

Analysis Concentration of *Toxoplasma gondii* on Anti-Toxoplasma IgG-IgM Antibody Levels, and the Outcomes of Pregnancy in Mice Balb/c

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Abstract

Introduction: *Toxoplasma gondii* is an obligate intracellular protozoan parasite that can infect any warm blood vertebrae, and if first trimester pregnant woman infected, it may cause abortion. The objective is to prove the effect of the *Toxoplasma gondii* concentration in anti-toxoplasma IgG-IgM antibody levels, and the outcomes of Balb/c mice pregnancies. **Materials and Methods:** The study was conducted in Balb/c mice with inclusion criteria, and was conditioned pregnant. The pathogen strains of *Toxoplasma gondii* tachyzoite injected intraperitoneally. The blood samples were taken serially to be tested for anti-toxoplasma IgG-IgM antibody levels. After the mice were injected with tachyzoite, they are assessed every day to observe their body weight, vaginal bleeding, and labor. Anti-toxoplasma IgG-IgM antibody levels examined using qualitative mouse IgG-IgM antibody ELISA KIT. **Results:** Anti-toxoplasma IgM antibody levels increased significantly after 24 hours of injection tachyzoites in all dose groups, and remained high through day 21. Anti-toxoplasma antibody IgG levels increased significantly after 72 hours post injection and remained elevated until day 21. The incidence of abortion is 100% in mice which injected tachyzoite levels 1×10^3 and 1×10^4 , and the incidence of abortion approximately 2 - 4 days post injection. 100% of mice that were injected with tachyzoites 1×10^1 and 1×10^2 have labor at term. Physical anomaly was found in baby mice from mice that were injected with tachyzoite 1×10^2 . **Conclusion:** There is a significant correlation between the concentrations of *Toxoplasma gondii* tachyzoite with anti-toxoplasma IgG-IgM antibody levels,

and there is a significant relationship between the concentrations of tachyzoite with abortion.

Keywords

Toxoplasma gondii Tachyzoite, Anti-Toxoplasma IgG-IgM Antibody Levels, Pregnancy, Abortion, Labor

1. Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan. There are three forms of *T. gondii*, they are tachyzoite (proliferative forms), cysts (containing bradizoite), and oocysts (containing sporozoite) [1] [2] [3] [4]. *T. gondii* consists of three types (type I, II and III) and two types from recombination (types IV and V) and has a different pathogenicity. Nowadays, there are at least more than 120 genetic markers that have been developed and used for the determination of *T. gondii* type [5] [6]. Toxoplasmosis is a zoonotic disease caused by *T. gondii* [4]. Toxoplasmosis is an important zoonotic disease caused by protozoan parasite *T. gondii*. The disease affects one-third of the total world population [7] [8]. In the world, *T. gondii* is one of the most common causes of abortion caused by parasite [9]. Limited data is available on the prevalence of *T. gondii* infections in Indonesia. Gandahusada, (1991) reported incidence of seroprevalence in Indonesia was around 2% - 63% [10]. In the field of obstetrics, toxoplasmosis becomes important because can cause bad pregnancy outcomes, such as abortion [11] [12], fetal death [13], hydrcephalus [14] [15] [16] and retinochoroiditis [17] [18] [19]. Humoral immune response associated with a form of active extracellular and invasive tachyzoite in the circulation system. Antibodies IgG and IgM play as the main role in the circulatory system, and IgA plays more dominant in the mucosal surface [20] [21] [22] [23] [24]. This study will prove the relationship between the number of *T. gondii* tachyzoite, with elevated anti-toxoplasma IgG and IgM antibody levels in pregnant mice, and pregnancy outcomes.

