The 1st International Conference On Health, Social, Sciences, and Technology (ICoHSST 2020)

Difference of Regulatory T Cells, Interleukin 10, Interleukin 6, Interferon (IFN)Γ, and Indoleamin Dioksigenase (IDO) Levels in Women With High and Low ASA: A Research Article

1st Nani Sari Murni
Department of Biomedical Science
University of Indonesia
Jakarta, Indonesia
Email: syauqi0809@gmail.com

2nd Indra Gusti Mansur
Department of Biology
University of Indonesia
Jakarta, Indonesia
Email: mansur.indra@gmail.com

Corresponding author: syauqi0809@gmail.com

***Abstract* - Unexplained infertility can be caused by various factors, including genetic, immunological, and idiopathic. One of the immunological factors that play a role in unexplained infertility is antisperm antibodies (ASA). About 10-30% of infertile couples are caused by ASA. This study wanted to analyze differences in cellular specific immune components namely regulator T cells and humoral immune components (cytokines IL10, IL6, IFNγ, and IDO) in women with high ASA and low ASA. Samples with high and low ASA were examined ASA titres using the husband's sperm auto-agglutination test (HSAaT) method. Each group of 6 samples were analyzed, so that the total is 12 samples. Tregs were evaluated using flow cytometry with the human forkhead box P3 (FoxP3) staining kit of Biotech and Device. Serum IL10, IL6, and IFNγ was determined using an Abcam ELISA kit. Serum IDO was determined using an RnD ELISA kit. The data were analysed using the Mann-whitney tests. There are differences in the Tregs population (p = 0.004), IL10 (p = 0.002), IFNγ (p = 0.002) and IDO (p = 0.041) but there is no difference IL6 levels (p 0.240) in women with high and low ASA. High ASA affects of the Tregs population, IL10, IFNγ, and IDO but has no effect on IL6 cytokines.**

Keywords* - *ASA, Tregs, IL10, IL6, IFNγ, IDO

I. INTRODUCTION

Immunity problems that occur in infertility are due to excessive immune response. Around 186 million people in the world experience infertility and 8-12% are partners of reproductive age. Conditions that cause infertility from a wife factor are 40-55%, from a husband factor of 30-40%, a combination of a husband and wife factor of 10%, and unexplained 10-25% [1], [2]. In infertility that is not explained there are genetic, immunological and idiopathic factors [3], [4]. Immunological factors that play a role in infertility that is not explained one of the indications that arises is the presence of antisperm antibodies (ASA). It is proven that ASA is one of the causes of infertility and miscarriage [5]. About 10-30% of infertile couples are caused by ASA [2]. ASA affects fertility, before or after the fertilization process. ASA inhibits sperm movement, capacitation, fertilization, and inhibits embryo implantation [6].

Immunological factors play a role in pregnancy. Non-specific and specific immune responses can be humoral and cellular immune responses. Cell-specific immune responses are mediated by T cells, while humoral-specific immune responses are mediated by B cells. However, the two immune responses cannot be viewed separately, both of which have close collaboration [7], [8]. T cells cooperate with B cells through a CD4+ subpopulation called Helper T cells(Th). Th1 cells secreted IFNγ [9]. IFNγ is also an inducer indolamin 2,3 dioxygenase (IDO). IDO is induced by IFNγ in large numbers, especially in macrophages in inflammatory and dendritic cells so as to produce IDO with its metabolites. Metabolites work on effector T cells and regulatory T cells [10]. Cooperation between regulator T cells and IDO enzymes will induce tolerance for pregnancy [11]. Th2 cells enhance the humoral immune response by secreting a large portion of IL10. Increased IL-0 and decreased pro-inflammatory cytokines such as TNFα, IL1β and IL6 occur during pregnancy [9], [12]. The maternal immune system requires tolerance for conception to occur. It is not only the balance of T helper (Th)1/Th2 that plays a role in pregnancy, but also the regulatory T cells (Tregs) that regulate the important role in pregnancy [12]. CD4+CD25+Foxp3+ immune cells are known as regulatory T cells to regulate alloreactive Th1 cells [13]. Regulatory T cells identified by FoxP3 expression have an important role in the success of gestation and implantation [14]. The aim of this study was to analyse difference of regulatory T cells, IL10, IL6, IFNγ, and IDO levels in women with high and low ASA.

