



An evaluation study of enzyme-linked immunosorbent assay (ELISA) using recombinant GRA1 protein for detection of igg antibodies againts *Toxoplasma gondii* infections

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ABSTRACT

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Reliable laboratory testing is important to detect *Toxoplasma gondii* infection and focuses on improving the low cost and easy to use the diagnostic instrument. Enzyme-linked immunosorbent assay (ELISA) method can be used to determine a large number of samples within a short period of time which based on antibody or antigen detection. We tested the sensitivity and specificity of GRA1 protein of as antigen using the ELISA method to toxoplasmosis diagnosis and compared it with commercial ELISA kit. Seventy sera samples were collected and tested using indirect ELISA, commercial ELISA kit and GRA1 protein-coated as antigen. The results showed 48 and 51 samples had positive IgG antibody using ELISA-GRA1 and commercial ELISA kit, respectively. The GRA1 sensitivity and specificity on ELISA were 100% and 86.36%, respectively. Whereas positive predictive value (PPV) was 94.11%. This result indicated that the recombinant GRA1 protein is a highly immunogenic protein in human toxoplasmosis and marker for toxoplasmosis screening.

ABSTRAK

Pengujian laboratorium yang handal penting untuk mendeteksi infeksi *Toxoplasma gondii* dan berfokus untuk meningkatkan instrumen diagnosis yang murah dan mudah digunakan. Metode *Enzyme-linked immunosorbent assay* (ELISA) dapat digunakan untuk menguji sampel dalam jumlah besar dalam waktu singkat dan berdasarkan pada deteksi antibodi atau antigen. Kami menguji sensitifitas dan spesifisitas dari protein GRA1 sebagai antigen pada metode ELISA untuk mendiagnosis toxoplasmosis dan dibandingkan dengan *commercial ELISA kit*. Tujuh puluh sampel serum dikumpulkan dan diuji menggunakan indirect ELISA, ELISA kit komersial dan protein GRA1 dilapisi sebagai antigen (ELISA-GRA1). Hasil penelitian menunjukkan, 48 dan 51 sampel positif memiliki antibodi IgG berturut-turut menggunakan ELISA-GRA1 dan ELISA kit komersial. Sensitivitas dan spesivisitas dari ELISA-GRA1 adalah 100% and 86,36%, positive prediction value (PPV) adalah 94,11%. Hasil ini menunjukkan protein GRA1 rekombinan adalah protein yang sangat imunogenik pada toksoplasmosis manusia dan *marker* untuk skrining toksoplasmosis.

Keywords:

Toxoplasmosis
recombinant GRA1 protein
ELISA
IgG antibody

INTRODUCTION

Toxoplasmosis is a disease caused by infection of obligate protozoan parasite, called *Toxoplasma gondii*. Human can be infected by *T.gondii* through congenital toxoplasmosis, consumption habits (raw meats, raw vegetables), activity with soil/meat without protection, blood transfusion, organ transplantation, etc. Oocyst become infective stage when passed out from definitive host and contaminated water sources, soil and plants. Most of toxoplasmosis is asymptomatic but can be serious problems in immunocompromised patients and newborns with congenital toxoplasmosis. Effect of *T.gondii* infection caused encephalitis in immunocompromised hosts, chorioretinitis on immunocompetent hosts or seriously congenital disease on fetus development if pregnant women become infected for the first time during pregnancy.¹ More than 60% world population are toxoplasmosis and 90% of it asymptomatic even they have *T. gondii* antibodies. It depends on the individual immune responses to prevent the symptoms.²