2. Materials and Method

Experimental procedures carried out in Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. This research use adult female mice strain Balb/c according to inclusive criteria (12 - 14 weeks old, BW 25 - 30 gram, no physical deformity). Exclusive criteria are non-active mice. Animal study determined based on the research guidelines for evaluating the safety and efficacy of herbal medicines in accordance with WHO standards. The mice had adaptation for one week, in the laboratory (in a standardized cage) with room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$), dark and light cycles (12/12 h), fed and watered ad libitum. The conceiving process is combining every two female mice and one adult male mouse in one cage. Mice is pro-

nounced pregnant if plug sign is found, which is mucous plug shows in introitus vagina, redder colour and thicker vagina compared to before mated. The time of pregnancy was decided by the as zero day finding of plug sign. The amount of sample is decided of 8 mice because this is a preliminary research. The pregnant mice was divided into 4 groups (K1, K2, K3, K4), each group consisting of two mice. Tachyzoite of pathogen *Toxoplasma gondii* strains, injected intraperitoneally into mice 1 day after pregnant. Group 1 (K1) injected with tachyzoite 1×10^1 , group 2 (K2) injected with tachyzoite 1×10^2 , group 3 (K3) injected with tachyzoite 1×10^3 , and group 4 (K4) injected with tachyzoite 1×10^4 . Blood samples were taken from the mice of 0.1 - 0.2 cc serially. The first blood was taken 1 hour before injected with tachyzoite, further blood samples were taken 24 hours, 48 hours, 72 hours, 14 days, and 21 days after injection. The blood was collected and stored in a refrigerator (freezer) at a temperature of -20°C . After the mice were injected with tachyzoite, they are assessed every day to observe their body weight, vaginal bleeding, and labor.

2.1. *Toxoplasma gondii* Tachyzoite

Toxoplasma gondii tachyzoite used in this study is a strain of the pathogen RH strain. Tachyzoite suspension was stored at -20°C . Tachyzoite concentration used was 1×10^1 , 1×10^2 , 1×10^3 , and 1×10^4 .

2.2. The Examination Procedures of Anti-*Toxoplasma* IgG-IgM Antibody Level

Blood samples examined collectively in Molecular Biology and Immunology Laboratory, Faculty of Medicine, University of Hasanuddin Makassar, Indonesia. Measurement of anti-toxoplasma IgM antibody levels using qualitative mouse IgM antibody (TP-IgM) ELISA KIT. Cat No: MBS9310461. Measurement of anti-toxoplasma IgG antibody levels using qualitative mouse IgG antibody (TP-IgG) ELISA KIT. Cat No: MBS109093. Mechanical antibodies are in accordance with procedures which have been determined in a catalog ELISA Kit.

3. Results

Anti-toxoplasma IgG antibody levels before and after injection of *T. gondii* tachyzoite. The sample groups (K1, K2, K3, K4) were injected respectively with tachyzoite 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 . Anti-toxoplasma IgG antibody level was examined serially (2 hr before injection, 24 hr post injection, 48 hr post injection, 72 hr post injection, 14 days post injection, and 21 days post injection). Anti-toxoplasma IgG antibody level was detected positively 72 hours post injection, with cut off point of 0.292 ± 0.037 (Table 1, Figure 1).

Anti-toxoplasma IgM antibody levels before and after injection of *T. gondii* tachyzoite. The sample groups (K1, K2, K3, K4) were injected respectively with tachyzoite 1×10^1 , 1×10^2 , 1×10^3 , 1×10^4 . Anti-toxoplasma IgM antibody level was examined serially (2 hr before injection, 24 hr post injection, 48 hr post injection, 72 hr post injection, 14 days post injection, and 21 days post injection).

Anti-toxoplasma IgM antibody level was detected positively 24 hours post injection, with cut off point of 0.332 ± 0.0045 (Table 2, Figure 2).

Mice that injected with 1×10^1 and 1×10^2 all samples gave birth at term (100%), while mice that injected with 1×10^3 and 1×10^4 tachyzoite all samples undergo abortion (found vaginal bleeding) about 2 - 4 days post injection. The sample group which injected with 1×10^1 tachyzoite gave birth to seven and nine neonatal mice and none of them are found with physical anomaly. Anyhow, the sample group which injected with 1×10^2 tachyzoite gave birth to five and seven neonatal mice. A physical anomaly was found; that is crooked tail bone in one neonatal mice from each both sample (Table 3).

Table 1. Anti-toxoplasma IgG antibody levels before and after injection of *T. gondii* tachyzoite.

