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SEROLOGICAL VERSUS ANTIGEN DETECTION METHODS FOR *GIARDIA DUODENALIS* DIAGNOSIS

By

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Abstract

Giardiasis constitutes an important public health problem in the world. Contamination of the water with fecal materials including viruses and pathogenic protozoa still represents an environmental health hazard, especially in rural areas. The survey study evaluated the relation between seropositivity and some risk factors. Moreover, the study compared between the serological IgG and IgM level and antigen detection methods for the diagnosis of giardiasis. The results indicate that sex distribution and age were the mean risk factors for seroprevelence. In this study, sera samples were employed in sandwich ELISA assay, to detect circulating *Giardia* antigens. None of the negative control serum samples gave a positive reaction, but cross reaction was encountered with 3 case of *Cryptosporidium*. The specificity of the assay was 94.83%.

On the other hand, the sensitivity of the *Giardia* patient's sera was 94.12% which was higher than that of IgG (86.25%) and IgM (87.50%) secretion measurements. In conclusion, antigen detection methods give better and earlier diagnosis for giardiasis can be performed quickly and do not require an experienced and skilled morphologist.

Key wards: Egypt, patients, *Giardia duodenalis*, ELISA, IgG, IgM.

Introduction

Giardia duodenalis (*G. lamblia* or *G. intestinalis*) is an intestinal flagellated protozoan and a major cause of diarrhea in humans and animals' worldwide (Flanagan, 1992) are caused by contaminated water (Karanis *et al,* 2007). Most infections are without symptoms, but sometimes, acquisition of infection is accompanied by acute diarrhea after an incubation period of 12-19 days (Andersen *et al,* 2006). While in developing countries, frequent exposure and endemic infections are common and are often associated with asymptomatic cysts excretion (Ortega and Adam, 1997). Several studies have examined the association between *G. duodenalis* infection and sociodemographic factors; however, the risk factors that are consistently found are age, sex and water supplies (Stuart *et al,* 2003). Usually parasitological diagnosis of giardiasis involves repeated examination of stool samples, and in certain cases examination of duodenal biopsy (Yakoob *et al,* 2005). However, these methods are not practical in large scale epidemiological studies. Instead, anti-*Giardia* antibodies have been used to detect parasite antigens in secretions and/or excretions (Nash and Keister, 1985), which demonstrated both by indirect immunofluorescence (IFA) and an enzyme linked immunosorbent assay (ELISA) (Soliman *et al,* 1998). But, these methods are not always suitable for epidemiological studies, as they are subjected to examine variation and require expensive apparatus. Instead antigen recognition (Trophozoites antigen) in *G. duodenalis* infected patients was used (Wenman *et al,* 1993). Specific antigens present in the cysts such as proteins with a molecular weight in the 66-103 kDa range are recognized by polyclonal antibodies (pAb) produced in immunized rabbits as immunologic diagnosis of giardiasis (Reiner *et al*, 1990; Guimarães *et al*, 1999).

This study was conducted on giardiasis patients in some endemic areas in Egypt.

It aims to evaluate the relation between some demographic data (sex and age) with G. *duodenalis* seropositivity. Furthermore, extraction of crude *Giardia* antigen (CGA) followed by immunization of rabbit to produce IgGpAb. Moreover, the produced specific anti-CGA IgGpAb was used in the detection of *Giardia* antigens in sera samples of thestudypopulation by sandwich ELISA; and the results were compared with that of the serological methods.

