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SANDWICH-ELISA DEVELOPMENT FOR THE DIAGNOSIS OF *TOXOPLASMA GONDII*

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Abstract

Toxoplasmosis is one of the most world-wide spread zoonosis representing a very serious clinical and veterinary problem. Although rare, congenital toxoplasmosis can cause severe neurological or ocular disease (leading to blindness) as well as cardiac and cerebral anomalies. Prenatal care must include education about prevention of toxoplasmosis. Thus, a standardized and approachable diagnostic tool for the serodiagnosis of toxoplasmosis is still needed. Serological tests are the most widely used biological tools for the diagnosis of toxoplasmosis worldwide. Sandwich-ELISA is a solid phase diagnostic method for detection of antigen or antibody that is used widely for diagnosis of protozoan and metazoan diseases of human and animals. In the present study, *T. gondii* SAG2 antigen was early detected in patient sera using Ssndwich-ELISA, PAb was prepared from anti-rabbit sera and used for coating and as conjugate in Sandwich-ELISA technique. 46 patients out of 50 were positive to *Toxoplsama spp.* with sensitivity and specificity 92% and 90%, respectively. The PPV was 90.2% and NPV was 83.3%. Finally, the result of our study showed that the Sandwich-ELISA designed in our study is easy to perform, not expensive, safe, and simple with good sensitivity and specificity.

Key words: Egypt, *Toxoplasma gondii*, pAb, Sandwich-ELISA, SAG2

Introduction

T. gondii is an intracellular protozoan parasite discovered in 1908 by Nicolle and Manceaux that causes toxoplasmosis (Nicolle and Manceaux, 2009; Dard *et al.*, 2016). Human infection generally occurs by the ingestion of cysts in under-cooked meat or oocysts from the environment (Robert-Gangneux and Dardé, 2012), then the single-celled, apicomplexan parasite capable of infecting nearly all nucleated cells within a vertebrate host displaying both phagocytic and non-phagocytic properties (Fentress *et al*, 2010; CDC, 2013).

Cats are the only definitive host with the infection of other species being purely accidental (Sterling, 2012). Naturally, the vast majority of infections in adults are asymptomatic due to the hosts' cellular immune responses (Tenter *et al,* 2000; Montoya and Liesenfeld, 2004). However, *T. gondii* can cause severe diseases in immune deficient individuals (Weiss and Dubey, 2009). Infection with *T. gondii* may lead to abortion or congenital toxoplasmosis in animals, especially in sheep and goats (Innes, 2010). Additionally, *T. gondii* IgG avidity can be agood marker of recent infection in sheep (Carneiro *et al*, 2009; Andrade *et al*, 2013). It is important to know whether the infection is recently acquired or chronic. Differentiation between acute and chronic infection has a dramatic impact, especially for the developing fetus (Boothroyd, 2009; Ali-Heydari *et al*, 2013); therefore, the human and animal significance of toxoplasmosis makes it urgent to search for alternative ways for the prevention and control of *T. gondii* infections (Innes and Vermeulen, 2006; Wang *et al,* 2007).

To prevent infection of human beings by *T. gondii*, people handling meat should wash their hands thoroughly with soap and water before going to other tasks (Lopez *et al,* 2005). Pregnant women should avoid contact with cats, cat litter, soil, and raw meat (Dubey, 2004). Strategies for early recogni-

tion of maternal or infant infection and the institution of effective treatment could have a substantial impact on the incidence and morbidity that are associated with this congenital infection (Lebech *et al,* 1999; Remington *et al,* 2001).

The clinical severity of infection during pregnancy is gestational time-dependent. Therefore, it is important to be diagnosed early and to distinguish between acute and chronic stages of infection for treatment and limitation of its effects (Rahbari *et al,* 2012). Diagnosis of *T. gondii* infection relies mainly on serological tests to determine specific antibodies such as IgG, IgM, IgE or IgA are currently the first-line methods of diagnosis to differentiate recent or chronic infections with *T. gondii* (Sensini, 2006) and directly by polymerase chain reaction (PCR), hybridization, isolation, and histology. Whereas indirect serological methods are widely used in immunocompetent patients, definitive diagnosis in immunocompromised people is mostly undertaken by direct detection of the parasite (Calderaro *et al,* 2006; Saadatnia and Golkar, 2012). The diagnostic challenge is differentiating between a primary and a chronic infection, and results of IgG & IgM testing can often be difficult to interpret. For this reason, it is important to consult with an expert in this area when confirming the diagnosis (Paquet and Yudin, 2013).