Time When Antibody Level Is Identified	The Concentration of Tachyzoite Anti-Toxoplasma IgG Antibody Level				Cut Off
	K1 (1×10^1)	K2 (1×10^2)	K3 (1×10^3)	K4 (1×10^4)	
2 hours before injection	0.121 ± 0.007	0.131 ± 0.007	0.218 ± 0.008	0.230 ± 0.003	
24 hours post injection	0.120 ± 0.005	0.130 ± 0.009	0.234 ± 0.011	0.226 ± 0.003	
48 hours post injection	0.126 ± 0.003	0.124 ± 0.007	0.249 ± 0.006	0.229 ± 0.006	
72 hours post injection	0.327 ± 0.033	0.368 ± 0.035	0.387 ± 0.021	0.377 ± 0.019	0.292 ± 0.037
14 days post injection	0.665 ± 0.011	0.699 ± 0.017	0.787 ± 0.028	0.829 ± 0.071	
21 days post injection	1.246 ± 0.012	1.302 ± 0.060	1.374 ± 0.020	1.488 ± 0.014	

Table 2. Anti-toxoplasma IgM antibody level before and after injection of *T. gondii* tachyzoite.

Time When Antibody Level Is Identified	The Concentration of Tachyzoite Anti-Toxoplasma IgM Antibody Level				Cut Off
	1×10^1	1×10^2	1×10^3	1×10^4	
2 hours before injection	0.166 ± 0.008	0.207 ± 0.003	0.179 ± 0.015	0.185 ± 0.009	
24 hours post injection	0.556 ± 0.022	0.554 ± 0.021	0.619 ± 0.030	0.548 ± 0.009	0.332 ± 0.045
48 hours post injection	0.928 ± 0.019	0.959 ± 0.029	1.040 ± 0.037	0.889 ± 0.012	
72 hours post injection	1.292 ± 0.033	1.326 ± 0.007	1.427 ± 0.017	1.480 ± 0.013	
14 days post injection	1.312 ± 0.022	1.331 ± 0.017	1.466 ± 0.030	1.477 ± 0.008	
21 days post injection	1.322 ± 0.022	1.354 ± 0.008	1.466 ± 0.088	1.495 ± 0.006	

Table 3. The relationship between *T. gondii* tachyzoite doses with pregnancy outcomes.

The Concentration of Tachyzoite	Outcome Pregnancy	
	Abortion (%)	Labor (%)
1×10^1		100
1×10^2		100
1×10^3	100	
1×10^4	100	

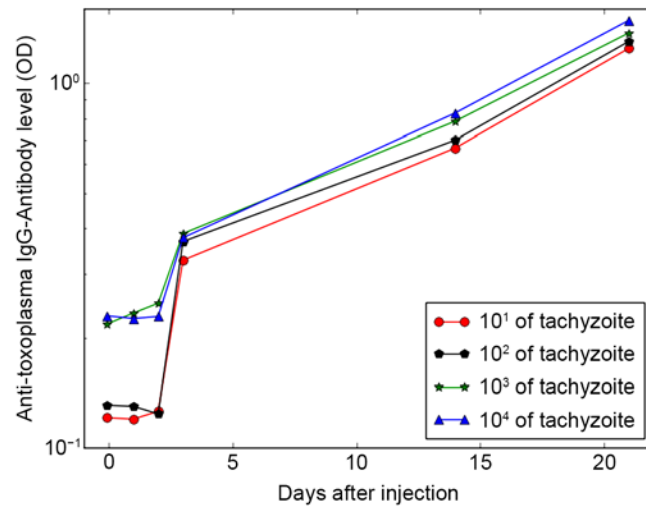


Figure 1. Anti-toxoplasma IgG antibody levels before and after injection of tachyzoite. Anti-toxoplasma IgG antibody levels increased significantly after 72 hours post injection tachyzoite. The highest levels of anti-toxoplasma IgG antibodies in mice injected with 1×10^4 tachyzoite.