Commonly, detection of toxoplasmosis using serological methods, such as a dye test (DT), modified agglutination test (MAT), enzyme-linked immunosorbent assays (ELISA), immunosorbent agglutination assay (ISAGA), indirect fluorescent antibody test (IFAT) and indirect haemagglutination assays (IHA) to detect *T. gondii* antibody. Parasite demonstrates in tissue can be done by culturing of parasite (*in vivo* and *in vitro*) and detection of specific nucleic acid using DNA probe, PCR and L-AMP methods. Enzyme-linked immunosorbent assays is a popular and easier method for detection of clinically toxoplasmosis and commercially available. Commercial ELISA kits using antigen from native tachyzoites which grown in mice or tissue culture and

probably contain varying amounts of extraparasitic material.³ Limitations of the tachyzoite antigen for serologic tests can be serious problems, another antigens should become an alternative test, such as using purified recombinant antigens which expressed by tachyzoites and bradyzoites. However, the whole tachyzoite native antigen test is difficult to standardize and some cases produce false positive reactions.⁴ Tachyzoites is not the only component caused activation of the immune response to produce antibody, expression of excreted-secreted antigen from bradyzoites can induce antibody production and IgG specific *T. gondii* which are always existed in an infected host lifetime.

GRA1 has been identified as excreted-secreted antigen (ESA) in tachyzoites and cross-reactive with bradyzoites.⁵ It located in the dense granule of both tachyzoites and bradyzoites and using as *T. gondii* secretory organelle marker. It always secreted in lumen and potentially can be identified in body fluid of the infected host. GRA1 can induce humoral and cellular immune responses in chronic infection phase such as increase production of antibody and IFN- γ on mice and humans. GRA1 epitope is presented on MHC class I molecules during infection and inducing specific CTLs.⁶⁻⁸ GRA-1 was secreted into the lumen of the parasitophorous vacuole as a soluble protein and associated with the membranous tubular network peripherally.⁹ GRA1 was needed for secretion of 3 secretory organelles of *T. gondii* and became a marker of dense granule proteins.¹⁰ GRA1 protein vaccination shows CD8⁺ T-cells activity against target cells infected with parasites and GRA1 transfected cell line.⁷

The costs of serologic test in developed countries is not prohibitively high and have low-cost alternative tests with the same sensitivity and specificity. The cost of developing the instrument depends on efficient production of recombinant

antigens.¹¹ The previous study in the same project had been developing an efficient system for the production and purification of GRA1 proteins and had tested for immunogenic activity. Based on the ability of GRA1 to stimulate immune response, we tried its ability as antigens to develop the diagnostic tools. Sensitivity and specificity of GRA1 as antigens in ELISA methods (ELISA-GRA1) compared to the commercial ELISA kit to the detection of IgG antibodies against *T. gondii*.

MATERIALS AND METHODS

Total of 70 human sera were obtained from a previous study in Central Java population and approved by Medical and Health Research Ethic Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada for study in human subject.¹² Sera was tested using ELISA methods and separated for ELISA kit test and ELISA-GRA1 coated protein as antigen. Preparation of recombinant protein GRA1 consists of isolation, characterization, cloning, expression and purification of GRA1 protein. Isolation, characterization, cloning and expression of GRA1 protein were carried by previous researcher in same project^{13,14} and the stored culture of *E. coli* inserted with GRA1 protein in 4°C until we used for this study. The recombinant *E. coli* was recultured and isolated GRA1 protein used to sonication for breaking the bacterial membrane. Purification of GRA1 protein using Ni-ted profino column chromatography and electrophoresis to confirmed the result.

Protein recombinant GRA1 was used

as antigen and coated overnight at 37°C using coating buffer (1:10). Dilution of human sera and anti-human IgG alkaline phosphatase were conjugated 10 times and 5000 times, respectively. Human sera was added and incubated at 37°C at an hour. Washing solution was added 3 times after each process to remove all unbounded particles. Antihuman IgG alkaline phosphatase, substrate and stop solution were added alternately to complete the methods and the quantitative result measured by ELISA reader. Cut off value was counted by mean of negative control. Seropositive of Tg-IgG antibodies measured by optical density and compared with cutoff value.

