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Conference Paper

Inhibition of Apoptosis in Retinal of Newborn Mice Due To Congenital Toxoplasmosis

Lucia Tri Suwanti^{1,3}, Mufasirin^{1,3}, and Hani Plumeriastuti²¹Department of Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga²Department of Pathology, Faculty of Veterinary Medicine, Universitas Airlangga³Researcher of Institute of Tropical Diseases, Universitas Airlangga

Abstract

Toxoplasma gondii infection in pregnant women cause defects in the newborn, such as hydrocephalus and eye damaged, even blindness. Histologically damage due to congenital infection of *T. gondii* need to be examined. Twenty pregnant mice were divided into two groups which are the treatment group and the control group. Each mouse in treatment group was infected with 10 takizoit by intraperitoneal. Each of the newborn were sacrificed, their head were taken and their eye tissue were fixed in 10% of buffered formalin and the histological sample were made in HE and TUNEL staining. The result showed that the retina of the eye of the newborn from infected mice damage. The damages include: hemorrhage, infected retinal cells, eye growth inhibition and decreased of apoptosis index of the retina cells.

Keywords: Apoptosis in retinal, Newborn, Congenital Toxoplasmosis, Ocular Toxoplasmosis.

Corresponding Author:

Lucia Tri Suwanti
tswant@gmail.com

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1. INTRODUCTION

Toxoplasmosis is a disease caused by *Toxoplasma gondii* infection. It is a zoonotic disease which can infect humans and all warm-blooded animals such as mammals and birds. The first infections in females during pregnancy can be transmitted to the fetus and leads to the congenital infection. Congenital toxoplasmosis clinical symptoms ranging from mild to severe such as visual impairment including retinochoroiditis, hydrocephalus, seizures, mental retardation and fetal death [1]. Although at the time of the birth of the newborn mice are asymptomatic, the symptoms will be emerged in few times later. The infection will become active again and is likely to cause retinocoroiditis and blindness [2]. Incidence of retinochoroiditis that it was due to *T. gondii* infection, reaching 30%-50% of all cases of posterior uveitis [3].

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According to [4], a lot of factors involved in the pathogenesis of *ocular toxoplasmosis*, they mentioned that *T gondii* infection increased apoptosis, nitric, IFN- γ , Fas and Fas-L, while according to [5] the damage of the eye due to *T. gondii* infection was caused by the increased expression of MHC 1 and TNF- α . The research held by Shen and Lyons used adult mice, they infected adult mice. Eye damage due to congenital toxoplasmosis has not been described. Therefore, through this research, in order to convey the occurrence of eye damage microscopically by observing the extent of histological damage.

2. MATERIALS AND METHODS

2.1. Propagation *T. gondii*

Isolates used in this study is the RH strain from the Department of Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga. Isolates were injected to intraperitoneal of healthy mice, 1×10^6 takizoit per mouse. Four days after infection, the mice were sacrificed. Intraperitoneal fluid was taken and the emergence of takizoit viewed under a microscope and the number of takizoit is measured by the haemocytometer improve Neubauer.

2.2. Mice Mating

A total of 20 pregnant mice were used in this study. In order to get the pregnant mice, 40 female mice mated with males. Each couple of mice were put in a cage. The next morning, mice were evaluated for the presence of vaginal plug and if it was found vaginal plug it means that mice were pregnant and with 0.5day gestation age [6]. Mice were maintained until the gestational age of 11.5 days.

2.3. Treatment

Twenty pregnant mice were divided into two groups which are the treatment group [I] which consists of 11,5 days pregnant mice infected by *T. gondii* and the other one the control group [II] which its are not infected. The infection dosage is 10 takizoit in 200 μ l buffer saline each mice intraperitoneally. All mice were maintained until delivery. Each of the newborn were sacrificed, their head were taken and their eye tissue were fixed in 10% of buffered formalin and the histological samples were made in HE staining in

