Effect of hyperbaric oxigen

by Arif Rahman Nurdianto

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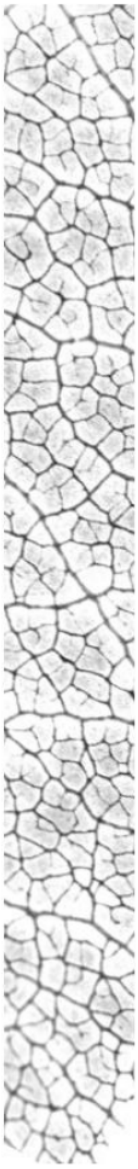

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Effect of hyperbaric oxygen therapy on ICAM-1 expression in artery spiralis of pregnant *Rattus norvegicus* infected by Tachyzoite from *Toxoplasma gondii*

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Abstract

Introduction: *Toxoplasma gondii* infection increases the production of Th1 cytokines that can cause fetal rejection. This study aims to determine the effect of hyperbaric oxygen therapy (HBOT) in the artery spiralis expressions of ICAM-1 in pregnant *Rattus norvegicus* infected by tachyzoite of *Toxoplasma gondii*.

Material and Method: The rats were divided into four groups randomly. Determination of ICAM-1 expressions was performed on day-5 after HBOT and measured through Immunohistochemistry examination.

Result: There was no significant difference in ICAM-1 expression among all groups (A and B [p <0.626], A and C [p <0.450], A and D [p <0.770], B and C (p <0.792), B and D [p <0.812], C and D [p <0.600]). However, the results showed that HBOT could not reduce ICAM-1 expression (6.4, 5.73, 5.4, 6.03, the mean ICAM-1 expression in HBO_2 treatment group was the highest).

Conclusion: This study concluded that HBOT could not reduce the expressions of ICAM-1 in artery spiralis with no significant difference among all groups.

Keywords: Toxoplasma gondii, Tachyzoite, Hyperbaric Oxygen Therapy, HBOT, ICAM-1

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INTRODUCTION

Toxoplasma gondii is a parasite obligate intracellular which has three type, tachyzoite, cyst and oocyst (Yuliawati et al. 2015) whereas associated with hygiene and sanitation as similiar with other infection such as *Helicobacter pylori* (Aftab et al. 2016, Miftahussurur et al. 2017). The infection increases the production of Th1 cytokines, such as interferon-γ (IFNγ) and interleukin-2 (IL-2) (Nurdianto et al. 2019). IFNγ is used to kill *T. gondii*, but the increase in Th1 cytokines is detrimental to the fetus because of the induction of strong Th1 cytokine responses to the fetal-maternal interface so that it can cause fetal rejection (Zhang et al. 2014). In *T. gondii* infection, IFNγ is produced by Th1, natural killer cells (NK) and cytolytic T cells, namely (CD8+ T cells). *T. gondii* infects macrophages producing Interleukin-12 (IL-12), which can activate NK cells to produce IFNγ and differentiate T helper (Th) lymphocytes into Th1 cells.

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Th1 cells produce IFNγ and IL-2 (Gigley et al. 2011, Simanjuntak et al. 2018, Yuliawati et al. 2015).

Increased tachyzoite, which infects tissues, is associated with increased IFN secretion (Ueno et al. 2014, Xu et al. 2013). Increased IFN secretion is associated with an increase in intercellular adhesion molecule-1 (ICAM-1). ICAM-1 can regulate the regulated on activation normal T cell expressed and secreted messenger RNA (RANTES mRNA) expression, which is a regulation of inflammatory cell chemotaxis (Hatibie et al. 2019). ICAM-1, and e selectin are involved in vasospasm in preeclampsia cases. In a state of pregnancy, blood vessels to the uterus are maximally dilated, but endothelial damage causes a decrease in blood flow to the uterus as a result of an increase in arterial tone leading to the uterus. The uteroplacental circulation is the circulation responsible for the delivery of nutrients and oxygen to the fetus. Normal uteroplacental circulation is very important for healthy fetal growth. An acute decrease in uteroplacental blood flow can result in threatening the survival of the fetus, while a decrease in chronic uteroplacental flow can lead to pathological processes of pregnancy, such as preeclampsia and fetal growth barriers (Frölich 2013, Leiva et al. 2011).

ICAM-1 is adhesion molecules that facilitate the migration of monocytes (Baba et al. 2017, Ueno et al. 2014). Monocytes are highly favored and predominantly infected by tachyzoite (Baba et al. 2017). Thus, it will facilitate the migration of tachyzoite infecting the placenta. In the case of chronic *T. gondii* infection, if IFNɣ is neutralized, it will cause transmission of fetomaternal tachyzoite (Abou-Bacar et al. 2004). Based on the research above, IFNɣ has a very important role in the process of eliminating *T. gondii* in the body in a hormone or optimum concentration because under excessive conditions, IFNɣ has a destructive effect and vice versa at low concentrations, which can also reduce elimination activities than *T. gondii*.