Effectiveness of GRA1 as promising-antigen was evaluated by ELISA kit commercial (GenWay BioTech) coated with native tachyzoites. Procedure of ELISA kit was followed the manual instruction. The kit consists of dilution buffer, washing buffer, negative and positive control, 4 types of calibrator to differentiate negative, low and high positive antibody concentration.

RESULTS

Serodiagnostic using recombinant proteins of *T. gondii* were evaluated by ELISA-GRA1 and compared with commercial ELISA kit. Sensitivity and specificity were measured for effectiveness detection of GRA1 protein as antigen. The result of GRA1-ELISA showed 51 positive and 19 negative samples, while the result of ELISA kit showed 48 positive and 22 negative samples (TABLE 1).

TABLE 1. Sensitivity and specificity of recombinant protein GRA1

No. ELISA kit No. ELISA-GRA1	Positive of IgG	Negative of IgG	Total
Positive of IgG	48	3	51
Negative of IgG	0	19	19
Total	48	22	70

DISCUSSION

Commercial ELISA kit usually using native tachyzoites antigens coated in microplate and worldwide distribution to toxoplasmosis diagnosis. This tool is a high-cost instrument among laboratories and not always accurate because often produces false positive reactions.⁴ Toxoplasmosis diagnosis is an important test for human in every social-economic status, development of low-cost diagnostic tools are seriously important to supporting of health status and epidemiological screening in infectious disease in populations.

The result of this study explicitly showed GRA1 antigen in ELISA was suitable for detection of serum antibody to *T. gondii* infections and clearly distinguished mean OD and 95% CI. The method was able to differentiate of seropositive and seronegative on *T. gondii*-IgG sera. The test of GRA1-ELISA showed 51 positive and 19 negative samples, while there were 48 positive and 22 negative samples tested by ELISA kit (GenWay BioTech).

All estimation of sensitivity and specificity observation were greater than 80%. Sensitivity of GRA1 is 100% and specificity reaches 86.36%. Based on 80% of sensitivity and specificity, the observed sample size was sufficient to estimate good sensitivity and specificity as diagnostic tools.

Dense granule proteins function is to manage modification of parasitophorous vacuole and intake nutrition from the cytoplasm of the infected cell.^{5,15-18} Modification needed for tachyzoites development in infected cell and replication of parasites on parasitophorous vacuole membrane.¹⁸ Most of dense granule proteins secreted in parasitophorous vacuoles and increased following the number of parasite infections. A molecule became potential antigen if have foreignness in the body a consist of molecule weigh

over 1kD, complex structure and stabil molecules.¹⁹ GRA1 protein have been reported have immunogenic and antigenic activity.^{6,7,20} Vercammen *et al.*⁶ reported the result of GRA1 vaccination induce humoral immune response in mice and produce IgG antibodies. Naturally, GRA1 in parasitophorous vacuoles lumen induce secretion of IgG spesific antibodies to GRA1 and can be diagnosed using recombinant proteins GRA1 and serologic methods with sera as samples.

However, there is a significant advantage on preparation of recombinant proteins than the preparation of crude *T. gondii* proteins. Recombinant *T. gondii* proteins can be produced economically in large quantities with *in vitro* *E. coli* culture, but crude *T. gondii* antigens must be extracted from *T. gondii* on animal models. Crude extracts contain large amounts of proteins, other macromolecules and most of them can affect test results.²¹

Purification of recombinant protein is an alternative to detection of serum antibodies and allow better standardization of immunoassays.²¹⁻²³ Furthermore, use combination of recombinant antigens may enhance the sensitivity of antibody-based assay. Several previous studies have found that recombinant antigens improve the serological diagnosis of *T. gondii* infection.²²⁻²⁶ Moreover, recombinant antigens have the potential to be used to create new tests that can distinguish newly acquired infections from those obtained in the past.

CONCLUSION

Our study showed high sensitivity and specificity of recombinant protein GRA1 as antigens for detections of toxoplasmosis using ELISA and specificity of GRA1 on ELISA are 100 and 86.36% to toxoplasmosis diagnosis.

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