The surface antigen (SAG) of *T. gondii*, that plays roles in the processes of host cell attachment and host immune evasion, is dominated by a SRS (SAG1-related sequence) family of proteins which includes the SAG1- and SAG2-like sequence branch (Jung *et al.*, 2004; Cong *et al*, 2013). Studies have indicated that SAG2 members participate in the process of parasite's invasion to the host, and their antibodies could block the further attachment of *T. gondii* on host cells (Lekutis *et al*, 2000; Machado *et al*, 2010; Cong *et al*, 2013).The SAG2 (P22), is protein that has been shown to be an attachment ligand and also to have good immunogenicity (Aubert *et al*, 2000; Khanaliha *et al*, 2014).

The study aimed to purify and characterize SAG2 antigen from *Toxoplasma* tachyzoites, evaluate and compare its diagnostic potential in human toxoplasmosis. The development of Sandwich-ELISA technique for the detection of *T. gondii* SAG2 antigen in human sera was performed.

Subjects, Materials and Methods

Animals: New Zealand white rabbits, weighting approximately 3 Kg and about 4 months age, were used in the production of the antibodies.

Preparation of parasite antigens: *T. gondii* antigen was prepared from peritoneal exudates of BALB/c mice infected 3 days earlier with tachyzoites of *T. gondii* RH strain according to Brooks *et al.* (1985). The peritoneal exudates of mice were centrifuged at 2000 g for 20 min, washed 3 times with phosphate buffer saline (PBS), the sonicated for twelve 5-10 periods, centrifuged at 12000g for 1 (hr) and supernatant was collected as soluble antigen. Protein content was determined by Bradford method and the soluble antigen was stored at -20˚C until use according to (Foroghiparvar *et al*, 2008; Azami *et al*, 2011).

Purification by DEAE-Sephadex G-5o and G-200 ion exchange chromatography: DEAE Chromatography is an effective method for separating proteins based on their charge. The DEAE group maintains a constant positive charge that is neutralized by counter ions, usually chloride ions. Other anions are capable of competing for the positive DEAE group (Sheehan and FitzGerald, 1996).

The specific fractions were analyzed for final max. purification by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), then protein content was colormetrically determined by the dye binding protein assay (Bradford, 1976) using commercially available Bio-Rad kits (Bio-Rad laboratories, Richmond, CA, USA).

Immunization of rabbits for production of polyclonal antibodies: Rabbits anti-*T. gondii* serum was obtained by immunizing New Zealand white rabbits intramuscularly with

1mg of SAG2 *T. gondii* antigen/each in equal volume of complete Freund's adjuvant (CFA). Two weeks later after the priming dose, each rabbit was received a booster dose of 0.5 mg antigen emulsified in incomplete FA (IFA). The following 2 boosting doses (0.5 mg antigen) were given at weekly intervals in IFA. The rabbit was bled for collection of serum one week later after a preliminary testing of titer by indirect ELISA. Rabbit serum which contained anti-*T. gondii* pAb was fractionated and kept at -20°C.

Purification of rabbit anti-*T. gondii* serum: Rabbit IgG purification steps were based on two different methods: Ammonium sulphate precipitation method (Nowotny, 1979) and Caprylic acid purification method (Mckinney and Parkinson, 1987). Reactivity assessment of the anti-*T. gondii* pAb was done by the indirect ELISA, with some modifications from that of Engvall and Perlmann (1971).