Subjects, Materials and Methods

A survey study was conducted on 512 patients from out-patients' Clinic at Health Centers in endemic areas; and 30 patients infected with other common parasites (*Cryptosporidium parvum, E. histolytica* and *Blastocystis hominis*). At the time of stool collection, medical sheets were filled out on each case included questionnaire covered demographic data, family life, education, job, age, sex and place of residence. This study was approved by the Ethics Committee of TBRI/ Institutional Review Board authorized by the license no. FWA00010609. Besides, 25 healthy volunteers' individuals served as parasite free-healthy negative control. Fecal samples were diagnosed at TBRI by merthiolate-iodine-formaldehyde concentration technique (MIFC) after Blagg *et al*. (1955). By parasitological examination, 80 *G. duodenalis* infected patients were divided into light, moderate and heavy infection groups. The groups were: Healthy volunteer cross matched persons GA: 25 negative controls. *G. duodenalis* infected patients (n=80): GB: 19 patients with light infection. GC: 27 patients with moderate infection.GD: 34 patients with heavy infection. Other parasites infected patients (n=30) was GE: 12 *Cryptosporidium parvum* infected patients, GF: 10 *E. histolytica* infected patients and GG: 8 *B. hominis* infected patients.

Blood samples: Blood samples were collected from all patients and sera were separated, then fractionated into small aliquots and stored at -70°C until needed.

Preparation of crude *Giardia* antigens (CGA): Feces samples were collected from patients with heavy infection, diluted with distilled water (D.W.) and filtered through 4 layers of gauze to remove the large materials. This was followed by the filtrate centrifugation at 800×*g* for 5 min., elimination of supernatant and washing of pellet many times. The pellet was resuspended in 20ml of D.W. and divided into 4 aliquots of 5ml each. Each aliquot was layered carefully over 3ml of 0.85-M cold sucrose; followed by centrifugation at 600×*g* for 10 min. The interfaces were recovered, diluted with D.W. 25-fold, and finally vacuum-filtered through a cellulose acetate membrane (Millipore; 47 mm in diameter, pore size of 5µm). The filter was washed with 3ml D.W. and the purified cysts were stored at 4°C with antibiotics (Penicillin and Streptomycin, 1,000 units and 1mg/ml, respectively) after Alvarado and Wasserman (2006). Protein content was measured by Bradford method (1976); and the reactivity of purified cysts antigen was evaluated by indirect ELISA according to Engvall and Perlman (1971).

Assessment of specific antibody responses: Anti-CGA IgM and IgG were measured using indirect ELISA. ELISA microtitre plates were coated with 100µl/ well of 3µg/ml of prepared CGA in carbonate buffer, pH 9.6, overnight at 4ºC. The plates were thoroughly washed with 0.02 M phosphate buffered saline with 0.05% Tween 20 (PBS/T), pH 7.4 (2 min/ wash), and unbound sites were blocked with 200µl/well of 0.1% bovine serum albumin (BSA) (Sigma) diluted in PBS/ T. After 2 hr. incubation at 37ºC, 100µl of diluted serum (1:250) was added and the plates were incubated for an hour at 37ºC. After washing, 100µl of 1/1000 anti-human polyvalent peroxidase conjugate (Sigma) was added and the plates were incubated for 2 hr. at 37ºC and washed with PBS/T. The substrate *O*-phenylenediamine-dihydrochloride (Sigma) was added and the plates were incu-

bated for 30 min in the dark at room temperature. The enzyme reaction was stopped with 50µl/well of 8 N H_2SO_4. Absorbance at 492 nm wavelength (A_{492}) of plates was read using a microplate ELISA reader (Bio-Rad, Richmond CA, USA).

Immunization of rabbits for production of anti-*Giardia* IgG pAb: Clean laboratory bred New Zealand white rabbits (~3kg weight) were examined before the study to ensure they were parasite-free and kept in the animal house in TBRI for 4 wk. (experiment duration) under standard laboratory conditions at 21°C, 16% moisture, filtered drinking water, diet 15% protein, 3% fat and 22% fibers. Each rabbit received 1mg of purified CGA by intramuscular injection at four sites. Two booster doses were given at 2 wk. Then, the other 3 booster doses were given at weekly intervals (Tendler *et al,* 1991).

Purification of anti-*Giardia* IgG pAb: Purification of anti-*Giardia* IgG pAb was done with: Ammonium sulphate precipitation method (Nowotny, 1979) and Caprylic acid purification method (Mckinney and Parkinson, 1987).

