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Discrepancy in Diagnosis of *Giardia Lamblia* Infection among Sudanese Population in Khartoum State-A Comparison between Direct microscopy, Formal Ether and Copro Antigen Elisa

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Abstract: *The objective of this case control study was to detect discrepancy in the diagnosis of Giardia lamblia among Sudanese patients by comparing the sensitivity and specificity of copro antigen ELISA aginst microscopy and FECT. Method: 100 patients diagnosed with Giardiasis, and100 healthy controls were included in this study. Fresh stool specimens were collected from each study participants and were examined microscopically for cysts and trophozoites by wet preparation, formal-ether technique and Copro antigen ELISA. Results: No differences between wet preparation and formal ether techniques. Out of 100 true positive samples after microscopy, only 90(90%) were positive by ELISA, while 96(96%) were true negative from 100 samples of control group. Copro-antigen ELISA for detection of Giardia lamblia results a sensitivity of 90% and a specificity of 94%. With 93.8% and 90.3% PPV and NPV. The obtained clinical cut off copro ELISA among Sudanese population infected with Giardiasis and non-infected group were [(.98±.21) and (.16±.18)] respectively. Higher cases of Giardiasis were detected in age group of 21-30 years 43% (30+13). Strong agreement between copro antigen ELISA and microscopy was obtained by Kappa test (P value 0.00). The level of accuracy was obtained by area under the curve 0.939, which represent excellent test also highly sensitive and specific.* Difference between these techniques was found to be statistically significant (p=0.001). *We recommend using ELISA in epidemiological studies also to confirm the diagnosis in patients with continuous symptoms of Giardiasis with no results by direct microscopy. Such technique would be helpful at early infection, when the level of parasite is quite low.*

Keywords: *Giardiasis, microscopy, Formol ether, Copro antigen Elisa.*

I. Introduction

Gastrointestinal infections consider the most causes of morbidity and mortality over the world and mainly in countries under developing. While diarrhea does not typically cause serious complications for most patients, it can be a fatal ailment for young children and elderly, especially those who are malnourished or have compromised immune systems [1].There were a variety of pathogens causing diarrhea as viruses, bacteria, and parasites. The most groups of parasitic diarrhea are *Entamoeba histolytica* and *Giardia lamblia* [2]. Some literature reported that 90% of the parasitic diarrhea is due to *Giardia* infections. G*iardia* is a parasite found in all parts of the world and in a large number of mammals, including humans, livestock, pets, wildlife, and aquatic animals [3, 4]. Several recent reports have also described *G. lamblia*in various birds and even fish, although true infections remain to be confirmed in these animals [5]. Different percentage of Giardiasis occurs between countries, and it is higher in areas with low sanitary condition. According to estimates from the WHO about 200 million people have symptomatic giardiasis, and around 500,000 new cases occur each year [6]. Studies in different European countries have indicated prevalence of 1–17%, and up to 100% of the population can be infected in certain highly endemic areas [7]. Microscopic detection of *Giardia* cysts in a stool specimen, either directly in a wet smear or after formol-ethyl acetate concentration, is the most frequently used method for diagnosis of giardiasis worldwide. Usually, most used is the basic technique for cysts and trophozoites in fecal specimens. Compared to identification of *Entamoeba* spp., microscopical diagnosis of *Giardiasis is* simple and cheap, but still with quite low the sensitivity due to the intermittent excretion of *Giardia* cysts, and thus it is recommended that at least three samples be examined in order to rule out giardiasis [8]. Quite number of commercial kits is available for detection of *Giardia* antigen. Two techniques that are often used are enzyme-linked immune-sorbent assay (ELISA) that assesses soluble antigens and a direct fluorescent antibody (DFA) test that detects intact organisms. Several studies have shown that these two methods offer greater sensitivity compared to light microscopy [9], but they are not available in all parasitology laboratories due to the high cost and substantial workload they entail, and also limited access to the required equipment. An alternative technique involves a solid-phase immune chromatographic test card system (Immuno Card STAT *Cryptosporidium /Giardia* rapid assay), which allows concurrent detection of *Cryptosporidium* and is also fast, easy to use, and does not require extra equipment but low sensitivity may occur [10, 11].

