19.66 % for detecting parasitic stages in stool samples compare to 7.91% by using double wet preparation method, $P<0.05$.

Regarding the relationship between *Giardia lamblia* distribution according to patient

gender, no significant difference was obtained between males and females, $P > 0.05$. Meanwhile according to patients ages high rate of giardiasis 5.99% was found in stool samples belongs to peoples aging from 1 year to 10 years compare to 0.47% of giardiasis among patients aging from 41 to 50 years, $P < 0.05$; table-4. The overall rate of intestinal parasitic infections

19.66 % in the present study was high, this reflects: lower educational level to health hygiene among children, poor experience in toilet use, overcrowded families, water contamination with *Giardia* and other waterborne parasites, and lack of insecticides that had role in mechanical transmission of the infective stages of intestinal parasites.

Table.1 Distribution of *Giardia lamblia* and other Intestinal Parasites by using Direct Microscopy

Parasites types	Positive		Negative	
	No	%	No	%
<i>Giardia lamblia</i>	43	10.31	347	89.61
Other intestinal parasites	39	9.35	378	90.65
Total	82	19.66 *	335	80.44

* $P > 0.05$

Table.2 Frequency of Other Intestinal Parasites by using Direct Microscopy

Type of parasites	Number positive	Percentages positive
<i>Blastocyst homonis</i>	17	4.176 *
<i>Entamoeba histolytica</i>	7	1.678
<i>Cryptosporidium parvum</i>	6	1.438
<i>Entamoeba coli</i>	3	0.719
<i>Cyclospora cayetanensis</i>	2	0.479
<i>Entamoeba hartmani</i>	1	0.239
<i>Iodamoeba butschili</i>	1	0.239
<i>Hymenolepis nana</i>	1	0.239
<i>Ancylostoma duodenale</i>	1	0.239
Total	39	9.352

* $P < 0.05$

Table.3 Comparison between the Employ of Direct Double Preparations Technique and Ether-Formalin Sedimentation Technique in Detecting *Giardia lamblia* in Stool Samples

Lab methods	<i>Giardia lamblia</i>				Other intestinal parasites				Total			
	Positive		Negative		Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%	No	%	No	%
Double wet preparations	20	4.79	397	95.21	13	3.12	404	96.88	33	7.91	384	92.21
Ether-formalin	23	5.51A	394	94.49	26	6.23B	391	93.27	49	11.75C	368	88.25
Total	43	10.31	335	89.68	39	9.35	378	90.65	82	19.66	335	80.44

A= $P > 0.05$

B and C= $P < 0.05$

Table.4 *Giardia lamblia* Distribution in Relation to Patients Age and Gender

Age groups in years	Total examined		Males		Females		Total		Positive rate from overall	
			Positive		Positive		Positive			
	No	%	No	%	No	%	No	%	No	%
≤1-10	163	39.08	11	6.74	14	8.48	25	15.15	25	5.99 *
11-20	43	10.31	2	4.65	1	2.32	3	6.97	3	0.71
21-30	75	17.98	3	4.00	4	5.33	7	9.33	7	1.67
31-40	49	11.75	0	0.0	3	6.12	3	6.12	3	0.71
41-50	44	10.55	1	2.27	1	2.27	2	4.54	2	0.47
51-60	43	10.31	2	4.65	1	2.23	3	6.97	3	0.71
Total	417	100	19	4.55	24	5.75	43	10.31	43	10.31

*P<0.05

The rate of *Giardia lamblia* 10.31% among IDPs in current study was not agree with the following ratios recorded in Iraq: 25.33 %,30.39 %,35.89 %, 37.5 %,44.59% and 45.9 % for giardiasis in Kirkuk Province, Kirkuk city, Erbil, Al-Tammen, Basrah and Tikrit Provinces by (Kadir et al., 2000); (Kader and Salman,1999), (Kadir et al., 1987), (Jar-Allah, 2012) and (Al-Somadayi, 2012) respectively. Also the rate of giardiasis 10.31 % in current study was lower than 18 %, 25.33%,62.2 %,67.6 % and 89.5 % recorded in Iran, Yemen, Egypt, Colombia and Chanaby (Taherkhan et al., 2009), (Al-Yousefi et al.,2013), (Yassin et al., 2000), (Jorge et al.,2009) and(Nkrumah and Nguash,2011) respectively. This finding reflects the degree of contamination with parasitic phases among this group of peoples in Kirkuk community. The reason to that most often might be related to nature of water consumption and water quality and water supply. It has been known, that a big construction for improving of roads and infra-structure in Kirkuk province from 2008 to 2014 was carried on. This action lead to breakdown of water pipes underground, because all of these pipes are very old, this lead to continue of water supply interruption in this Province. The second reason to this high rate might be attributed to uncontrolled