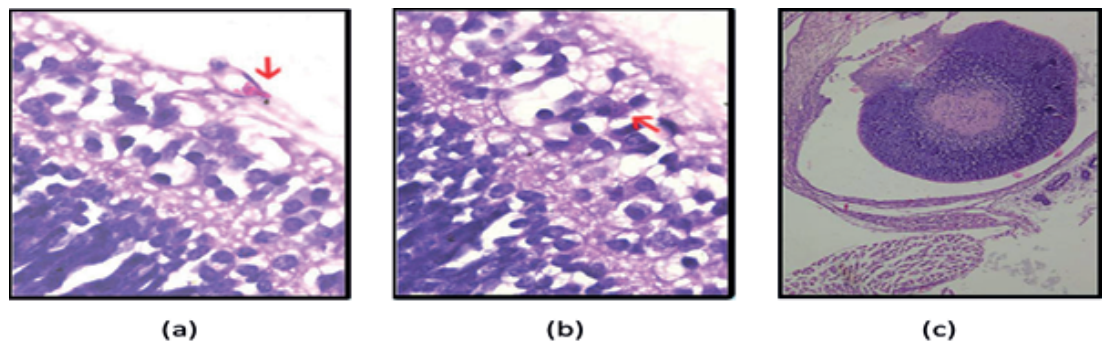


Figure 1: Retinal cell infected of *T. gondii* infected newborn mice. (a) Retinal hemorrhage, (b) infected cell, (c) stunted eye .HE staining, magnificate 1000x (a and b) and 100x (c).

TABLE 1: Mean of Apoptosis Index of Retinal Cells of Newborn Mice.

Treatment	Apoptosis Index [%]
Control	15.7 ^a ± 3.56
<i>T. gondii</i> Infection	5.6 ^b ± 1.65

^{a,b}, different superscrib in the same row shows significantly different [p<0,05].

order to reveal the level of necrotic damages and TUNEL staining to reveal the apoptotic cells. The data were analyzed by t-test [with significance level $\alpha = 0.05$].

3. RESULTS

3.1. Eye damage as the effect of *Toxoplasma gondii* infection

The focus of this observation is on the retina. On the eyes of a neonatal from *T. gondii*-infected mice were found hemorrhage in the retina of the eye [Figure 1a] and retinal cell was infected by group of tachyzoites [Figure 1b]. From all 10 newborn mice that were observed, there was one neonatal which that eyes stunted [Figure 1c].

3.2. Retinal Apoptosis

The result of this observation shows the retina of newborn mice which are infected by *T. gondii* were decreased compared with newborn from the control group mice [uninfected]. the result above means that *T. gondii* infection inhibits the apoptosis process in the retina [Tabel 1 and Figure 2].