The administration of Hyperbaric Oxygen Therapy (HBOT) can increase IFNɣ without causing abortion in pregnancy that is infected by tachyzoite *T. gondii*. In addition, HBOT can reduce ICAM-1 expression through eNOS induction in vivo endothelial cells conditioned to experience hypoxia and reperfusion injury (Buras et al. 2000, Ilyas et al. 2019, Widiyanti 2011). Therefore, through this study, we tried to find out how the effect of HBOT on ICAM-1 expression in pregnant *Rattus norvegicus* infected by tachyzoite *T. gondii*.

MATERIALS AND METHODS

This was an experimental study with four groups of rats, including 37 pregnant *Rattus norvegicus*. Rats were infected after getting a vaginal plug, which means the mice had been pregnant for 0.5 days (Clark et al. 2001). Duration time from first infection to animal sacrifice was seven days. This research was performed after obtaining ethical clearance from Animal Care and Use Committee (ACUC) of Faculty of veterinary medicine, Universitas Airlangga No.777-KE.

The HBOT treatment group A was pregnant rats infected by tachyzoite receiving 10 sessions of HBOT 2.4 ATA in 3x30 minutes; the HBOT treatment group B was uninfected pregnant rats receiving 10 sessions of HBOT 2.4 ATA in 3x30 minutes; group C was pregnant and infected rats by tachyzoite but did not received HBOT; and group D was pregnant rats only without infection and not receiving HBOT. Each infected pregnant rat was given with a 103 tachyzoite of *T. gondii* via intraperitoneal injection. Examination of ICAM-1 artery spiralis expressions was performed on day-5 after HBOT (HBO twice a day). From the sacrificed rats, the uterine spiral artery was taken and sent for immunohistochemistry assay to evaluate the ICAM-1 expression (Susi Ari Kristina, Ni Putu Ayu Linda Permitasari 2019).

This histopathological examination was intended to determine the expression of myometrial ICAM-1. The ICAM-1 expression in each sample was assessed semi quantitatively according to the modified Remmele method (Nowak et al. 2007) in which the Remmele scale index (Immunoreactive Score/IRS) is the result of multiplication between the percentage of positive immunoreactive cells with color intensity scores on immunoreactive cells. Data for each sample were averaged in IRS value observed in 5 (five) different view fields at 400x magnification. All of these examinations used ordinary light microscopes from Nikon H600L brand (Nikon Corp., Tokyo, Japan), equipped by a Fi2 DS 300-megapixel digital camera (Nikon Corp., Tokyo, Japan) and Nikkon Image System image processing software (Nikon Corp., Tokyo, Japan). All immunohistochemistry were processed using ImageJ application to count the scale bar. All results from Immunohistochemistry expression of ICAM-1 myometrium were checked using One way ANOVA through SPSS 21 (IBM corp., New York, United States).

RESULTS

The results of IRS calculation of ICAM-1 expression in 5 (five) different view fields at 400x magnification from spiral artery samples that have been carried out immunohistochemical staining showed the following results. The mean ICAM-1 expression in the spiral arteries of infected rats and HBOT therapy was 6.4. The mean of ICAM-1 expression in the spiral arteries of normal pregnant rats and receiving HBOT therapy was 5.73. This result was lower than the values in Group A. The mean of ICAM-1 expression in the spiral arteries of rats infected and not receiving HBOT therapy was 5.4, and the value was lower compared to Group A and Group B. The mean of ICAM-1 expression in the spiral