Characterization of anti-*Giardia* IgG pAb: The protein content of purified antibodies was measured according to Bradford (1976); molecular weight was determined by 12.5% SDS-PAGE under reducing condition after Harlow and Lane (1988). Purified reactivity of IgG pAb was tested by indirect ELISA (Engvall and Perlman, 1971).

Labeling of anti-*Giardia* IgG pAb with Horseradish Peroxidase (HRP) (Periodate Method) (Tijssen and Kurstak, 1984): Five mg horse raddish peroxidase (HRP) (Sigma) was resuspended in 1.2ml D.W. and 0.3ml freshly prepared sodium periodate and incubated at room temperature for 20 min. HRP solution was overnight dialyzed against 1mM sodium acetate buffer (pH 4) at 4ºC. HRP was added to 0.5ml of IgG pAb solution (5mg/ml in 0.02M carbonate buffer, pH 9.6). The mixture was incubated at room temperature for 2 hr. followed by addition of 100 µl sodium borohydride and incubation for 2 hr. at 4°C. The HRP conjugate IgG pAb was dialyzed against 0.01 M PBS (pH 7.2).

Detection of *Giardia* antigens in patients' sera by ELISA: Sandwich ELISA was performed as plates were coated with 100µl/well of purified pAb (1/100 for IgG in carbonate buffer 0.06 M, pH 9.6) and incubated overnight at room temperature. Then, plates were washed 3 times with 0.1 M PBS/T, pH 7.4, and were blocked by 200 µl/well of 0.1% BSA/PBS/T and incubated for 2 hr. at 37°C and washed 3 times with PBS/T. 100µl stool elution samples, or purified water samples were pippetted in duplicate and incubated for 2 hr. at 37°C and the wells were washed 3 times and 100µl/well of peroxidase-conjugated pAb of 1/50 for IgG was then added and incubated for 1 hr at room temperature. The assay was completed with those of De Jonge *et al*. (1990) and Qiu *et al*. (2000)

Key Features in Reliability of Test Results: Data were presented as mean ± standard deviation of mean (X±SD). Assay or test specificity and sensitivity can be selected and adjusted to meet the needs of a clinician for the diagnosis and monitoring of a disease. This may be accomplished by changing the selection of the reference value (i.e., cut -off or upper limit of normal) for the particular test (Zane, 2001). Also, positive predictive value (PPV) and negative predictive value (NPV) were assessed for all groups.

Results

The rate of infection with *Giardia* was described according to several parameters; sex distribution, age and clinical data.

The patients (Tab. 1) were 43 male patients (53.75%) and 37 female patients (46.25%). The male patients form a low percentage of heavy infected group (41.18%) and a high percentage of moderate and light infected patients (66.67% & 57.89%, respectively), while female sector constituted a high rate (58.82%) of

heavy infected patients (GD), low percentage (33.33%) of moderate infected patients (GC) and 42.11% of light infected patients (GB) (33.33% and 42.11%, respectively).

Table 1: Sex distribution among groups

Groups	Male		Female		Total
	No.	%	No.	%	No.
GB	11	57.89%	8	42.11%	19
GC	18	66.67%	9	33.33%	27
GD	14	41.18%	20	58.82%	34

The age in all groups ranged from 6-75 years. It was obvious (Tab. 2) that the heavy infection (GD) was common in adults between 35 & 75 years with mean age of 53.33 while, the younger ones constituted moderate (6-40 y) (GC) and light infection (12-35y) (GB) with mean age of 20.53 and 27.68, respectively.

Table 2: Age distribution among groups

Groups	Minimum	Maximum	M±SD
GB	12	35	27.68±0.432
GC	6	40	20.53±0.087
GD	35	75	53.33±0.612

X: mean, SD: standard deviation

Clinical data: Most cases were asymptomatic (n=46, 57.50%); but diarrhea was the commonest complaint among them as 34 patients (42.50%) suffered from diarrhea.