The study was conducted on 50 *T. gondii* infected patients from Alexandria University Hospitals, Out-patients clinic of TBRI Hospital and El-Azhar University Hospitals diagnosed clinically & parasitologically. Cross-matched thirty patients infected with *Entameba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum* were included as positive control. Also, 20 individuals of the medical staff at TBRI served as parasite free-healthy negative control. In sterile polypropylene tubes, 5ml blood was taken from each individual by vein puncture, and was allowed to clot at room temperature for 2 hr. Serum was separated by centrifugation at 2000 g for 10 min. and then fractionated into small aliquots and stored at -70°C until use.

Conjugation of polyclonal antibodies with Horse-Radish Peroxidase: Periodate treatment of carbohydrates opens the ring structure and allows these moieties to bind free amino groups. Schiff's bases that have been formed are reduced by sodium borohydride (Nakane and Kawaoi, 1974; Tijssen and Kurstak, 1984). Five mg of horse-radish peroxidase (HRP) enzyme was resuspended in 1.2ml. distilled H_2O. 0.3ml of freshly prepared sodium periodate was added and incubated at room temperature, for 20min. The HRP solution was dialyzed versus 1mM sodium acetate buffer, pH 4 at 4°C with several changes overnight. After dialysis the pH was increased to 9.5 by adding approximately 20µL of 0.2 M $NaHCO_3$ buffer, pH 9.5. The HRP was removed from dialysis tubing and was added to 0.5ml of the Ab (protein content 3mg/ml) solution. This was left to incubate at room temperature for 2hr. Hundred µl of 20 mM sodium borohydride (in 0.01 M PBS, pH 7.2) solution was added and the solution was incubated at 4°C, for 2hr. The HRP-mAb conjugate was dialyzed versus 0.1 M borate buffer at pH 7.2 overnight at 4°C then collected in aliquots and saved at -20°C.

Sandwich-ELISA (Antigen detection assay): The microtitration plates were coated with 100µl/well of purified anti-*T. gondii* pAb (20µg/ml for IgG in carbonate buffer 0.06 M, pH 9.6) and incubated overnight at room temperature. Plates were washed 3 times with 0.1 M PBS/T, pH 7.4. The remaining sites in the wells were blocked by 100µl/well of 2.5% FCS/PBS/T and incubated for 2hr. at 37°C. The plates were washed 3 times with PBS/T. Hundred ul of serum samples was pippetted into the wells in duplicate and incubated for 2hr. at 37°C. The wells were then washed 3 times as before. Hundred µl/well of peroxidase-conjugated pAb of 1/250 for IgG was added and incubated for an hr. at room temperature. The plates were washed 5 times with washing buffer. Hundred µl/well of substrate solution [one tablet of O-phenylene diamine dihdrochloride (OPD, Sigma) dissolved in 25 ml of 0.05 M phosphate citrate buffer, pH 5 with urea hydrogen peroxidase (Sigma)] was added to each well and the plates were incubated in the dark at room temperature for 30 min. 50µl/well of 8 N H_2S0_4 was added to stop the enzyme substrate reaction. The absorbance was measured at 492 nm by using ELISA reader (Bio-Red).

Key Features in Reliability of Test: Test specificity and sensitivity was 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).

Results

Purification of 37 kDa SAG2 antigen of *T. gondii* by DEAE-Sephadex G-50-ion exchange chromatography. The OD_{280} profile of the antigen fractions obtained following purification by DEAE Sephadex G-50 ion exchange chromatography. The eluted antigen was represented by 2 peaks one at fraction number 5 with maximum OD at 3.24 and the other at fraction number 25 with maximum OD value equal to 2.88.

Purification of 37 kDa SAG2 of *T. gondii* by DEAE-Sephadex G-200-ion exchange chromatography. The OD 280 profile of the antigen fractions obtained following purification by DEAE Sephadex G-200 ion exchange chromatography. The eluted antigen was represented by a single peak with maximum OD value equal to 2.92 at fraction number 40.

