Immunodiagnosis and molecular validation of *Toxoplasma gondii*-recombinant dense granular (GRA) 7 protein for the detection of toxoplasmosis in patients with cancer

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**A B S T R A C T**

Serological assays for the diagnosis of toxoplasmosis mostly rely on the tachyzoite specific antigens of *Toxoplasma gondii*, which are difficult to produce by conventional methods. The aim of this study was to clone and express of GRA7 protein of *T. gondii* and evaluate its potential for immunodiagnosis of toxoplasmosis in cancer patients. As well as validate the results using a new molecular assay, LAMP technique. The GRA7 gene was successfully cloned, expressed and purified by affinity chromatography and the production was evaluated by SDS PAGE, dot blot and western blot analyses. The rGRA7 was used for developing an ELISA based on the rGRA7 using sera from patients with toxoplasmosis and healthy controls. Furthermore, 50 serum samples from leukemic children infected with toxoplasmosis and 50 seronegative controls were included to evaluate the sensitivity and specificity of rGRA7 based ELISA. Finally, the LAMP technique was used to assess the accuracy and validity of the results obtained by rGRA7 based ELISA. The consistency of the results of two tests was determined by using the Kappa coefficient of agreement. The rGRA7 showed higher and optimum immunoreactivity with 1:100 dilution of serum from *Toxoplasma* infected patients. The sensitivity and specificity of test were calculated as 92 and 94\%, respectively. According to the Kappa coefficient of agreement, there was a significant conformance between the results obtained by ELISA based on the rGRA7 and the results of LAMP technique (κ=96, \(P<0.001\)). Findings of the present study showed that rGRA7 can be used as a potential immunogenic antigen for developing immunodiagnostic tools for immunodiagnosis of toxoplasmosis in patients including patients with cancer.

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1. Introduction

*Toxoplasma gondii*, an obligate intracellular parasite, is the causative agent of toxoplasmosis, and if left untreated, may bring about serious complications or death in immunocompromised patient (Rieffer and Wallon, 2013; Asgari et al., 2013; Stajner et al., 2013). *T. gondii* is usually transmitted through the placenta, ingestion of food or water contaminated with oocysts and consumption of materials contaminated with *Toxoplasma* cysts; raw meats from mammals and birds can be a potential food source of *T. gondii* infection for human (Tenter, 2009; Jones and Dubey, 2012; Campos et al., 2014). In individuals with normal immunity, the acute form of the disease recovers spontaneously without treatment, but in patients with a suppressed immunity (immunocompromised people), such as those taking certain types of chemotherapy (leukemic patients), those with HIV/AIDS or those who have recently received an organ transplant, the risk of reactivation of brain cysts and host death is very high (Fallahi et al., 2014a). The prevalence of *Toxoplasma* encephalitis in AIDS patients can reach up to 40\% in which 10–30\% will die from this parasitic disease (Yuan et al., 2007). Toxoplasmosis has often been described in some specific malignancies such as lymphoma, acute and chronic leukemia

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or multiple myeloma (Yazar et al., 2004; Fallahi et al., 2014a). Endochin-like quinolones, sulfonamide, clindamycin, spiramycin, pyrimethamine, and atovaquone are available medications for the treatment of toxoplasmosis or prevention of related complications (Djurkovic-Djakovic et al., 2002; Doggett et al., 2012) which often due to lack of drug effect on tissue cyst are unsuccessful and in case of ocular toxoplasmosis continue to end the patient’s life.

Laboratory diagnosis of toxoplasmosis is made by histological examinations, serological assays and a variety of biological and molecular methods (Ho-Yen et al., 1992; Montoya, 2002; Casartelli-Alves et al., 2014). Detection of IgG antibodies produced against various antigens of T. gondii that usually exist in serum of patients with toxoplasmosis (Dickerson et al., 2014), is possible by several immunoassays including enzyme-linked immune sorbent assay (ELISA), latex agglutination test (LAT), western blotting, indirect fluorescent antibody assay (IFA) etc. (Bahir et al., 2005; Basso et al., 2013). However, detection of toxoplasmosis is not possible by immune staining (Hill and Dubey, 2002). Antigens of tachyzoites (an infectious form of T. gondii) are essential elements for developing serological assays for the detection of toxoplasmosis. For instance, dense granule (GRA) proteins of T. gondii exhibit higher immunogenicity and are abundantly expressed in the hosts infected with tachyzoites, and therefore have higher potential to develop tools for laboratory diagnosis of toxoplasmosis (Velmurugan et al., 2008; Kotresha et al., 2012). Antigens used in serological techniques are usually prepared from cultured parasites which often have impurities and contain varying amounts of material culture, other cells and organisms that affect the sensitivity of these techniques. Furthermore, conventional methods to produce these antigens are not economical in terms of money, time, and safety. Emergence of recombinant DNA technology has led to cost effective, time saving and safer procedures for cloning of T. gondii recombinant antigens (Indrasantri et al., 2011; Ching et al., 2014).