migration and inhabitation of IDPs to old buildings, in complete building, old schools. Moreover 4 to 5 families live in one house (highly crowded).All of these factors have had role in increasing the rate of intestinal parasites particularly giardiasis in current study. This finding was not agreed with those recorded in the same Province by (Salman et al., 2015), whom they record 7.05 % of giardiasis among IDPs in the same governorate. It is not clear how much these differences may be explained by differences in study design, geographical location, population group, sensitivity of laboratory methods, stage of disease or type of laboratory tests.

Regarding common intestinal parasites records in current study particularly *Blastocystishominis* 4.17 % as high rate rather than with other 8 parasites recorded, this finding was highlighting the alarm of the bad conditions of IDPs within areas whom they live. Moreover it has been found that there is relationship between Irritable bowel syndrome (IBS) and *Blastocystis hominis* infections (Ustun and Turjay, 2006). So this group of peoples(IDPs) may have bowel diseases predisposed by other psychiatric condition due to they were living under low level of poor hygienic conditions.

This rate was lower than 1.66 % that recorded among the IDPs in the same province by (Salman, et al 2105), whom they record 1.66%). Also was not agreed 41.05 % of *Blastocystis hominis* recorded by (Salman, 2015). Variances in the rate of *Blastocystis* mostly due to employee of ELISA copro-antigen test by the later author, who assess the efficacy of direct microscopy and ELISA in detecting *Blastocystis hominis*.

Comparison between the employ of direct double preparations technique and ether-formalin sedimentation technique in detecting *Giardia lamblia* in stool samples, in this regard the obtaining of low efficacy of stool concentration method(formalin-ether technique) might be due to the light weight of *Giardia lamblia* parasites, which most often undergo flotation when it was in solution with high specific gravity as the used formalin-ether technique cannot permit that(WHO,1991). While obtaining 6.23 % of other intestinal parasites by using formalin-ether technique higher than 3.12 % by using direct double wet preparations. This finding reflect that other intestinal parasites involve some helminthes recorded in current study, which have heavy weight that draw any heavy parasitic forms to the bottom of the tested tube containing stool sample plus parasites in this method(WHO,2003). This finding was agreed with those recorded by (Kadir and Salman,1999), (Salman and Ali,2013) and (Salman and Mustafa, 2013).

The result of the current study in regard of patient gender in relation to *Giardia lamblia* particularly and other intestinal parasites frequencies show no impact of gender in parasites distribution among IDPs. This finding was agreed with those reported in few studies done among different localities of Kirkuk governorate (Hayder, 1993) and (Al-somaydayi, 2012) and (Kader and

Salman,1999). And with that recorded by (Kadir and Al-Barzanji,2000) in Arbil and with that recorded by (Al-Hanoon,1976) in Mosul whom they did not find significant difference in the rate of infection between males and females. While not agreed with that recorded by (Salman and Mustafa,2013),whom they recorded of giardiasis among males than in females in the same Province. No differences between two sexes are probably due to the different in technique used, or could be due to socioeconomic status.

Considering the age, recording of 15.55 % of giardiasis and 5.99 % from overall rate among children in age group from 6 months to 10 years compare to low rates in other age groups might be attributed to fact that this aged group peoples were spend most times out of the door so they were highly exposed to infectious agents including parasitic forms particularly *Giardia* parasite stages (Salman,2001). Malnutrition, due to poverty, which leads to immune diminishing had role in increasing the susceptibility of acquiescing infectious agents including *Cryptosporidium* and *Giardia* (Striepen, 2013) and (Watson et al.,2007). Conclusions: The overall rate of intestinal parasites, particularly giardiasis among young aging of IDPs in Kirkuk Province was high. Sedimentation (formalin-ether technique) of concentration method have had specific role in detecting intestinal parasites in stool samples of infected peoples than direct double wet preparation method.

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How to cite this article:

Yahya Jirjees Salman, Abdul-Rahman Aziz Al-Tae and Ali Mohammed Abid. 2016. Prevalence of *Giardia lamblia* among Iraqi Displaced Peoples in Kirkuk Province. *Int.J.Curr.Microbiol.App.Sci*. 5(1): 753-760. <http://dx.doi.org/10.20546/ijcmas.2016.501.076>