The eluted protein fractions resulted from the DEAE Sephadex G-50 purification method was analyzed by 12.5% SDS-PAGE under reducing condition and showed 3 major bands at 80, 30, 27 and many minor bands ranged from 110 kDa to 17 kDa. The eluted protein fractions resulted from the DEAE Sephadex G-200 purification method was analyzed by 12.5% SDS-PAGE under reducing condition and showed only one band at 37 kDa which representing 37 kDa SAG2 (Fig. 1). The crude antigen obtained from positive *T. gondii* blood sample contains 7mg/ml of total protein as measured by Bio-Rad protein assay while it was 3.5 mg/ml after purification DEAE-Sephadex G-50 gel filtration chromatography, and it was 1.5mg/ml after DEAE-Sephadex G-200 gel filtration chromatography.

The antigenicity of the purified target antigen was tested by indirect ELISA technique. Blood samples from human-infected with *T. gondii* gave strong reaction against 37 kDa SAG2 with mean OD reading equal to 1.99 and no cross reactions were recorded with sample of animals or patients infected with other parasites e.g., *E. histolytica* with mean OD reading equal to 0.36, *G. lamblia* with mean OD reading equal to 0.12 and *C. parvum* with mean OD reading equal to 0.14.

Production of pAb against target 37 kDa SAG2: Blood samples were withdrawn from New Zealand white rabbit before the injection of each immunizing dose. They were tested for the presence of the specific anti-*Toxoplasma* antibodies by indirect ELISA. An increasing antibody level started one week after the 1st booster dose. Three days after the 2nd booster dose immune sera gave a high titer against 37 kDa SAG2 with OD of 1.5 at 1/100 dilution.

The IgG fraction of rabbit anti-*T. gondii* pAb was purified using different purification steps including ammonium sulfate precipitation method followed by 7% caprylic acid precipitation method. The total protein content of crude rabbit serum with anti-*T. gondii* pAb was 12.5 mg/ml. The yield of purified anti-*T. gondii* IgG pAb following each purification step was determined by the assessment of protein content. Using the 50% ammonium sulfate precipitation method, the protein content was 8 mg/ml, while following 7% caprylic acid precipitation method the content dropped to 4 mg/ml.

The purity of IgG pAb after each steps of purification was assayed by 12.5% SDS-PAGE under the reducing condition. Purified pAb IgG was represented by H- and L-chain band at 50 & 31 kDa respectively. The pAb was free from other proteins (Fig. 2).

Specificity of polyclonal antibody against 37 KDa SAG2: Reactivity of anti- *T. gondii* pAb against 37 kDa SAG2 and other parasite antigens (*Entameba histolytica, Giardia lamblia, Cryptosporidium parvum*) was determined by indirect ELISA. The produced

anti- *T. gondii* pAb diluted 1/100 in PBS/T buffer gave strong reactivity to 37 kDa SAG2. OD readings at 492 nm for *T. gondii* were 2.95 compared to 0.63, 0.35, and 0.21 for *E. histolytica, G. lamblia* and *C. parvum*, respectively.