Differentiation of acute and chronic infections in cancer patients is extremely important and helpful for taking preventive measures and appropriate treatment. The potential use of ELISA method based on the recombinant GRA (rGRA) antigens of T. gondii in distinguishing between recently acquired and chronic infections has been verified by some studies, showing significant higher absorbance values with human serum (Nigro et al., 2003). For instance, Ching et al. (2013, 2014) have shown that serodiagnosis of toxoplasmosis and differentiating present and past infections of T. gondii is effectively possible using western blotting based on the rGRA2 and rGRA5. A recombinant GRA7 pTZ57RT plasmid vector was constructed and the GRA7 gene was cloned in Escherichia coli strain TOP10 competent cells (Arab-Mazar et al., 2014). This study aimed to clone and express recombinant GRA7 (rGRA7) by E. coli strain BL21 using pGEX-6p-1 expression vector, and also to verify immunoreactivity of rGRA7 as antigen for developing immunoassays such as ELISA for immune diagnosis of toxoplasmosis in patients with cancer. Since molecular techniques such as different kinds of PCR, have good sensitivity and specificity in the diagnosis of toxoplasmosis and can be considered as a kind of gold standard, to verify the results obtained by rGRA7 based ELISA, a newly described DNA amplification technique, loop mediated isothermal amplification (LAMP) targeting the 35-repeat B1 gene of T. gondii genome was used. LAMP technique with extremely high sensitivity and specificity using a DNA polymerase with strand displacement activity (Bst DNA Polymerase) and four to six specially designed primers (which eliminates non-specific binding) can amplify up to 10^6 copies in less than an hour under isothermal (63–67 °C) conditions (Notomi et al., 2000; Mori et al., 2001; Nagamine et al., 2002; Fallahi et al., 2014b Fallahi et al., 2014b, Fallahi et al., 2015). Amplification and detection of a gene can be completed in a single step. DNA amplification can be easily detected by visual inspection of the turbidity or fluorescence of the reaction mixture or by a Loopamp real-time turbidimeter (Notomi et al., 2000; Mori et al., 2001; Fallahi et al., 2014b; Fallahi et al., 2015). These characteristics enable the LAMP to be applied as an accurate and rapid molecular diagnostic tool for the detection of T. gondii.

2. Materials and methods

2.1. Construction of recombinant expression vector

The GRA7 gene fragment was cloned in pTZ57RT (Novagen, purified and confirmed as previously described (Arab-Mazar et al., 2014), it was then digested by BamHI (Fermentas, Germany) restriction enzyme. After thermal inactivation of the restriction enzymes and analysis by agarose gel electrophoresis, the linearized pTZ57RT plasmid and the GRA7 gene were extracted from the 1% agarose gel by agarose gel DNA extraction kit (Qiagen, USA). The purified GRA7 gene fragment was cloned into pGEX-6p-1 (Novagen) expression plasmid at the specified restriction site. Recombinant plasmid was confirmed by both colony PCR and enzyme digestion method.

2.2. Sub-cloning of pGEX-GRA7 recombinant vectors

In order to transform the recombinant plasmids into expression hosts, 2 µl of the recombinant pGEX-GRA7 plasmid was added to 50 µl of E. coli strain BL21 competent cells on ice, gently mixed by stirring with the pipette tip, incubated on ice for 30 min, heat shocked for 1 min at 42 °C water bath, and then cultured into Luria-Bertani (LB) agar medium (Sigma–Aldrich, USA) containing ampicillin (100 µg/ml), followed by an overnight incubation at 37 °C. Finally, the white-color colonies recognized as pGEX-GRA7-positive were chosen, and the successful sub-cloning of pGEX-GRA7 recombinant plasmid was confirmed by polymerase chain reaction (PCR), BamHI restriction enzyme, and gene sequencing analysis as previously described for pTZ57RT-GRA7 recombinant plasmid (Arab-Mazar et al., 2014).