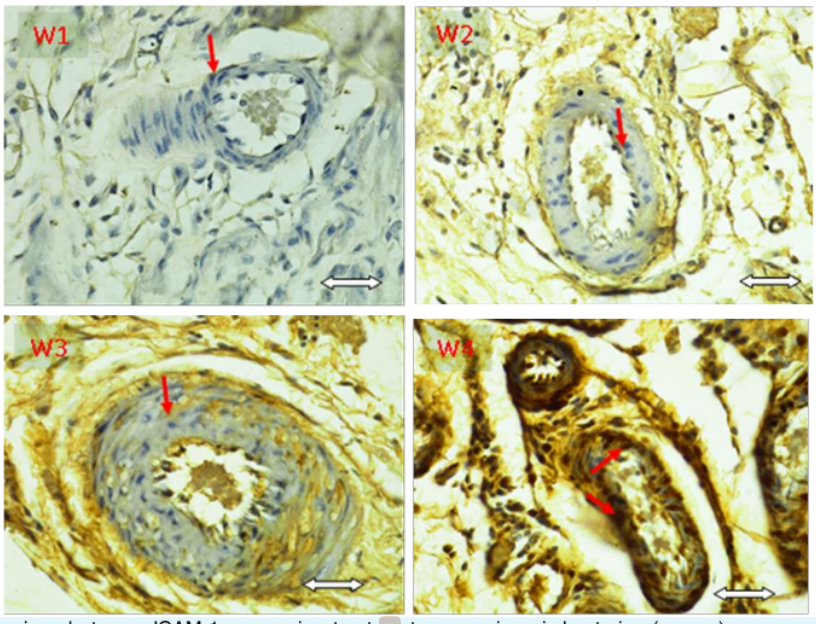

Fig. 1. Comparison between ICAM-1 expression treatment groups in spiral arteries (arrows) expressed both in the endothelial and vascular walls (immunohistochemical staining, 400x magnification; Nikon H600L microscope; 300 megapixel DS camera Fi2). White marker scale is in 1 µm with image J application

Table 1. The results of IRS calculation of ICAM-1 expression

Groups	ICAM-1 Expressions in Artery spiralis (Immunoreactive Score / IRS)
A	6.4
B	5.73
C	5.35
D	6.03

arteries of Group D rats as a normal pregnant control group without infection and without HBOT was 6.03, and that value became the benchmark (**Table 1**).

From One-Way ANOVA test, there was no significant difference from ICAM-1 expression in artery spiralis among all groups with A and B ($p < 0.626$), A and C ($p < 0.450$), A and D ($p < 0.770$). B and C ($p < 0.792$), B and D ($p < 0.812$), as well as C and D ($p < 0.600$).

DISCUSSION

The HBOT could not reduce ICAM-1 expression and there was no significant difference in ICAM-1 expression among all groups. The results of this experiment indicated that ICAM-1 spiral artery levels in Group A appeared to be higher than that in Group C. Besides, ICAM-1 expression in Group A was higher compared to those in group B and D. We postulated that such event occurred because in Group A, HBOT administration increased the IFNɣ level in the group infected by tachyzoite *T. gondii* and received HBOT, whereas in Group C, the expression of IFNɣ was lower than that in Group A (Nurdianto et al. 2019). The results of ICAM-1 expression in Group D or normal pregnant group showed higher ICAM-1 expression compared to that in Group B, indicated that administration of HBOT in cases of normal pregnancy could suppress ICAM-1 expression in spiral arteries. The unusual results were also in accordance with the experiment of administration of HBOT in normal *Rattus norvegicus*, which received HBOT, and normal pregnant mice where lower IFNɣ levels were obtained in the normal pregnant group and received HBOT therapy.

T. gondii in pregnancy can stimulate IFNɣ production excessively so that it can cause abortion in pregnancy which will be marked by hemorrhage, spiral artery dilation, hypocellularity of the decidua basalis, apoptosis of placental cells, a decline in uterine natural killer cell (NKC) numbers, increased indoleamine 2,3-dioxygenase mRNA and reduced IL-15 mRNA (Irianti et al. 2017, Senegas et al. 2009).

T. gondii inhibits IFN-γ and the encryption activity of IFN-β-Induced host cell signal transducer and activator of transcription 1 (STAT1) by increasing the association of STAT1 with DNA. Toxoplasma type II prevents the recruitment of complex chromatin remodeling containing Brahma Related Gene-1 (BRG-1) to the promoters of IFN-γ inducing secondary genes, such as Ciita and Major Histo Compatibility Complex II (MHC II) genes in macrophages, thus inhibiting its expression. Type I toxoplasma inhibits the expression of the primary IFN-γ

response genes 1 (IRF1) gene through a different mechanism that does not depend on histone deacetylase activity. In contrast, infections with type I, II, or III types of *T. gondii* inhibits the separation of STAT1 from DNA, preventing the recycling and continuation of the transcription cycle mediated by STAT1. This causes an increase in binding of STAT1 to the promoter IRF1 (Rosowski et al. 2014).

Resistance to *T. gondii* is mainly mediated by T helper 1 cell (Th1) by producing IFNɣ, whereas type 2 cytokine that is produced by Th2, such as IL4, and IL10 was associated with increased susceptibility to infection. Strong Th1 response in toxoplasmosis in pregnancy will induce abortion in early pregnancy (Nurdianto et al. 2019, Wibowo et al. 2015).

HBOT reduces CD18 function, which is directly related to the actin cytoskeleton and binding of actin proteins to neutrophils and decreased ICAM-1 expression in endothelial cells (Lavrnja et al. 2015, Uzun et al. 2010). This also applied to Group C with the same mechanism. The administration of HBOT could increase NO and high NO concentrations could cause an actin cytoskeleton disruption. Cytoskeletal relationships allows integrins (CD18) to mediate cell adhesion and regulate cell shape. Because polarization of the CD18 molecule is needed to form strong adhesion, NO can interfere CD18 function through the actin disruption of the cytoskeleton. NO mediates inhibition of the cyclic guanosine monophosphate of Polymorphonuclear leukocytes (c-GMP PMN), inhibition of membrane guanylate cyclase, or reduction of F-actin, which could contribute to cytoskeleton actin disorders and prevent CD18 polarization (Jones et al. 2010).