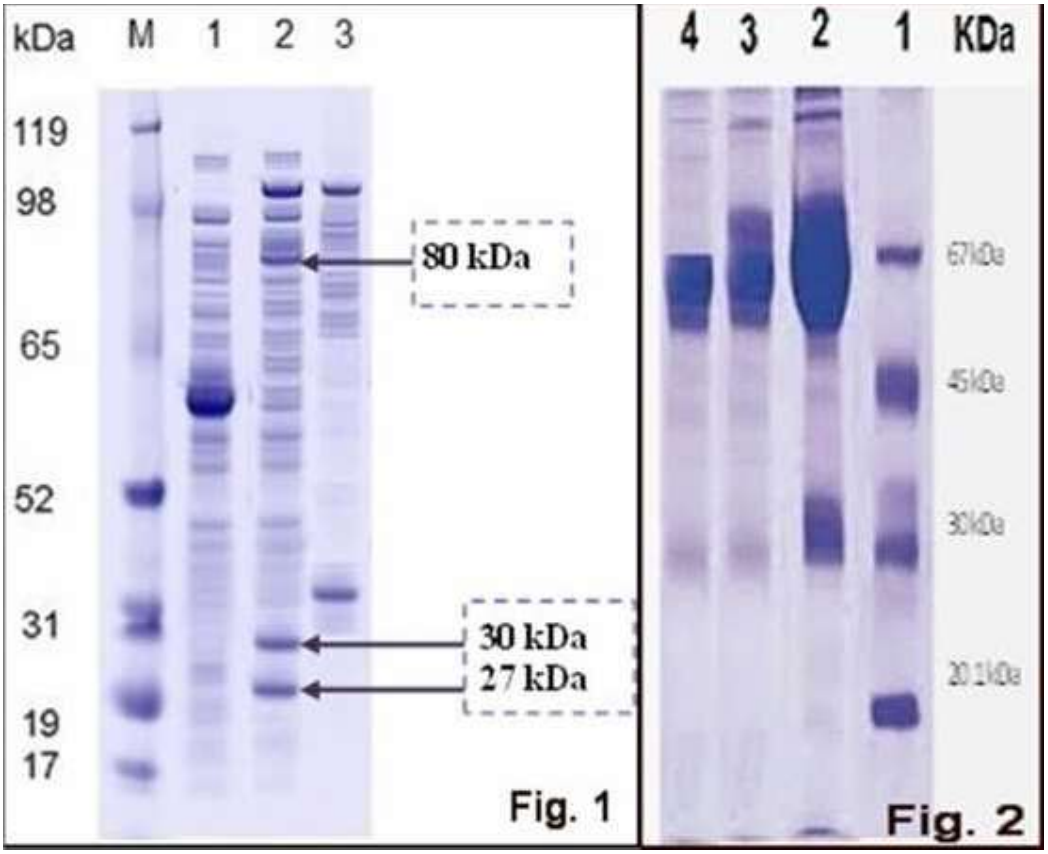
Table 1: Sensitivity, specificity, PPV &NPV percentage of Sandwich-ELISA to detect SAG2 antigen in sera

Sandwich-ELISA	%Sensitivity	%Specificity	%PPV	%NPV
	92%	90%	90.2%	83.3%

Explanation of figures

Fig. 1: 12.5% SDS-PAGE (1mm & reducing) of target antigens eluted from ion exchange chromatography column. Lane M: Low molecular weight standard. Lane 1: Crude *T. gondii* antigen Lane 2: Purified antigen after DEAE sphadex G- 50 and Lane 3: Purified antigen after DEAE sephadex G-200.

Fig. 2: 12.5% SDS-PAGE of anti-*T. gondii* IgG pAb (Stained with coomassie blue). Lane 1: Molecular weight of standard protein. Lane 2: Crude anti-*T. gondii* IgG pAb. Lane 3: Precipitated proteins after 50% ammonium sulfate treatment and Lane 4: Purified IgG pAb after 7% caprylic acid treatment



Standardization of sandwich ELISA used for detection of *T. gondii* SAG2 antigen: The anti-*T. gondii* IgG pAb used for detection of 37 kDa SAG2 was employed as antigen capture and HRP antibody conjugate in sandwich ELISA. It was necessary first to do some preliminary standardization and optimization work before the application of the technique on individuals' blood sample.

An ELISA plate was coated with coating pAb in 0.1 M carbonate buffer, pH 9.6, as well as 1% BSA to exclude non-specific binding of the conjugate. Serial dilutions of the conjugate (1/10, 1/20 & 1/40µg/ml) were added, the optimum concentration of anti-*T. gondii* IgG pAb conjugate was at 1/40.

The application of anti-*T. gondii* IgG pAb as antigen capturing antibody was evaluated by coating an ELISA plate with various dilutions of purified anti-*T. gondii* IgG pAb (1/20, 1/40 & 1/80) with coating buffer using 1/40 dilution of conjugate. The optimum

concentration of coating anti-*T. gondii* IgG pAb displayed in the highest OD reading was found to be 1/80.

Sandwich-ELISA: The sensitivity and specificity of sandwich ELISA which is 92% and 90%, respectively and the PPV and NPV shows percentage 90.2% and 83.3%, respectively (Tab. 1).