Assessment of specific antibody responses by ELISA in sera: Circulating IgM in serum Cut off value for positive was 0.284. OD values of *G. duodenalis* infected groups were 1.671±0.151 for GD; 0.735±0.061 for GC & 0.471±0.451 for GB (Tab.3). Results were significantly higher than both control group (0.142±0.071) and other parasite groups. Ten out of 80 *G. duodenalis* infected samples showed false negative results and the assay sensitivity was 87.50%. All negative controls were below cut off value while 7 out of other parasites groups (GsE, F & G) showed false positive results recording 87.27% specificity. The PPV and NPV were 91.95% and 84.61%, respectively.

Table 3: Detection of circulating IgM in serum of different groups by ELISA

	Positive cases		Negative cases	
Groups	*X± SD*	No	*X± SD*	No
GA			0.142 ± 0.071	25
GB	0.471 ± 0.451	13	0.251 ± 0.041	6*
GC	0.735 ± 0.061	23	0.391±0.032	4*
GD	1.671 ± 0.151	34		
GE	0.485 ± 0.025	2**	0.321 ± 0.076	10
GF	0.522 ± 0.041	3**	0.117 ± 0.062	7
GG	0.385 ± 0.031	2**	0.246 ± 0.162	6

X: mean OD reading at 492 nm, SD: standard deviation, * False -ve results, ** False +ve results.

Detection of circulating IgG in serum: Cut off value for positivity was mean±2 SD and equaled 0.375 (Tab.4). OD values of *G. duodenalis* infected groups (GD, 2.691±0.165; GC, 1.351±0.089 & GB, 0.581±0.101) were significantly higher than both healthy control GA (0.237± 0.069) & other parasites groups (GE, 0.512±0.138; GF, 0.453±0.091 & GG, 0.398± 0.031).

Eleven out of 80 *G. duodenalis* infected groups (GC & GB) showed false negative results and the assay sensitivity was 86.25%. All the 25 negative controls were below the cut off value; while 9 out of other parasites groups (GsE, F & G) showed false positive results recording 83.64 % specificity. PPV and NPV were 89.89% and 83.33 %, respectively.

Table 4: Detection of circulating IgG in serum of different groups by ELISA

Groups	Positive cases		Negative cases	
	X± *SD*	No	*X*± *SD*	No
GA			0.237 ± 0.069	25
GB	0.581 ± 0.101	12	0.253 ± 0.032	7*
GC	1.351 ± 0.089	23	0.351 ± 0.041	4*
GD	2.691 ± 0.165	34		
GE	0.512 ± 0.138	4**	0.253 ± 0.160	8
GF	0.453 ± 0.091	3**	0.118 ± 0.081	7
GG	0.398 ± 0.031	2**	0.330 ± 0.112	6

X: mean OD reading at 492 nm, SD: standard deviation, * False –ve results, ** False +ve results.

Preparation of CGA: CGA was prepared with 7mg/ml protein and its strong antigenicity was detected by ELISA as 0.924±0.102 compared to 0.157±0.124, 0.208±0.094 and 0.105±0.072 for *C. parvum, E. histolytica and B. hominis* respectively.

Preparation of anti-CGA IgG pAb: Antibody level increased from 1 wk. after the 1st booster dose. High titer of antibody against CGA was (OD=3.2) at 1/100 dilution after the 2nd booster dose by 3 days. Total protein content of crude rabbit serum containing anti-GCA antibody was 9.2 mg/ml; then dropped to 3.5 mg/ml after purification steps. IgGp Ab was represented by H- and L-chain band at 53 & 31 kDa (Fig. 1), respectively, when analyzed by 12.5% SDS-PAGE under reducing condition. The pAb appears free from other proteins. The produced anti-CGA IgG pAb, diluted 1/100 in PBS/T buffer, showed a strong reactivity against CGA; where the OD reading at 492 nm for *Giardia* was 1.725 compared to *C. parvum* (0.284), *E. histolytica* (0.135) and *B. hominis* (0.189).

