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Comparison between Specificity and Sensitivity of Intestinal Giardialamblia Assays in AL-Door District, Salahdin Province, Iraq

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Abstract: **The Giardia lamblia is known as a considerable cause of diarrhea in human. Difficulties are confronted in the detection of that parasite in patients' faces because of intermittent excretion of the parasite.In this study it was determined specificity and sensitivity of floatation methodby zinc sulphate solution and ELISA assay for Giardialamblia detection compared with direct iodine stain methodAmong 82 patients who were attending AL-Door hospital through period of September to December 2018. The prevalence of Giardiasis was 32.9% and 67.1% was negative. As well this study showed that the percentage of G. lamblia infection was 31.7% by direct iodine stain method, 29.3% was by floatation methodand the sensitivity ratio was 92.3%, specificity was 100%,While 32.9% was positive by ELISA assay and the sensitivity ratio was 92.3%, specificity was 94.6%. Also our study found that the prevalence for Giardiasis depending on gender in this study was 67% for male, 33.3% for female. The results of study were showed the prevalence of infection was 23.2% in age group 8-25 years, 7.3% in 26-35 years and 2.4% in age group 36-45 years. The distribution of infection depends on residencewhich was 20.7% for rural and 12.2% for urban.**

Keywords: Giardia intestinalis, floatation method, ELISA,AL-Door district,Salahdin province.

I. Introduction

Giardialamblia or G. intestinalisis flagellated parasite that infects alimentary tract of a variety of the mammalian hosts involving human(Berrilli *et al.*, 2010). Because of G. lamblia has a fecal-oral circulation and is transmitted by consumption of contaminated food or water or by infected person to person contact, the highest infection rates is presentin the regions where hygienic conditions are bad(Shah *et al.*, 2008)). The highest averages of infection are observed in developing countries, where infections occur mainly among individuals populating in closed communities, immigrants and travelers coming back from endemic countries(Omar *et al.*, 2013). Infected person could be asymptomatic while other suffer diarrhea, bloating and abdominal pain, malaise as well as weight loss and that is because of themalabsorption (Salim *et al.*, 2013).This cause changes in enteric epithelial function with microvilli shorting(Mank *et al.*, 1997). For technicians, it is difficult to diagnose Giardiabecause the parasite's cysts are introduced intermittently besidesimilarity of those cysts with other microorganisms such as yeast(Payne *et al.*, 2005).

In the current study, specificityand sensitivity of techniques (including direct iodine stain method, floatation method by Zinc sulphate solution and enzyme-linked immunosorbent assay for antigenic detection in fecal samples) are compared for G. lamblia diagnosis.value of each Sensitivity, Specificity and predictive were calculated as following:-

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100 =$$

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100 =$$

$$\text{Positive predictive value or PPV} = \frac{TP}{TP+FP} \times 100 =$$

$$\text{Negative predictive value or NPV} = \frac{TN}{TN+FN} \times 100 =$$

$$\text{Accuracy of the test} = \frac{TP+TN}{TP+TN+FP+FN} \times 100 =$$

II. Materials and methods

This study carried out in period of September to December 2018, Among 82patientswith abdominal symptoms and diarrhea whose ages ranged from 8 to 45 years, who were attending AL-Door model hospital. From each patient fecal samples were collected at the same time andcomplete information were revealed in special questionnaire designed for this purpose.Each sample is divided into three parts, the first part was diagnosed by direct microscopical method after preparing 3slides which was stained bylugol- iodine stain for immediately trophozoite or cysts detection(Gupta, 1979).The second part was diagnosed via floatation method byzinc sulphate solution(John and Petri, 2006) and the last part was added formalin solution 10% to it and stored for

ELISA assay (for detect trophozoites& cysts of G. lamblia antigens in feces samples (DRG/Germany)) which was done later depending on manufacturer instructions.

III. Results

Among 82 feces samples diagnosed,67.1% was negative and32.9% was positive for Giardiasis. Table (1).

Table (1):- Giardiasis among infected & non infected patients and a comparison between three methods for Giardiasis diagnosis.