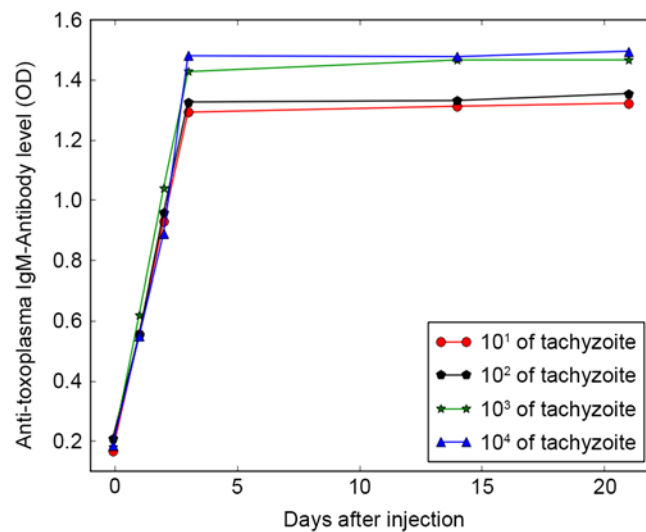


Figure 2. Anti-toxoplasma IgM antibody levels before and after injection of tachyzoites. Anti-toxoplasma IgM antibody levels increased sharply after 24 hours post injection tachyzoite. After 72 hours after injection, anti-toxoplasma IgM antibody levels increased not significantly. The highest levels of anti-toxoplasma IgM antibodies in mice injected with 1×10^4 tachyzoite.

4. Discussion

In this study, we analyzed the outcomes of pregnancy, and the anti-toxoplasma IgG-IgM antibody levels, with doses of injected toxoplasma tachyzoites in early pregnant mice. In this study, we found that the amount/concentration of tachyzoites that infect mice in early pregnancy is associated with the outcomes. This study concluded abortion occurred in the group of injected mice with dose 1×10^3 and 1×10^4 , and abortion occurs about 2 - 4 days after intraperitoneal injection, while a group of tachyzoites injected doses of 1×10^1 and 1×10^2 are all born at term. Castano, *et al.*, (2014) reported that the incidence of abortion around 91.6% and occurred between day 7 and 11 after the 2000 sporula oocytes given orally in sheep. The incidence of abortion around 58.3%, and occurred between day 9 and day 11 post-administration of 500 sporula oocytes [25].

In this study, the IgG antibody level increase significantly 3 days post tachyzoite injection and keep increasing until day 21. Mean while, the IgM antibody level increase significantly 1 day post tachyzoite injection and keep increasing until day 21. De Carlo, *et al.*, (2008) reported that the IgM antibody level increase at day 5 - 7 and maximized until 1 - 2 months after acute infection, and decreased faster than IgG antibody level. However, in some cases, the IgM antibody level persisted after acute infection of toxoplasma. The IgG antibody level usually detected in 1 - 2 weeks post injection, and peaked at 12 weeks to 6 months post infection and prolonged for years and usually persist live long [26].

Several animal studies reported that abortion can occur due to acute infection of *T. gondii*. Abortion occurs because of damage to the products of conception due to parasite replication in the placenta or fetus [27] [28]. The incidence of thrombosis and infarction in the placenta also can occur on the event that have nothing to do with the replication of the parasite. Castario, *et al.*, (2014) reported thrombosis and infarction occurs in the placenta and sheep fetus without antigen or parasites replication, and also reported elevated levels of interferon-gamma (IFN- γ) was significantly in abortion group than the control group [25].

In this study, the physical anomaly was a crooked tail bone found in neonatal mice from the group which injected with tachyzoite 1×10^2 . The cause of this anomaly is unclear, since infection of *T. gondii* in pregnancy usually cause congenital defect such a hydrocephalus and retino choroiditis [29] [30].

5. Conclusion

Anti-toxoplasma IgM antibody levels increased significantly after 24 hours of injection of *T. gondii* tachyzoites in all dose groups, and remained high until day 21, whereas anti-toxoplasma IgG antibody levels increased significantly after 72 hours post injection tachyzoites and remained elevated through day 21. The incidence of abortion is 100% in the group of mice with injected tachyzoites doses of 1×10^3 and 1×10^4 , and abortion occurs about 2 - 4 days post-injection. Mice were injected tachyzoites 1×10^1 and 1×10^2 have labor at term 100%. Physical anomaly was found in neonatal mice from mice that were injected with tachyzoite 1×10^2 . There is a significant correlation between the concentrations of

Toxoplasma gondii tachyzoite with anti-toxoplasma IgG-IgM antibody levels, and there is a significant relationship between the concentrations of tachyzoite with abortion.

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Conflicts of Interest

The authors declare no conflict of interest.

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