2.3. GRA7 protein production

A single lately transformed pGEX-GRA7-positive colony was inoculated into 5 ml of LB broth with ampicillin (100 µg/ml), followed by an overnight incubation at 37 °C and shaking at 200 rpm, and sub-cultured to reach an optical density of 0.600. Protein production in the cells was induced by 1 mM IPTG (Merck, Germany) followed by additional 6–8 h incubation at 37 °C and shaking at 200 rpm.

2.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) analysis

Purified rGRA7 protein was examined by SDS PAGE (Laemmli), using 15% acryl amide stacking, and separating gels. The gels were stained and analyzed with Coomassie Brilliant Blue dye. Protein contents from recombinant GRA7 in E. coli were compared with those from E. coli strain BL21 and pGEX-6p-1 without insertion.

2.5. Western blot analysis

For western blot analysis following SDS PAGE, the protein content of gel was transformed on nitrocellulose papers, and stabilized by a UV Cross Linker (UV Tec, EEC). The nitrocellulose papers were then cut into vertical strips and blocked using bovine serum albumin (BSA), as blocking solution, for 2 h at room temperature by constantly shaking. After washing, the nitrocellulose strips were incubated with human positive serum (1:200) for 1 h, washed and
treated with the Horse Radish Peroxidase (HRP)—conjugated rabbit anti serum antibodies IgG, at room temperature for 1 h. Afterwards, the strips were washed and treated with solution containing Tris, H2O, H2O2, and DAB (3, 3’-diaminobenzidine) (Merck, Germany) followed by incubation at room temperature for 15 min. Finally, the reaction was stopped by washing in H2O and analyzed for brown bands.

2.6. Protein purification by affinity chromatography

Purification of rGRA7 protein was achieved using Glutathione Sepharose 4B kit according to the manufacturer’s guidelines. Briefly, the gel was packed in Pharmacia chromatography column, washed with 7 bed volume of phosphate-buffered saline (PBS) to remove the preservative (alcohol 20%), and the gel bed was then equilibrated with 3 bed volume of PBS. The sonicated samples, following protein expression step as mentioned above, were applied to the column and the eluent was discarded, followed by washing of the column with 7 bed volume of PBS. Finally, the bound material was eluted with 5 bed of elution buffer containing 10 mM Glutathione in 50 mM Tris–HCl (pH 8.0), and the fractions were collected.

2.7. Immunologic characterization of purified rGRA7

To verify the immunologic value of purified recombinant protein, immuno-serologic tests were performed using human sera from normal healthy controls and patients with T. gondii infection.

2.7.1. Dot blot analysis

Three groups of dot spots applied on nitrocellulose papers were used including: 20 μl of purified recombinant protein (elution), and 20 μl of each lysates of pGEX-GRA7-positive and pGEX-GRA7-negative bacteria, and stabilized by UV Cross Linker. After washing (twice with PBS, 10 min shaking), blocking (by adding blocking buffer and incubation at 37 °C for 75 min), and washing again, 1:100 and 1:200 dilutions of a serum from a patient with toxoplasmosis was separately added, and incubated at 37 °C for 90 min. After washing twice, Horse Radish Peroxidases (HRP)—conjugated rabbit anti-serum antibodies were added and incubated at 37 °C for 1 h. Finally, the papers were washed and placed into a chromogenic substrate solution containing Tris, H2O, H2O2, and DAB (3, 3’-diaminobenzidine) for 10 min, and was then examined.

2.7.2. Western blot analysis

Western blotting for verification of purified rGRA7 protein was performed according to the protocol as mentioned above.