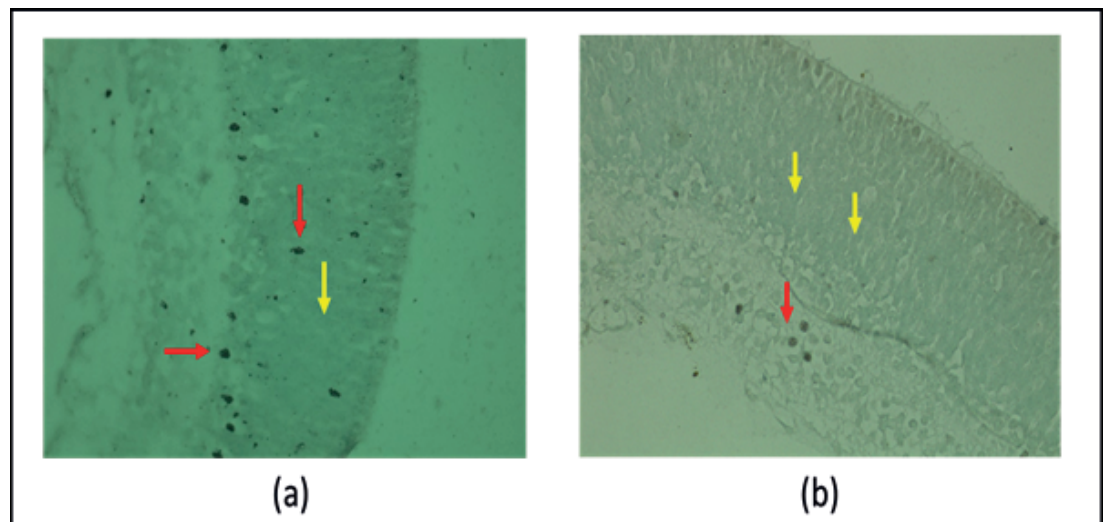


Figure 2: Retina of Newborn Mice with Tunnel staining (400x), (a) Control Group, (b) *T. gondii* Infection Group. Yellow arrows (→) are alive retinal cells and Red arrows (→) are apoptotic tetinal cells.

4. DISCUSSION

Toxoplasma gondii is a intracellular protozoan parasite that can invade and infecte all nucleated cells of birds and mammals, including humans and the disease called toxoplasmosis. The development and severity of toxoplasmosis are highly ranging from asymptomatic to cause the lymphadenopathy, encephalitis and retinochoroiditis.

Incidence of retinochoroiditis that it was due to *T. gondii* infection, reaching 30%-50% of all cases of posterior uveitis [3]. Furthermore, according to [3], that the disease happens because of various factors, including genetic and immune status of host, genetic parasites and time getting an infection [congenital or postnatal]. The first infection during pregnancy, it can be transmitted to the foetus and cause congenital infection. Although at the time of birth of the newborn mice were seen normal, sometime later the symptoms will be appeared. The infection will become active and is likely to cause retinocoroidhitis and blindness. Wallon et al. [2] only found one newborn with lesions in the eye and the lesions just appeared after infant at the age of 3.1 years and some even appear after 12 years later. According to [5] the damage of the eye due to *T. gondii* infection were caused by increasing of the expression of MHC 1 and cytokine TNF- α .

According to the result research [5], although the retinal damage of result of these research only showed mild damage, hemorrhage [Figure 1a], it is predicted that someday the damage will get worse since takizoit *T. gondii* is also found in the retina cell [Figure 1b]. Although unusual, case of hemorrhage in eye that caused congenital toxoplasmosis was also reported [7].

The apoptotic of retina cells of newborn mice which are infected by *T. gondii* were decreased compared with newborn from the control group mice [uninfected]. It means that *T. gondii* infection inhibits the apoptosis process in the retina [Tabel 1 and Figure 2]. The decreasing of apoptosis index in this research may be the explanation about the failed development of the newborn mice retina [Figure 1c] and it is different with the result of the previous research that *T. gondii* infection caused increasing apoptosis in the skull and brain of newborn mice [8, 9].

Apoptosis in eye is tightly related to the development of the eye. There are two stages of the apoptosis during the retina development [10]. The first stage is when the embryo is 12,5 days and reaching the pitch in 14,5 and 16,5 days. The second stage is when the apoptosis happens during the synaptogenesis which is two weeks after the birth. A slightly different argument expressed by [11], which is in the development of the vertebrata eye, particularly the retina, there are three stages of apoptosis periodically changes. In the case of mice, the early development of the mouse embryo ages 9-11 days, apoptosis occurs in the optic vesicle and lens placode. When the embryo reach ages 13-15 days, the second stage occurs, which is the time when the neurogenesis in the retina, where the differentiation of retinal neuron occurs in the retina. The last stage is during the synaptogenesis which occurs between embriyonic age of 17 days until the second week after delivery.

T. gondii infection in this research has been held in mice to 11, 5 days of gestation old, where it is the time when the first stage of apoptosis is happening in the eye development [11]. Further research has to be done in order to determine whether it is considered as a *T. gondii* mechanism to avoid the apoptosis. According to [12], in the case of *T. gondii* infection, apoptosis only happens to the uninfected cells while the infected ones are avoiding the apoptosis.

5. CONCLUSION

Congenital Toxoplasmosis causing the damages in newborn mice retina. The damages are infected retinal cells, hemorrhage and eye growth inhibition and decreased of apoptosis index of the retina cells.

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