II. Materials And Methods:

2.1. Study design: Case control study was conducted during period from August 2014 to June 2016 in Khartoum state, Sudan.

2.2. Subjects: A total sample size of 200 patients were divided into two groups, 100 patients with Giardiasis as study group and 100 healthy individuals as control group, confirmed by microscopy after formal ether as gold standard method for diagnosis .Permission of this study were obtained from the Research Committee, College of Medical Laboratory Science at Sudan University. The aim of the study was explained to all participants in this study. Informed consent was obtained from each participant. Also, a questionnaire was designed to collect data from the patients.

2.3. Sample: Stool samples from all individuals were collected in clean, leak proof container and were divided into two parts, one part in preservation media of 10% formalin was used for standard microscopic for O&P and confirmed by formal ether concentration technique, a second part was stored in frozen condition at -20º C until used for Elisa technique.

2.4. Measurements of *Giardia* copro antigen: All faecal samples were analyzed for qualitative determination of *Giardia* specific antigens according to the manufacturer instructions. ELISA kits from Demeditec Diagnostics GmbH. It is an enzymometric two step immune assay based on polyclonal peptide antibodies. OD was measured at 450 nm / ≥ 620 nm using semi automated Elisa. 0.08 OD and above indicates the samples contains *Giardia* antigen. All samples were analyzed in the laboratory of Alrayan centre.

2.5. Statistic evaluation: Statistical analysis was performed using SPSS 16 (Statistical Package for Social Sciences).The obtained mean of the case and control group represent the clinical cut off level of Giardiasis in Sudanese population. Since microscopy test was reported as a reference standard test. Sensitivity, specificity, PVP, NPV and accuracy were calculated with the following formula to analyze data: sensitivity: sensitivity: A/(A+C) × 100]; Specificity: D/(D+B) × 100]; PVP: A/(A+B) × 100]; NPV: D/(D+C) × 100], and accuracy:(TN + TP)/(TN+TP+FN+FP)], where a = true positive samples, b = false positive samples, c = false negative samples and d = true negative samples. Kappa test was done to measure the agreement between the methods. P value ≤ 0.05 was statistically significant. while the Kappa value explains the strength between the two tests, when the value equal 0.5 it represent mirror agreement, above 0.7 represent good agreement and above 0.8 represent very good agreement. Receiver Operating Characteristic (ROC) curve was obtained to determine the excellent, good, and worthless tests plotted on the same graph (validation of ELISA). Accuracy is measured by the area under the ROC curve. An area of .90-1 represents an excellent test; .80-.90 = good .70-.80 = fair .60-.70 = poor an area of .5 represents a worthless (fail) test. p-value ≤ 0.05 was considered as statistically significant.

III. Results

Within the100 positive cases detected by microscopy after formol ether, as gold standard method, Giardia was detected in 90(90%) in ELISA technique, whereas 100 samples in control group was free of Giardia by both wet preparation and concentration technique, 96(96%) were negative by ELISA, while only 6(6%) from control were detected as false positive by ELISA [Table1]. Out of 200 participants, 96 (48%) were detected positive cases of *Giardia* and 104 (53.5%) were negative by copro ELISA [Table1]. The clinical cut off copro ELISA among Sudanese population of Giardiasis infected and non-infected group [(0.98±.21) and (0.16±.18)] respectively [Table3]. Out of 100 cases of Giardiasis 65(72.2%) were males and 25(27.8%) were females [Table4]. Age of patients ranged from 2-80 y and mean age was 26.84± 12.9y. Among studied variables 80% of cases were acute phase of disease. Highest cases of Giardiasis diagnosed by ELISA test were detected in age group of 21-30 years, 43% (30+13) [Table5]. Microscopical appearance of stool samples among case study group of Giardiasis, 88 (88%) with acute phase of the disease. cysts were detected in15 (15%) (n = 100). 40% of Giardia positive cases had pus in their stool, while no RBCs were detected in all cases. Accuracy of Elisa was obtained by Kappa test (P value 0.001) [Table 6]. The validation and sensitivity of Elisa was measured by ROC curve, area under the curve 0.939 represent excellent test with high sensitivity and specificity [fig 1].