No. of examined samples 82									
No. of positive samples		No. of negative samples		Diagnosis method					
				direct iodine stain method		floatation method		ELISA assay	
No.	%	No.	%	No.	%	No.	%	No.	%
27	32.9	55	67.1	26	31.7	24	29.3	27	32.9

The prevalenceof Giardiasisdepending ongender in this study was distributed to67% for male and33.3% for female. However the prevalence depends on age groups was 23.2% in age group 8-25 years, 7.3% in age group 26-35 years and 2.4% in age group 36-45 years. Whilethe distribution of infection depends on residence was20.7% for rural and 12.2% for urban. Table (2).

Table (2):- Demographic data of Giardiasis infected patients

	No. of patients	No. of positive patients	%	Chi-square X^2	P-Value
Gender			67		
Male	49	18		0.799^{ns}	0.371
Female	33	9	33.3		
Age					
8-25	41	19	23.2	9.158**	0.01
26-35	28	6	7.3		
36-45	13	2	2.4		
Residence			20.7		
Rural	50	17		10.055**	0.002
Urban	32	10	12.2		

The percentage of G. lambliainfection was 31.7% by direct iodine stain method and 29.3% by floatation method. The whole samples which were positive byfloatation method were positive by direct iodine stain method and 2 samples which were negative byfloatation method were positive by direct iodine stain method. Table (1&3)and fig. (1)

Table (3):- Results of flotation method against direct iodine stain

		direct iodine stain method		Total
		+	-	
Floatation method	+	24^{TP}	0^{FP}	24
	-	2^{FN}	56^{TN}	58
Total		26	56	82

While 32.9% was positive by ELISA assay,24 samples which were positive bydirect iodine stain were positive by ELISA assayand 3 samples which were negative bydirect iodine stain were positive by ELISA assay. As well as ELISA test failed to detect 2 samples were positive bydirect iodine stainTable (1&4) and fig. (1).

Table (4):-Results of ELISA assay against direct iodine stain method

		direct iodine stain method		Total
		+	-	
ELISA assay	+	24^{TP}	3^{FP}	27
	-	2^{FN}	53^{TN}	55
Total		26	56	82

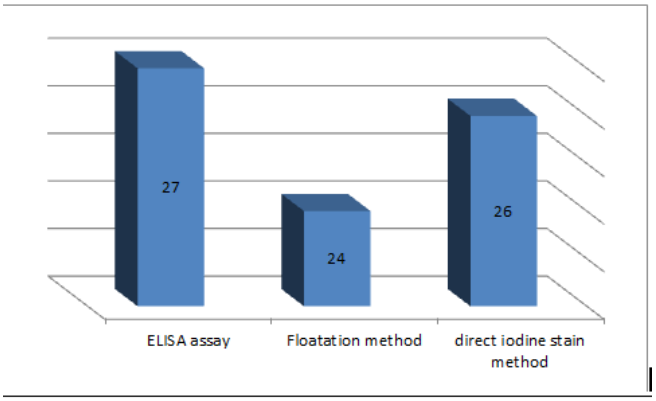

Figure (1):- *G. lamblia*infection diagnosis rate by direct iodine stain, floatation method and ELISA assay.

In comparison with direct iodine stain method, our study found that floatation method sensitivity was 92.3%,specificity was 100%, PPV was 100%, NPV was 96.6% and accuracy of the method was 97.6%. while ELISA sensitivity was92.3%, specificity was 94.6%, PPV was 88.9%, NPV was 96.4% and accuracy of the method was 93.9%. Table (5).

Table (5):- Prevalence of giardiasis depending on diagnosis method

methods	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
Floatation	92.3	100	100	96.6	97.6
ELISA assay	92.3	94.6	88.9	96.4	93.9

IV. Discussion

Routine microscopy of recurrent 3 fecal samples is up to the present time being the commended gold standard assay for Giardia lamblia detection, however the sensitivity of that method is still established to be low (Jahan *et al.*, 2014; Beaver and Jung, 1985).Thus, through this study,we estimated the performance of flotation method and ELISA

assay as diagnostic methods in comparison to traditional microscopy for G. lamblia diagnosis.