Discussion

T. gondii, an obligate intracellular protozoan parasite causing toxoplasmosis in humans and animals, is one of the most common chronic diseases throughout the world affecting one third of the world's human population (Jones *et al*, 2001; Zhou *et al,* 2011; Jones and Dubey, 2012; Saki *et al,* 2015). The sero-epidemiological evaluations indicate that toxoplasmosis is one of the most prevalent human diseases in so many countries (Elsheikh, 2008; Saki *et al.,* 2015). High prevalence of the infection were reported among pregnant women and women of child bearing age from different foci in Latin America, parts of Eastern/Central Europe, the Middle East, parts of south-east Asia and Africa (Pappas *et al,* 2009).

Transmission of *T. gondii* is by ingestion of cysts in undercooked or raw meat or by accidental ingestion of oocysts from contaminated soil, water and/or food. Meat is one of the most important sources of the infection to individuals (Dubey *et al*, 2005; Nardoni *et al,* 2011). In many studies, contaminated drinking water and close contact with cats were implicated as sources of *Toxoplasma* infection in humans worldwide (Sukthana *et al*, 2001; Lin *et al*, 2008; Saki *et al*, 2015). Primary maternal infection with toxoplasmosis during pregnancy is frequently associated with transplacental transmission to the fetus. However, it is not certain whether *Toxoplasma* infection can cause recurrent abortion (Saki *et al,* 2015).

Most cases of toxoplasmosis are asymptomatic or mild and influenza-like, but immunocompromised patients often develop fulminating life-threatening symptoms as pneumonia and encephalitis (Montoya and Liesenfeld, 2004; Weiss and Dubey, 2009). Although toxoplasmosis is largely asymptomatic in the majority of women, primary infection during pregnancy can result in disease transmission through the placenta and lead to hazardous consequences such as abortion, still birth has different degrees of mental or physical retardation, hydrocephalus, and blindness (Elsheikha, 2008).

Since treatment of the mother can reduce serious fetal sequelae, rapid and accurate diagnosis with precise estimation of the time of infection is critical for the proper management and healthy gestational outcome (CDC, 2000). Also, the *Toxoplasma* effects responded to appropriate antibiotic therapy, thus, early diagnosis was important (Rogers *et al*, 2008).

Early and accurate diagnosis using sensitive and specific diagnostic tests is essential to prevent and treat severe toxoplasmosis (Nogareda *et al*, 2014; Dard *et al*, 2016). The tests used to diagnose toxoplasmosis depend on the immune status of the patient and the clinical setting (Murat *et al*, 2013). Two biological approaches are currently used: (i) the direct detection of *T. gondii* using molecular biology approaches or, less frequently, by mouse inoculation or microscopic examination (parasitological methods); and (ii) indirect detection using serological assays (Robert-Gangneux and Dardé, 2012; Dard *et al*, 2016). Serology is the most convenient tool in most cases, but some clinical situations, such as suspicion of disseminated disease or prenatal diagnosis of congenital toxoplasmosis (CT), also require PCR analysis (Villard *et al,* 2016). So the early diagnosis of toxoplasmosis allows earlier treatment to limit clinical outcomes.

The diagnosis of *T. gondii* infection before conception is very essential especially in population with low seroprevalence rate but is usually not possible and therefore testing for antibodies to *Toxoplasma* in pregnancy is performed only in suspected cases. Seroprevalence studies showed *Toxoplasma* infection in pregnant women was between 7

and 51.3% throughout the world and the results of anti-*Toxoplasma* antibodies in women with abnormal pregnancy varied from 17.5 to 52.3% (Saki *et al*, 2015). The seroprevalence of *T. gondii* infection in women with first trimester abortion in Qena Governorate of Egypt was high. Pregnant women living in rural area were at a higher risk for acquiring infection during pregnancy (Tammam *et al*, 2013)

In spite of the advances developed in diagnosis of bacterial, viral and protozoan diseases, methods have to be renewed to be more rapid, sensitive and specific. During the past few years, there has been increased interest in the diagnosis of parasitic diseases using techniques, which are rapid, simple and inexpensive. Conventional serological tests such as indirect haemagglutination (Jacobs and Lunde, 1957), complement fixation (Kent and Fife, 1963), counter immunoelectrophoresis (GentiliniMaP, 1972) and immunofluorescence (El-Metanawey *et al*, 2009) are tedious, difficult to standardize, conduct and interpret. Also, the reagents are consumptive and require highly trained technicians as well as expensive instruments such as the fluorescent microscope (Soliman *et al*, 2014).