The ICAM-1 expression in Group A was higher than that in Group C. If we look at previous events, tachyzoite *T. gondii* infection in tissues increased along with the increase of IFNɣ (Nurdianto et al. 2019, Ueno et al. 2014, Xu et al. 2013) since increased IFNɣ could trigger the expression of ICAM I adhesion molecules that facilitated the migration of monocytes (Furtado et al. 2012, Ueno et al. 2014). Monocytes are cells that are often infected by tachyzoite *T. gondii* (Baba et al. 2017). Thus, *T. gondii Tachyzoite* has a great opportunity to infect tissues, such as spiral arteries, using monocytes as its vehicle and ICAM-1 on spiral arterial tissue as its anchor. So that, if there is excessive tachyzoite infection in the spiral arteries and cause excessive spiral artery cell death and possibly cause abortion, this did not occur in this experiment.

This possibility can occur because IFNɣ is able to provide protection against *T. gondii* infection related to STATI molecules on the JAK/STAT pathway (Hunn et al. 2010, Schneider et al. 2013). IFNɣ induces INDO formation, which made tryptophan in tachyzoite-infected cells degraded and induced increased secretion of reactive oxygen intermediates (ROI), nitric oxide (NO) and intermediate reactive nitrogen (RNI) in phagocytic cells. The degradation of tryptophan causes the replication of *T. gondii* to be inhibited (Schneider et al. 2013). In addition, IFNɣ can also induce the synthesis of IFN-γ-inducible GTP-binding protein (IGTP) and The IFN-inducible protein Irgm1 LRG-47, which can control *T. gondii* tachyzoite replication (Hunn et al. 2010).

If IFNɣ is neutralized in cases of chronic infection, it will cause transmission of fetomaternal tachyzoite *T. gondii* through the placenta (Abou-Bacar et al. 2004). In excessive levels, IFNɣ will have destructive effects so that IFNɣ is still needed in balanced concentration.

The HBOT can reduce ICAM-1 expression through endothelial NO synthase (eNOS) induction in vivo endothelial cells conditioned to experience hypoxia and reperfusion injury (Buras et al. 2000, Ilyas et al. 2019, Widiyanti 2011). This is consistent with Group B results in which administration of HBOT in a normal pregnancy caused a decrease in ICAM-1 expression compared to group D or normal pregnancy.

The HBOT can reduce apoptosis by reducing the expression of Hypoxia-inducible factor 1-alpha (HIF-1α), Protein 53 (P53) and Bcl-2 nineteen kilodalton interacting protein 3 (BNIP3). Besides, it increases the expression of B cell lymphoma 2 (Bcl2) and Caspase 3. HBOT can reduce apoptosis in mice with spinal cord injury by suppressing Apoptosis-associated speck-like protein (ASC) and Caspase-3 HBOT could also reduce ICAM-1 expression through eNOS induction in vivo studies of endothelial cells conditioned to experience hypoxia and reperfusion injury.

Death due to *T. gondii* tachyzoite infection is caused by rupture of cells due to tachyzoite, which is multiplied in cells and immunological factors. ICAM-1 is widely distributed in all cells and its expression can be regulated by cells. ICAM-1 expression can be triggered by interleukin 1 (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-gamma (IFN-γ) which increase due to Th1 activated by *T. gondii* infection. With the high ICAM-1 in the endothelial spiral arteries, it makes it easier for tachyzoite to infect cells and multiply in them and made the spiral artery cells ruptured so that the blood flow to the endometrium and others are disrupted and can cause increased risk of abortion (Hooks et al. 2008). It did not occur in Group A in which IFN-γ rats raised and ICAM-1 also raised but no abortion occurred (Nurdianto et al. 2019).

High ICAM-1 expression in spiral artery can increase the likelihood of tachyzoite-infected monocytes being able to infect spiral arteries. However, this event still needs to be investigated with further research because in another study, it was found that IFN-β could reduce ICAM-1, the chemokine (C-X-C motif) ligand 9 (CXCL9) and CXCL10 production (Hooks et al. 2008). The reason is because the reduction in CXCL9 will lower excessive cell inflammation. So, further research is needed to answer this.

CONCLUSION

Administration of HBOT 2.4 ATA in 3x30 minutes could not reduce the ICAM-1 expression in artery spiralis from rats that were infected by tachyzoite *T. gondii*. There was no significant difference from ICAM-1 expression in artery spiralis among all groups.

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