Table (1): Analysis of Copro Ag ELISA results in case and control group:

			Giardia copro ELISA		
			possitive	negative	Total
Status	Case	Count	90	10	100
		% within Status	90.0%	10.0%	100.0%
		% of Total	45.0%	5.0%	50.0%
	control	Count	6	94	100
		% within Status	6.0%	94.0%	100.0%
		% of Total	3.0%	47.0%	50.0%
Total		Count	96	104	200
		% within Status	48.0%	52.0%	100.0%
		% of Total	48.0%	52.0%	100.0%

Table (2): Accuracy for Copro Ag Elisa among study population using microscopy as a reference standard:

Parameters	Accuracy
Sensitivity	90%
Specificity	94%
PPV	90%
NPV	93.75%
Accuracy	92%

Table (3): Mean cut off Copro Ag ELISA among case and control groups:

Group	N	Minimum	Maximum	Mean	Std. Deviation
Giardia case group	100	0.04	0.65	0.13	0.009
Giardia control group	100	0.02	0.13	0.07	0.08
Disease prevalence (purposive)	47%				

Table (4): Sex distribution among study population:

			SAE		Total
			MALE	FEMALE	
remarktestg	possitive	Count	65	25	90
		% within remarktestg	72.2%	27.8%	100.0%
		% of Total	65.0%	25.0%	90.0%
	negative	Count	7	3	10
		% within remarktestg	70.0%	30.0%	100.0%
		% of Total	7.0%	3.0%	10.0%
Total		Count	72	28	100
		% within remarktestg	72.0%	28.0%	100.0%
		% of Total	72.0%	28.0%	100.0%

Table (5): Distribution of *Giardia* infection according to age group:

Age groups:	sex		Total
	MALE	FEMALE	
> 10 years	9	4	13
10 – 20 years	7	2	9
21 – 30 years	30	13	43
31 - 40 years	10	3	13
41 - 50 years	3	2	5
51 - 60 years	3	2	5
More than 61+	1	1	2
Total	63	26	90

Table (6): Kappa agreement between Copro Ag Elisa and microscopy results in case and control group:

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Measure of Agreement	Kappa	0.830	0.039	11.767	0.001
N of Valid Cases		200			
a. Not assuming the null hypothesis					
b. Using the asymptotic standard error assuming the null hypothesis.					

Figure (1): ROC curve for diagnosis of Giardiasis by copro Ag ELISA:

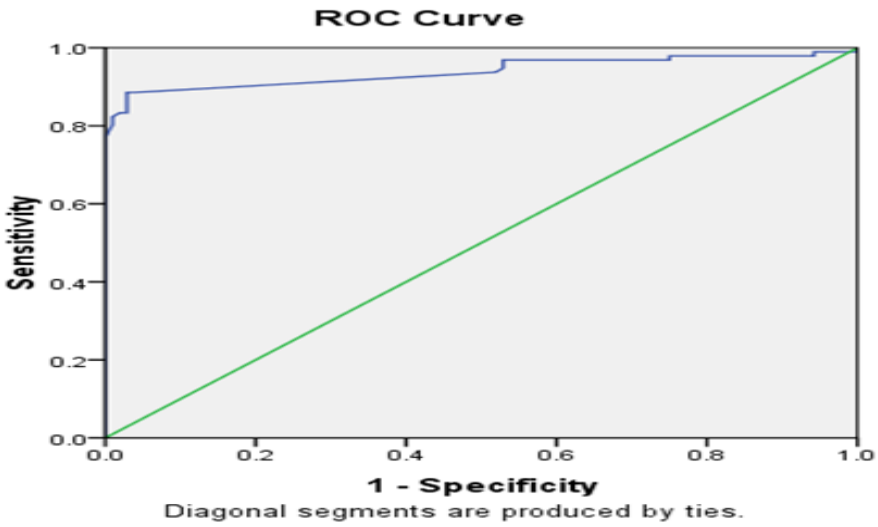

Table (7): Accuracy Area under the Curve (AUC):

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
0.939	0.020	.000	0.900	0.978
a. Under the nonparametric assumption				
b. Null hypothesis: true area = 0.5				

IV. Discussion

G. lamblia is one of the most common intestinal protozoan parasites which affect about 200 million people in Asia, Africa and Latin America with 280 million infections per year [12, 13]. Epidemiological studies have shown that most cases of parasitic diarrhoea in children are due to *G. lamblia* infection, especially in areas with poor sanitation. [14].The diagnosis of giardiasis is based primarily on microscopic examination of stool samples through the identification of motile trophozoites or the cyst phase. [15]. Microscopic examination requires examining three consecutive stool samples in order to obtain higher sensitivity (over 90 %). Lower sensitivity (approximately 50 %) of a single sample examination may be attributed to low parasite density, sporadic excretion of cysts or the possibility of the parasite being masked with bile pigments. [17]. The need for a more robust diagnostic techniques lead to the development of rapid, sensitive and specific diagnostic methods [16]. ELISA is a rapid, sensitive and cost effective method for detection of specific antigens in stools and confirmation of certain infection. Copro antigens of a parasite could be traced and diagnosed even if the live parasite is absent in the fecal samples [18]. The present study showed that the percentage of positive rates of G. lamblia that were detected by using direct wet mount was (45%), while it was increased to reach more than (50%) when using formal either concentration technique (FECT).These results were similar to results obtained by (Eltayeb et al. 2012) [19] and disagreed with the result of Gabbad and Elawad (2014) [20] in Khartoum State. The current study revealed that the prevalence of *G. lamblia* infection among males was higher (30 %) than in females (13%), these results were in agreement with *Yakoob et al*. (2005) [21] who found that the prevalence of G. lamblia was 38.9% higher in males than in females in Pakistan. The present study showed that the prevalence rate of G. lamblia was higher (43%) in the age group 21-30 years old; these results were not in line with Iraqian study which was done by Raza and Sami (2009) [22] who showed that the highest rate of infection (17%) was among the age group (6-10) years old. Our study demonstrated that the sensitivity and specificity of the copro ELISA test for detection of *G. lamblia* versus microscopy were 90% and 94%. These results were similar to an Iraqi study conducted by Souhaila, [23], 76.4% and 100%. *Giardia* cysts or trophozoites are difficult to recover from infected patients. Quality of the diagnosis can be confirmed by examining at least three stool samples over several days. Copro antigen ELISA test is more sensitive and specific. Several immunological tests can detect *Giardia* antigens in stool specimen but until now does not replace the simple microscopy. We recommend using ELISA in epidemiological surveys in Sudan, same as a result obtained by Al-Saeed, [24] and to confirm the diagnosis in patients with typical clinical symptoms of giardiasis but with negative results by direct microscopy

V. Conclusion

The study concluded that diagnosis of *G. lamblia*by copro ELISA has high sensitivity and specificity, compare to microscopy that required highly qualified microscopist. No apparent differences between the three techniques in diagnosis of giardiasis. The benefit of copro ELISA is valuable in epidemiological study and in diagnosis, especially for those who had low parasitessecretionin their stool samples and was negative by microscopy with continuous sign and symptoms. The clinical cut off obtained is considered as a reference range for diagnosis of Giardiasis by copro ELISA in Sudan.

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