The classical diagnosis of toxoplasmosis relies on serological methods and detecting of specific immunoglobulin antibodies. Several studies have suggested the role of *T. gondii* in the causation of abortions. Several serological assays have detected the immunoglobulin (IgG and IgM antibodies) against *T. gondii* in the serum. Among the assays, ELISA showed high sensitivity and specificity (Saki *et al,* 2015).

Anti-*T. gondii* IgG and IgM are the most commonly used, Although IgM could be eliminated a few weeks after the infection, and it may be detected even after one year, its presence does not necessarily mean a recent infection. IgG titers peak within 1-2 months after infection but remain elevated for the whole life (Grant *et al*, 1990; Baratloo *et al*, 2015). However, Moustafa *et al.* (1998) reported that antigen detection assays may facilitate earlier diagnosis than antibody tests, as production of detectable levels of specific immunoglobulin needs time. Moreover, antigen detection could be used in routine screening for case detection in low transmission areas or detection of residual infections in very low transmission areas in order to eliminate the parasite reservoir and aid interruption of transmission. The variety of antigens were secreted and excreted by the parasites present in the blood, feces, urine and other fluids of the infected host. These antigens have potential for use in immunodiagnosis and vaccine development (Abdel-Rahman *et al*, 1999).

The demonstration of *T. gondii* antigens in serum samples of experimentally infected animals suggests that antigens are detectable in acute phase of toxoplasmosis (Lindenschmidt, 1985; Luft and Remington, 1998). For example, Raizman and Neva (1975) showed presence of CAgs in mice sera by counter-current electrophoresis and agar gel diffusion on day 2-4 of infection. Huskinson *et al.* (1989) demonstrated *T. gondii* antigens in sera and urine of mice as early as 5 days post infection by Western blot. Moreover, Shojaee *et al.* (2007) detected antigenemia from the 4th day post infection in mice sera by immunoblotting.

To increase diagnostic potency, the isolation of their immunogenic fractions could be useful (Luo *et al,* 2011). Tachyzoites stage was thought to be responsible for acute infection and expresses immunodominant antigens that induce strong immune responses (Gross *et al,* 2004). Abdel-Rahman *et al.* (2005) used *T. gondii* crude and affinity purified tachyzoites antigens isolated from slaughtered sheep in the diagnosis of toxoplasmosis in horses. Conde de Felipe *et al.* (2007) found that the *T. gondii* fractions 29-35 kDa detected the specific peak of IgG in goats 2 weeks earlier than crude extract.

Surface, SAG1, SAG2A, SAG2B, SAG3, and SAG related sequence antigens, SRS1,

SRS2, and SRS3, are mainly expressed on the tachyzoite surface (Manger *et al*, 1998; Cong *et al.*, 2013). Studies have indicated that SAG2 members participate in the process of parasite's invasion to the host, and their antibodies could block the further attachment of *T. gondii* on host cells (Lekutis *et al*, 2000; Machado *et al*, 2010; Cong *et al*, 2013). The SAG2 (P22) is protein that has been shown to be an attachment ligand and also to have good immunogenicity (Aubert *et al*, 2000; Khanaliha *et al*, 2014). So, the-SAG2 was chosen as target antigen in the present study.

The SAG2 antigen was purified from *Toxoplasma* fluid by DEAE-SephadexG-5o and G-200 ion exchange chromatography. The eluted bands were analyzed by 12.5% SDS-PAGE under reducing condition, SAG2 antigen appeared at 37 kDa, and had 1.5 mg/ml protein content. The results were reasonable with those of Villavedra *et al.* (2001) who purified, and isolated a fraction of 30-33 kDa from crude tachyzoites antigen that used successfully in the diagnosis of human toxoplasmosis by ELISA.