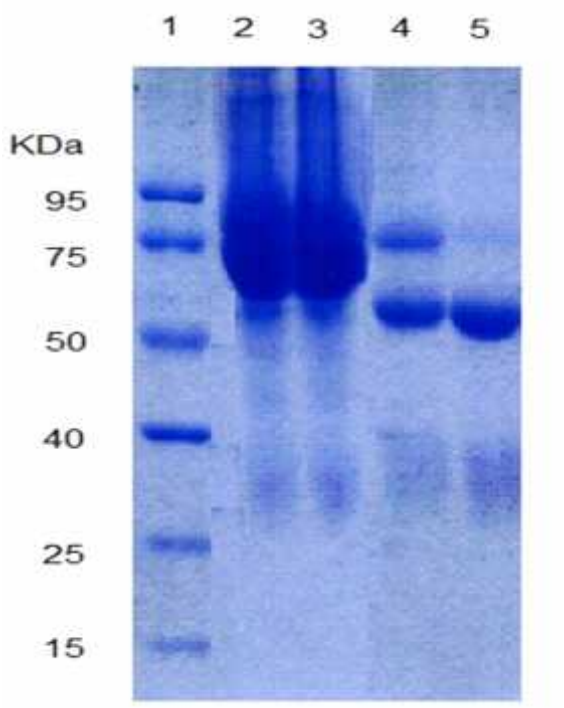

Fig. 1: 12.5% SDS-PAGE of anti-*G.duodenalis* IgG pAb before and after purification: Lane 1: Molecular weight of standard protein. Lane 2, 3: Crude anti-*G. duodenalis* IgG pAb. Lane 4: Precipitated proteins after 50% ammonium sulfate treatment & Lane 5: Purified IgG pAb after 7% caprylic acid treatment.

Detection of CGA in serum samples: After optimization of sandwich ELISA, 1/100 and 1/50µg/ml, for coating and HRP conjugated antibody, was chosen as working dilution for subsequent assay. Cut off value for positivity was 0.344;

according to our results (Tab. 5), the OD values of *G. duodenalis* infected groups were 0.487±0.019, 0.794±0.058 & 1.451 ±0.085 for GB, C & D, respectively. The results were significantly higher than both healthy control group (0.248±0.048) and other parasite groups. Two cases of *G. duodenalis* moderate infected samples (GD) showed false negative results and the sensitivity of the assay was 94.12%. All negative controls were below the cut off value; while 3 cases of *C. parvum* infected patients (GE) showed false positive results recording 94.83% specificity. The PPV and NPV were 96.39% and 91.67%, respectively.

Table 5: Detection of CGA in sera of infected human by ELISA technique.

	Positive cases		Negative cases	
Groups	*X*± *SD*	No	*X*± *SD*	No
GA			0.248 ±0.048	25
GB	0.487 ± 0.019	16	0.114±0.014	3*
GC	0.794 ±0.058	25	0.237±0.042	2*
GD	1.451 ±0.085	34		
GE	0.501 ±0.106	3**	0.285 ±0.091	9
GF			0.274 ±0.121	10
GG			0.195 ±0.089	8

X: mean OD reading at 492 nm, SD: standard deviation, * False –ve results, ** False +ve results.

Table 6: Sensitivity, specificity, PPV and NPV of IgG, IgM and CGA in serum samples

Method	Sensitivity	Specificity	PPV	NPV
IgM detection	87.50%	87.27%	91.95%	84.61%
IgG detection	86.25%	83.64%	89.89%	83.33%
CGA detection	94.12%	94.83%	96.39%	91.67%