2.7.3. ELISA

Concentration of purified rGRA7 was measured using biophotometry, and was calculated as 8 μg per 1 ml of solvent. Various dilutions of the rGRA7 were prepared in carbonate buffer and were used as antigens for developing an ELISA test. Briefly, 100 μl of each concentration was separately added into 96-well polystyrene micro-plates and incubated at 4 °C for 18 h, then washed (three times with 200 μl of PBS) and blocked using 300 μl of blocking buffer (BSA 0.1%) for 90 min. After washing (three times with 200 μl of PBS), 1:100 and 1:200 dilutions of a serum from a patient with toxoplasmosis were separately added into the wells, incubated at room temperature for 90 min, and washed (three times with 200 μl of PBS). Afterwards, 100 μl of HRP-conjugated antibodies (1:5000; was added into the wells, incubated at room temperature for 90 min in a dim-light condition, and washed (three times with 200 μl of PBS). Finally, 100 μl of substrate solution containing α-phenylenediamine (OPD; Sigma–Aldrich, USA), acetic acid, and citric acid was added to each well, incubated at room temperature for 15 min in a dim-light condition, the reaction was terminated by 50 of 1 M H2SO4, the optical density (OD) of the wells was read at 450 wavelength using an ELISA reader (Sunrise Tecan, Austria), and the cut-off point, specificity and sensitivity of the test were calculated.

2.8. Study population

Demographic characteristics and clinical manifestations of 50 seropositive and 50 seronegative patients for toxoplasmosis admitted to the Medical Center for Children with Cancer, Tehran, Iran, included in the study previously were described (Fallahi et al., 2014b).

2.9. LAMP technique

To verify the accuracy and reliability of the results obtained by ELISA based on the rGRA7, molecular analysis of 50 seropositive and 50 seronegative blood samples for the presence of T. gondii, DNA was performed by LAMP technique. The blood samples were previously collected from leukemic children. DNA was extracted from theuffy coat, using a commercial DNA extraction kit (DNG-Plus™, DN8118C, Cinnacol, Iran) according to the manufacturer’s instructions. The LAMP assay using designed primers for B1 gene was carried out as previously described (Fallahi et al., 2014b). As a positive and negative control, T. gondii RH strain DNA and double distilled water were included in each run, respectively. Visual endpoint judgment of turbidity of the resulting magnesium pyrophosphate in the reaction mixture and visual inspection of the LAMP amplicons in the reaction tube by adding 1 μl of 1/10 dilution of SYBR Green I (Invitrogen lot: 49743A, USA) after the reaction, and observe the fluorescent signals of the solutions under UV light, were used to detect the LAMP positive reactions. The consistency of the results obtained with rGRA7 based ELISA and LAMP technique was determined by using the Kappa coefficient of agreement.

2.10. Ethics statement

The present study involving human subjects is in accordance with the Helsinki declaration of 1975 as revised in 2000, and it has been approved by and carried out under the guidelines of the Ethical Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran. Also, this study involving animals followed the institutional and national guides to the care and use of laboratory animals.

3. Results

3.1. Confirmation of successful sub-cloning of the GRA7 gene

The GRA7 gene and pGEX-6p-1 plasmid (both digested by BamHI restriction enzyme) were ligated and transformed into BL21 competent cells (Fig. 1a and b). The PCR assay using primers specific for GRA7 gene (Fig. 1b), and also BamHI restriction enzyme analysis (Fig. 1c) revealed a successful sub-cloning of the gene into expression vector and host. In addition, comparison of gene sequence of the cloned gene with the wild type (consensus) of the gene, obtained from Gen Bank, showed that only 24 bases were changed in the cloned gene. This evidence further confirmed the successful cloning of the GRA7 gene of T. gondii into pGEX-6p-1 expression plasmid.
Fig. 1. Agarose 1% gel electrophoresis analysis of: (a) pGEX6p-1 plasmid used for sub-cloning, lane 1; linear pGEX6p-1 plasmid (5000 bp), lane 2; non-recombinant supercoil pGEX6p-1 plasmid, lane 3; DNA ladder, (b) recombinant pGEX-GRA7 plasmid, lane 1–6; PCR products using primers specific for GRA7, lane 7; DNA ladder, and (c) recombinant pGEX-GRA7 plasmid following BamHI enzymatic digestion, lane 1; recombinant plasmid, lane 2; DNA ladder, and lane 3; GRA7 fragment.

Fig. 2. The SDS PAGE (a) and western blot (b) analysis of the expression of 55-kD rGRA7 protein in E. coli BL21: (a) SDS PAGE analysis following Coomassie brilliant blue staining, lane 1; BL21 without plasmid, lane 2; BL21 containing recombinant pGEX6p-1 plasmid following 6h after induction, lane 3; BL21 containing pGEX6p-1 plasmid without insert, lane 4; BL21 containing recombinant pGEX6p1 plasmid following 8h after induction; and lane 5, 50-kD GRA7 protein as marker, (b) Western blot analysis using human serum (1:200), lane 1; BL21 lysates without GRA7 insert, lanes 2–4; BL21 lysates containing recombinant pGEX4t-1 plasmid following respectively, 4, 6, and 8h after induction, and lane 5; 50-KD GRA7 protein as marker.