II. METHOD

This research method was observational. The study group consisted of 6 married women with ASA titres ≤1:128 and 6 married woman with ASA ≥1:262,144 who came to Sayyidah Mother and Child Hospital in Jakarta from July 2018 to April 2019 with unexplained infertility problems, total 12 married women were enrolled. The study was approved by the Research Ethics Committee of Faculty of Medicine University of Indonesia (Number: 0437/UN2.F1/ETIK/2018), and all 12 participants signed an

informed consent before enrolment. Each patient was examined for ASA titres using the husband's sperm auto-agglutination test (HSAaT) method.

III. RESULTS

The results for the 12 study group samples are shown in Table 1. Significant differences in regulatory T cells populations were found in women with high and low ASA (p 0.004), IL10 (p = 0.002), IFNγ (p = 0.002) and IDO (p = 0.041) however there is no difference IL6 levels (p 0.240). Univariate analysis illustrates that the median regulator T cell population in low ASA is higher (9%) compared with high ASA women (3.35%). Median levels of IL10 (93.51 pg/ml) in women with high ASA are higher than that of low ASA (1.45 pg/ml). Median levels of IL6 (2.25 pg/ml) in women with low ASA are higher than that of high ASA (1.51 pg/ml). Median levels of IFNγ (9.61 ng/ml) in women with low ASA are higher than that of high ASA (3.69 ng/ml). Median IDO levels (5.11 ng/ml) in women with low ASA are higher than high ASA (0.54 ng/ml).

Table 1. Difference of regulatory T cells, IL-10, IL-6, IFNγ, and IDO levels in women with high and low ASA

	Group	n	Median (min-max)	p
Regulatory T cells (%)	High ASA	6	3.35 (1.5-4.8)	0.004
	Low ASA	6	9 (4.1-22.6)	
IL-10 levels (pg/ml)	High ASA	6	93.51 (9.02-298.85)	0.002
	Low ASA	6	1.45 (0.71-2.81)	
IL-6 levels (pg/ml)	High ASA	6	1.51 (0.93-4.90)	0.240
	Low ASA	6	2.25 (1.8-5.4)	
IFN-γ levels (ng/ml)	High ASA	6	3.69 (3.50-5.62)	0.002
	Low ASA	6	9.61 (7.12-48.85)	
IDO levels (ng/ml)	High ASA	6	0.54 (0.22-41.55)	0.041
	Low ASA	6	5.11 (2.77-42.85)	

*data normality and Mann-whitney test

IV. DISCUSSION

A significant difference (p = 0.004) the regulatory T-cell population, IL10 (p = 0.002), IFNγ (p = 0.002) and IDO (p = 0.041) were found between women with high and low ASA, but no significant difference (p = 0.240) the IL6 levels was found between women with high and low ASA (Table 1). The low regulatory T cell population in women with high ASA compared to low ASA, this shows that ASA affects the development of the regulator T cell population. As we know that regulatory T cells are known to play a role in protective immune responses through increases in Th1 or Th17 responses [15]. Regulatory T cells serve as immunoregulators and induce immune tolerance. About 5–10% of CD4+ T cells are regulatory T cells that express CD25+FoxP3+(14). Regulatory T cells inhibit the proliferation and production of cytokines by CD4+ and CD8+ cells [16]., the production of immunoglobulins by B cells, the cytotoxic activity of natural killer (NK) cells and dendritic cell maturation. These responses then lead to immune tolerance [17]. However, besides cytokines (in this case, IL10), other factors, such as adipokines, pregnancy hormones and seminal fluid, also have immunoregulatory activity and influence the success of pregnancy by increasing the number and activity of regulatory T cells [18]. Univariate analysis illustrates that the median regulator T cell population in low ASA is higher (9%) compared with high ASA women (3.35%) showed that the development of regulator T cell population in women with low ASA was better than in high ASA, this supports the possibility of pregnancy for women with low ASA.

A significant difference IL10 (p = 0.002) in women with high and low ASA, median levels of IL10 (93.51 pg/ml) in women with high ASA are higher than that of low ASA (1.45 pg/ml) show that IL10 as an anti-inflammatory cytokine produced by Th2 cells will inhibits the action of pro-inflammatory cytokines such as IL1, IL6, IL12 and TNF. IL10 also inhibits APCs by inhibiting major histocompatibility complex (MHC) class II expression and inhibiting the action of co-stimulator molecules, such as CD 80 and CD 86. Biologically, IL10 binds to the IL10R1 and IL10R2 receptors that specifically initiate cascade signals. IL10 activates the Janus kinase (JAK) and STAT pathways. In a normal pregnancy, IL10 increases during trimesters I and II, but not in trimester III [19], [20], [21]. In the absence of a high serum level of IL10, regulatory T cells will decrease if ASA increases, whereas a high serum level of interleukin 10 can inhibit this increase in the regulatory T cell population, this will inhibit the occurrence of maternal tolerance for pregnancy.