Discussion

In the present study, three other protozoa were selected; *E. histolytica* affects anyone, and commonest in people who live in tropical areas (CDC, 2010), pathogenic *Blastocystis hominis* (El Deeb and Khod-eer, 2013) and zoonotic cryptosporidiosis (Massoud *et al*, 2008) were reported in many Egyptian Governorates more or less side by side with giardiasis. *Giardia* is a pathogenic protozoan with a worldwide distribution having a more relevant prevalence in warm climate and in children (Calderaro *et al,* 2010). In many countries of Asia, Africa, and Latin America, approximately 200 million people had symptomatic Giardiasis (Thompson *et al*, 2000; Yason and Rivera, 2007), with 280 million infections per year (El-Nahas *et al*, 2013). The regional infection prevalence differs greatly; and accounts more than 30% in children in the African and the Eastern Mediterranean region (Thompson and Smith, 2011). Yoder and Beach (2007) and Sponge *et al.* (2009) reported that *G. intestinalis* infected infants early in life and being a major cause of epidemic childhood diarrhea, with a rate of 15-20% in children less than 10 years.

Several authors studied the relation between gender and giardiasis; Raza and Sami (2009) reported that females gave highest infectivity rate due to the household activities. While other reported that males had the highest infectivity rate, and attributed this to the fact that males being more activities outdoors in contact with environmental conditions than females (Al-Saeed and Issa, 2006). Also, Bernawi *et al.* (2013) found that the percentages of *G. lamblia* infected males were higher than females, but without significant differences. The same results were reported in Egypt (El-Shazly *et al*, 2004), in Libya (Kassem *et al,* 2007) and in Yemen (Al-Qobati *et al*, 2012). Besides Al-Warid (2012) found no significant differences between male and female infection rates (52.32% & 47.68%, respectively).

The present study measured the infection rate and several parameters as sex, age distribution, and clinical data. Accordingly, males constituted a higher percentage (53.75%) than females (46.25%); this may be due to exposing to infected

water in farming work, and contact with infected canal water due to swimming and washing costumes. On the other hand, females constituted a high percent (58.82%) of heavy infected GD, 33.33% of gp C and 42.11% of GB.

In Latin American endemic intestinal parasites were persistent and prevalent in all aged natives; mainly soil transmitted helminths and protozoa as *C. parvum*, *G. lamblia* and *E. histolytica* (Kaminsky *et al*, 2004). In the present study, the infection degree increased with increase in age. Heavy infection (GD) was found in ages from 35 to 75 years, moderate infection (GC) from 6 to 40 and light infection (GB) was from 12 to 35 years. Diarrhea was the commonest complaint among 34 patients (42.50%), others were symptomless. El-Mohammady *et al*. (2012) found high giardiasis infection rate in Egyptian schoolchildren. Sherief *et al*. (2012) reported that diarrhea was a complication in neutropenic cancer children due to *Giardia*, gram -ve bacteria and *Candida*, the incriminated pathogens

Serological tests, although not always positive, but acceptable to patients and in epidemiological studies (Al-Tukhi *et al*, 1994). El Shazly *et al.* (2007) in Egypt reported highly significant positive correlation between symptoms, oocysts count and ELISA-OD copro-antigen. Rivera *et al.* (2009) reported that detection of serum antibodies against *G. duodenalis* by ELISA was a useful in epidemiologic studies. They documented a seroprevalence of 55.3% for *G. duodenalis* in Mexico. Anti-*Giardia* IgG antibody secretion was detected in patients; but failed to distinguish between present and past infection. Comparing IgG with IgM antibody response was shorter and more indicative of active infection and differentiated current from past or asymptomatic infection (Dutt *et al*, 1991). Also, anti-*Giardia* IgA proved a useful indicator of active infection; but absence did not exclude infection as about two thirds of patients did not produce a measureable IgA response (El-Gebaly *et al,* 2012). Miotti *et al*. (1985) reported that 79% of lactating women had IgA antibodies in breast milk and 77% had serum IgG against *Giardia* spp. Faubert (2000) reported that IgM, IgG, & IgA in sera and IgA levels in intestinal fluid were 100, 70, 60, & 50%, respectively in the patients infected with GS/M isolate.