3.2. SDS PAGE and western blot analysis prior to purification of the rGRA7 protein

Purified rGRA7 protein was analyzed by SDS PAGE, using 15% acryl amide gels followed by Coomassie-blue staining. As shown in Fig. 2a, in SDS PAGE analysis rGRA7 was found to resolve at 55 kD, which indicated successful expression of the protein in recombinant GRA7 of E. coli (Fig. 2). Western blot analysis of the gel’s protein content was also transferred on nitrocellulose paper using human serum which further verified the production of 55-kD rGRA7 protein (Fig. 2b).

3.3. Immunoserologic tests after purification of the rGRA7 protein

Results of dot blotting on purified rGRA7 protein using two different dilutions of human serum revealed spots as rGRA7 in purification buffer, as well as in BL21 lysates containing recombinant pGEX-6p-1 plasmid, but not in lysates without GRA7 insert, thereby confirming the accuracy of the rGRA7 purification process (Fig. 3a). Western blotting following purification also detected purified rGRA7 protein as 55-kD brown bands (Fig. 3b), which showed successful purification of rGRA7, and also the antigen-antibody interaction between rGRA7 and anti-GRA7 antibodies from human serum of patients with toxoplasmosis.

Moreover, result of the ELISA test using various concentrations of rGRA7 and positive human sera for toxoplasmosis demonstrated high reactivity of rGRA7 protein with sera containing human anti-GRA7 antibodies. The optimum concentration for rGRA7 as coating antigen was calculated as 4 μg/ml; and the suitable dilution for serum was calculated as 1:100. In addition, using a commercial ELISA kit (EUROIMMUN, Germany) and the sera from 50 seronegative and 50 seropositive leukemic children, the cut-off point sensitivity and specificity of the test were calculated as 0.6, 92% and 94%, respectively.

3.4. Consistency of the results of ELISA based on the rGRA7 and LAMP

Molecular analysis of blood samples using the LAMP technique revealed that 92% (46/50) of seropositive and 6.0% (3/50) of seronegative samples from leukemic patients were positive for the presence of Toxoplasma DNA (Fig. 4). According to the Kappa coefficient of agreement, there is a significant conformance between the results of the ELISA based on the rGRA7 and results obtained by LAMP technique (κ=96%, P<0.001). In other words, results of the ELISA based on the rGRA7 were highly compatible with the results obtained by LAMP technique.

4. Discussion

T. gondii is an obligate intracellular opportunistic parasite that affects one third of the human population around the world, causing death in some immunocompromised individuals. Early detection of the infection is of great importance in the prevention or worsening of the disease, and also in planning for effective treatments, especially in immuno-compromised individuals such as children with leukaemia. Serological assays based on GRA proteins are actually used in laboratory diagnosis of T. gondii infections (Velmurugan et al., 2008). Cloning and expression of several GRA proteins, particularly GRA2, GRA5 and GRA7, in heterologous sys-
**Fig. 3.** The dot blotting (a) and western blotting (b) after purification of rGRA7 protein: (a) lane 1; purified rGRA7 protein, lane 2; lysates pGEX-GRA7-negative bacteria, lanes 3; Flew Through, and lane 4; lysates of pGEX-GRA7-positive bacteria. (b) lane 1; rGRA7 protein before purification, lane 2; rGRA7 protein after purification, lane 3; 50-KD GRA7 protein as marker, lanes 4-5; Flew Through.

**Fig. 4.** Comparison of ELISA based on the rGRA7 and LAMP technique on seropositive and seronegative blood samples from children with cancer (a) LAMP on seropositive samples (b) LAMP on seronegative samples (c) ELISA based on the rGRA7 test on seropositive samples (d) ELISA based on the rGRA7 test on seronegative samples. P, positive control; N, negative control; 1–10 represent ten accidentally selected seropositive and seronegative blood samples taken from children with cancer.
tems has received diagnostic and clinical attractions in the last two decades (Jacobs et al., 1998; Igarashi et al., 2008; Ching et al., 2013). For instance, DNA products of cloned GRA7 have been claimed to efficiently provoke remarkable and polarized Th1/Th2 immune responses against T. gondii, characterized by augmented production of IgG2 and IFNγ (Jongert et al., 2008; Liu et al., 2014). However, a limited number of recombinant GRA proteins have been tested as antigens for developing ELISA and other immunological assays.