No significant difference (p = 0.240) IL-6 levels was found between women with high and low ASA, median levels of IL6 (2.25 pg/ml) in women with low ASA are higher than that of high ASA (1.51 pg/ml) shows that the inflammatory response occurs in both high ASA and low ASA. Increased IL6 is often seen in cytokine profiles of unexplained infertility cases, recurrent miscarriages, preeclampsia and preterm labor. The excess of IL6 will inhibit of regulatory T cells [22]. The increase in antibodies produced by B cells as a means of self-defense against antigens also increases the signal against proinflammatory cytokines, IL6. IL6 is a key cytokine that blocks the development of regulatory T cells and induces differentiation of Th 17 cells[17]. IL6 is a proinflammatory cytokine and antibody produced by B cells and acts as an important link in several cellular reactions. A coordinated immune response involves cues between the various leukocytes and tissue cells that play a role in that response [23], [24].

A significant difference IFNγ (p = 0.002) between women with high and low ASA, median levels of IFNγ (9.61 ng/ml) in women with low ASA are higher than that of high ASA (3.69 ng/ml) shows that at the beginning of the decline in ASA there is an immune response to tolerance in the maternal body. Increased of IFNγ as a response to tolerance from high anti-inflammatory when ASA is high in maternal. The theory explains that IFNγ activates macrophages to kill foreign cells and stimulate the further development of Th1 and inhibit the development of Th2 and Th17 cells. IFNγ will increase polarization response in the Th1 subset [25]. IFNγ is one of the cytokines produced by Th1 and plays a role in failure of implantation and recurrent miscarriage [26]. Significant differences IDO (p = 0.041) in women with high and low ASA, median IDO levels (5.11 ng/ml) in women with low ASA are higher than high ASA (0.54 ng/ml) showed that similar to the response IFNγ which increased at the onset of decreased ASA in response to tolerance in the maternal body. IDO connection with IFNγ because IDO inducing compounds are IFNγ. IFNγ stimulate macrophages/

dendritic cells produce IDO with its metabolites. Metabolites work on effector T cells and regulatory T cells [27], [28], [29], [30]. IDO under IFNγ transcription control catalyzes and limits tryptophan degradation. Tryptophan is an important stimulus for effector T cell proliferation. If tryptophan is limited, apoptosis will occur [31]. IDO plays a role in maternal tolerance by controlling the presence of tryptophan in situ T cells in the uterine microenvironment [32]. Changes in tryptophan concentration and IDO activity are associated with pregnancy outcomes, including pregnancy failure and preeclampsia [33], [34]. The first functioning APC is dendritic cells. Dendritic cells are very heterogeneous and become part of stimulation that activates T cells, and tolerogenic dendritic cells express CD8α and IDO. IDO plays an important role in inducing apoptosis activated by effector T cells, such as Th1 and Th17 [14]. As previous research has found that IDO protects the fetus from the maternal immune response through two mechanisms, namely: 1) IDO reduces the availability of tryptophan as an essential amino acid. The amount of tryptophan in the maternal circulation decreases during pregnancy, and 2) IDO produces a series of kinurenin pathway metabolites that support the proliferation and differentiation of regulatory T cells [27], [28], [29], [30].

ASA stimulates cellular specific immune response and humoral immune response, this is indicated by the results of univariate analysis that regulatory T cells, IL6, IFNγ, and IDO levels are higher in women with low ASA compared to high ASA. Bivariate analysis did show significant differences between regulatory T cells, IL10, IFNγ, and IDO levels in women with high and low ASA.

V. CONCLUSION

High ASA stimulates cellular immune response, that is, regulatory T cell population, and has effect on humoral immune responses, namely cytokines IL10, IFNγ, and IDO but no effect on IL6.

VI. ACKNOWLEDGMENT

This project was supported by Beasiswa Unggulan Dosen Indonesia-Lembaga Pengelola Dana Pendidikan (BUDI-LPDP) 2016.

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