Among 82 feces samples diagnosed for Giardiasis,67.1% was negative and 32.9% was positive.depending on gender, the infection in this study was 67% for male, 33.3% for female. Those results differ from that study established by AL-Bayati (AL-Bayati, 2015) and salmanet. al. (Salmanl *et al.*, 2014).High activity and frequent exposure to the external environment make male more affected by parasite than female (Salmanl *et al.*, 2014).

Giardiasis prevalence depending on age groups washigherin age group 8-25. Our results differ from that conducted by butty in Nineveh(Buty, 2011) and AL-Bayati(AL-Bayati, 2015).Usually, younger persons are more infected by Giardiasis because they have more contact with external contaminated landas well as factors related to the immune system(Alam *et al.*, 2011).While the prevalence of infection depending on residence was 20.7% for rural and 12.2% for urban.Several factors cause the spread of Giardiasis among residents of rural areas including:-the reduction of educational level, bad experiment in toilet use, contaminated water with parasites, crowded families and lack ofinsecticides used for killing mechanical transportation of the infected stages of intestinal parasites(Salmanl *et al.*, 2014).

This study showed that infection of Giardiasisby direct iodine stain method was higher than by floatation method, the whole samples which were positive byfloatation method were positive by direct iodine stain method and 2 samples which were negative by floatation method were positive by direct iodine stain method, Those results almost agreewith study conducted by salman *et al.*, in Kirkuk city (Salmanl *et al.*, 2014) and by Gotfred-Rasmussen et. al. (Gotfred-Rasmussen *et al.*, 2016). Italso differsfrom that studyestablished inTikrit district (Muhsin and Daoud, 2015).

In this study Giardiasis percentage using direct iodine stain method is higher than the rate of infection using floatation method and this may be due todestruction of parasite's trophozoites by Centrifugation (Salmanl *et al.*, 2014). However, direct iodine stain method and floatation technique needs proficient staffand is work intense.

While 32.9% of Giardiasis was positive by ELISA assay, 24 samples which were positive bydirect iodine stain were positive by ELISA assay and 3 samples which were negative by direct iodine stain were positive by ELISA assay. As well as ELISA test failed to detect 2 samples were positive by direct iodine stain,those 2 false negative results could be related to low Giardia parasite densities and intermitentGiardia excretion with stool(Ali and Hill, 2003).

In comparison with direct iodine stain method, our study found that the sensitivity and specificity of thefloatation methodare similar to those of the ELISA test.Those resultsalmost agree with study conducted by Wilson and Hankenson (Wilson and Hankenson, 2010). In addition, the value of eachPPV, NPVand accuracy offloatation method are higher than those ofthe ELISA test, those result roughly agree with study conducted by Uchoa and Almonsny (Uchoa and Almosny, 2018).Several studies have shown different results for sensitivity, specificity, PPV, NPVand accuracy for the ELISA test(Mohammad and Moawad, 2016; Al-Saeed and Issa, 2010; Ozekinci, 2005).The difference among the previous studies is due to the difference in the number of samples examined in each study,while the ELISA assay sensitivity has been based to be improved against increasing number of samples(Addiss *et al.*, 1991).

V. Conclusions

1-The prevalence of Giardiasis was 32.9% and 67.1% was negative.

2-The percentage of infection was 31.7% by direct iodine stain method, 29.3% was by floatation method (sensitivity ratio was 92.3%, specificity was 100%), While 32.9% was positive by ELISA assay (sensitivity ratio was 92.3%, specificity was 94.6%).

3-The prevalence of infection depending on gender in this study was 67% for male, 33.3% for female. Also The results of study showed the prevalence of infection was 23.2% in age group 8-25 years, 7.3% in 26-35 years and 2.4% in age group 36-45 years. The distribution of infection depending on residence was 20.7% for rural and 12.2% for urban.

VI. Recommendations

1- To conduct laboratory floatation methodsfor investigating parasites in addition to direct microscopic diagnosis with more than one slide per a sample.
2- To conduct immunological tests such as ELISA to investigate Giardia in the faeces. Laboratory microscopes should be provided with micrometers to accurately diagnose the parasite.
3- Spreading health awareness among the population, especially the rural population, to avoid infection by adhering to sanitary and hygiene conditions, enjoying eating with street vendors and drinking from non-sterilized water.

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