The GRA7 gene of T. gondii, using pTZ57R plasmid, previously has been cloned and amplified (Arab-Mazar et al., 2014). In the present study, the GRA7 protein in E. coli strain BL21 was successfully subcloned and expressed, using pGEX-6p-1 expression plasmid. In this study, the rGRA7 protein, purified by affinity chromatography was evaluated by SDS PAGE analysis and immunological assays including dot blotting and western blotting. Furthermore, using a devised ELISA, this rGRA7 protein showed high reactivity with sera from patients with toxoplasmosis. Also, high specificity and sensitivity was assessed for rGRA7 based ELISA.Various approaches have been suggested for facilitating the cloning process of a DNA fragment into vectors/plasmids (Sambrook et al., 1989; Hotten et al., 1994). In the present study, a T-vector was preferentially constructed, since cloning of a gene fragment into a T-vector has been shown to have some advantages over others such as cloning end blunts (Zheng et al., 2005). For example, cloning of a gene into a T-vector provides the ability to maintain it in a gene library for future use etc. Also used in this study, is the pGEX-6p-1 expression plasmid belonging to the gene-fusion category of vectors, and expressed rGRA7 as glutathione S-transferase (GST) fusion, which facilitated the purification process.

Very few studies exist regarding cloning, expression and immunoreactivity characterization of recombinant GRA7 protein of T. gondii, and these studies have revealed similar results to the findings of this study (Jacobs et al., 1998, 1999: Zheng et al., 2005). For example, the recombinant GRA7 protein cloned and expressed by Jacobs et al. (1998, 1999) exhibited high reactivity with IgG antibodies and sera from patients with acute stage infection of T. gondii in a devised ELISA with approximately similar specificity and sensitivity, but lower cut-off value compared to those in this study. However, Jacobs et al. used a different strain of E. coli, MC1061 (pAC1) and a different fusion partner, a short mTNF fragment and hexahistidine, in comparison with present study. In addition, Wang et al. cloned the GRA7 gene into the pTE28a plasmid and expressed it in E. coli. Similar to the findings of this study, the results of their studies showed high sensitivity and specificity for ELISA based on the rGRA7, thereby introducing this protein as a potential diagnostic tool for T. gondii infection (Wang et al., 2014). Moreover, to assess the validity of the results obtained by ELISA based on the rGRA7, the LAMP technique was conducted on DNA extracted from the buffy coat of positive and negative samples for toxoplasmosis.

The LAMP method utilizing a DNA polymerase with strand displacement activity (Bst DNA polymerase) and four to six primers which target six to eight distinct internal regions on the target DNA, amplify a few copies of genetic material with high sensitivity and specificity to 10^9 under isothermal conditions (63–65 °C) in less than an hour (Mori et al., 2001; Fallahi et al., 2014b: Fallahi et al., 2015). The high sensitivity of LAMP is due to the primers used in the reaction and the auto-cycling amplification can produce very large amounts of DNA of various sizes (Nagamine et al., 2002). The significant conformance between the results of ELISA based on the rGRA7 and LAMP technique (~96%, P < 0.001) shows that these assays have consistency with each other and the results obtained by ELISA based on the rGRA7 are reliable and had acceptable accuracy. Finding a positive LAMP result in patients with negative ELISA based on the rGRA7 is an important outcome shows that a negative result (seronegative) obtained from enzyme immunoassays, does not indicate a virtual negative result for toxoplasmosis and more sensitive supplementary assays should be done.

This study showed that rGRA7 cloned and expressed by E. coli using pGEX-6p-1 plasmid, exhibits high immunoreactivity, and the ELISA method developed on the basis of this recombinant protein is highly specific and sensitive. Therefore, rGRA7 protein can be considered as a potential tool for serodiagnosis and detection of toxoplasmosis, and also for studying the stage of the disease and immune response against T. gondii in infected patients, as well as in children with leukaemia.

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