In this study, prevalence of ELISA antibodies showed that IgG & IgM antibodies against *G. duodenalis* antigens were detected in 69 & 70 patients, respectively. The overall seroprevalence of *G. duodenalis* was age-specific. In IgG, GD (patients from 35 to 75 years old) recorded the highest secretion (2.691±0.165), followed by GC (1.351±0.089), and then GB (0.581±0.101). The same was seen with IgM secretion; where OD values of *G. duodenalis* infected groups were 1.671± 0.151 for GD; 0.735±0.061 for GC and 0.471±0.451 for GB. Again GD recorded the highest Igs secretion. This shwed that the mean titers of CGA antibodies against *G. duodenalis* increased with age particularly in giardiasis rural endemic areas, infection occurred in preschool and schoolaged children, but re-infection could frequent in adulthood (Bilenko *et al*, 2008). This finding may explain the high antibodies levels in the present study in adults. These results also agreed with Rivera *et al.* (2009) who reported increased IgG titers against *G. duodenalis* with age, 0.241±0.042 in children 1-4 years old and 0.379±0.042 in adults 40-59 years old (P<0.0001).

This quantification of anti-CGA antibodies by ELISA proved useful for diagnosing *G. duodenalis* more than stool examination, with sensitivity of 86.25% & 87.5% and specificity of 83.64% & 87.27% for IgG & IgM, respectively. Dependable method for *Giardia* early diagnosis with higher sensitivity is needed. Addiss *et al.* (1991) reported that ELISA antigen detection showed more sensitivity than detection of cysts by microscopy (95.0% vs. 83.2%). Kamel *et al.* (2013)

found that the 65 kDa glycoprotein *Giardia* specific antigen (GSA-65 kDa) was a better antigen, and recommended sandwich ELISA, using purified anti-GSA-65 kDa IgG pAb, as a diagnostic test for *Giardia* spp. more than light microscopy.

In this study, sucrose floatation method was used for purification of CGA from stool samples (Alvarado and Wasserman, 2006). The total protein content (7 mg/ml) was measured by Bio-Rad Protein assay. This agreed with Duque-Beltrán *et al.* (2002) who purified *Giardia* cysts from human fecal samples by sucrose and percoll gradients. Rabbits were immunized with CGA according to Tendler *et al.* (1991). Anti-CGA IgG pAb was purified by ammonium sulphate and caprylic acid purification methods. IgG pAb was represented by H- and L-chain bands at 53 and 31 kDa, respectively, by 12% SDS-PAGE under reducing condition indicating that the purified antibody appears free from other proteins. The measured protein content of anti-CGA IgG pAb was 3.5 mg/ml from starting 9.2 mg/ml (Bride *et al*, 1995; Yang and Harrison, 1996). The reactivity of the purified pAb, against CGA and other parasite antigens was determined by indirect ELISA. This was followed by labeling of purified IgG pAb with HRP and its application as coating and conjugate antibody for the detection of CGA antigens in infected patients' sera samples by sandwich ELISA. Optimum dilution of purified IgG pAb as a coating layer was 1/100 whereas a peroxidase conjugated layer was 1/50. The prepared anti-CGA IgG pAb succeeded in detection of *Giardia* antigens (CGA) in 75 patients' sera samples (34, 25 & 16 from GsB, C & D, res- pectively) with high sensitivity (94.12%), specificity (94.83%), PPV (96.39%) and NPV (91.67%).

Conclusion

The purified CGA obtained from *Giardia* cysts could be introduced as a suitable candidate antigen for immunodiagnosis of *Giardia* spp. infected human using sandwich ELISA for antigen detection and serodiagnosis. Moreover, the purified IgG pAb successfully detected CGA in the sera of *Giardia* infected patients even those with light giardiasis infection.

The outcome data proved to be accurate and early diagnostic tool for giardiasis, including case detection and resulting community treatment, assessment of the morbidity and evaluation of control strategies.

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