The reactivity of SAG2 antigen was tested by indirect ELISA and gave a strong reaction at mean OD reading equal to 1.99 with *T. gondii*. This purified antigen was utilized for immunization of rabbit for the development of pAb. It should be noted that pAbs have clear technical advantages. They are inexpensive to produce relative to the cost of monoclonal antibody technology. In addition, large quantities of pAb (~10 mg/ml) can be produced from the serum of an immunized animal. Finally, high affinity pAb can be isolated merely 2-3 months after the initial immunization, so facilitates their rapid study. In addition, pAbs contain the entire antigen-specific antibody population; thus they offer a statistically relevant idea for the overall picture of an immune response. The anti-*T. gondii* IgG pAb was represented by H- and L-chain bands at 53 and 31 kDa, respectively, and had a protein content of 4mg/ml. The results were reasonable in compared with the yield of purified immunoglobulin from any biological fluid following similar purification procedures (Bride *et al,* 1995; Yang and Harrison, 1996). The purified IgG fraction of \rabbit sera was employed as both antigen capture (1/80ug/ml) and peroxidase conjugated (1/40ug/ml) detecting antibody in sandwich ELISA. The cut-off value for positivity in sandwich ELISA for SAG2 was equal to 0.323 in serum. All values equal to or above these cut-off values were considered positive.

Suzuki *et al.* (2000) examined the efficiency of detection of IgM antibodies to a 35-kDa antigen (P35) of *T. gondii* for serodiagnosis of acute infection in pregnant women by using a double-sandwich ELISA with recombinant P35 antigen (P35-IgM-ELISA). The results showed that 18 (90.0%) of 20 serum samples from recently acquired infected patients were positive, whereas none of the 33 serum samples from patients acquired infection in the distant past were positive. Only four (25.0%) of the 16 serum samples from group suggestive of persisting IgM antibodies were positive. On the other hand, all these serum samples were positive by the conventional IgM ELISA. These results indicate that demonstration of IgM antibodies against P35 by the P35-IgM-ELISA is more specific for the acute stage of the infection than demonstration of IgM antibodies by the ELISA that used the whole-lysate antigen preparation.

Studies with sera obtained from four pregnant women who seroconverted (IgG & IgM antibodies) during pregnancy revealed that two of them became negative by the P35-IgM-ELISA between the 4^{th} and 6^{th} months post the seroconversion, whereas the conventional IgM-ELISA titers remained the highly positive. However, owing to the IgM long half-life in many patients, and the high percentage of non-specific results, the *Toxoplasma*-specific IgM assay is no longer such test of benefit in diagnosing acute *Toxoplasma* infection (Gras *et al,* 2004; Kaul *et al,* 2004).

The present study was conducted on *T. gondii* infected patients, other parasites infected patients and healthy controls. The Sandwich-ELISA was carried out with the sensitivity of 92%, specificity of 90%, PPV of 90.2% and NPV of 83.3%.

Generally speaking, the endemicity of toxoplasmosis in Egypt was known long ago. It was reported in man (Rifaat *et al,* 1963), edible animals (Rifaat *et al,* 1968), congenitally in new-borne (Wishahi *et al*, 1971) and among the childbearing aged females (Saleh *et al*, 2916) as well as in the healthy blood donors (Elsheikha *et al*, 2009). Also, reported in the renal transplant recipients from *Toxoplsama* infected blood donors (Barsoum, 2006) and cats (Al-Kappany *et al*, 20011).

Conclusion

Generally speaking, *Toxoplasma gondii* and toxoplasmosis are not only encountered in Egypt, but also worldwide.

The antigen detection assay is superior and more sensitive than antibody detection assay specially in diagnosing the active infection where *T. gondii* cysts are predominant. Antigen detection assay might be a useful approach for assessment of the efficacy of treatment especially after removal of the cyst. Further studies are recommended to improve the diagnostic efficacy of antigen based ELISA method by using a highly purified recombinant antigen. Sandwich ELISA technique proved to be sufficiently sensitive assays for the detection of human toxoplasmosis. Sandwich-ELISA is a simple fast and cheap method. Moreover, sandwich-ELISA was more acceptable with respect to its higher sensitivity and simplicity in practice.

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