

# 18. Correlation of Hematological Parameters on Maternal and

*by* Muhammad Yulianto Listiawan

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# Correlation of Hematological Parameters on Maternal and Pediatric Leprosy Immunity Ratio: A Study in Endemic Areas in East Java, Indonesia

Muhammad Yulianto Listiawan<sup>1</sup>, Flora Ramona Sigit Prakoeswa<sup>2,3</sup>, Anang Endaryanto<sup>3</sup>,  
Cita Rosita Sigit Prakoeswa<sup>4</sup>

<sup>1</sup>Associate Professor, Department of Dermatology and Venereology, Faculty of Medicine, Airlangga University / Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, <sup>2</sup>Doctoral Candidate, Doctoral Program, Faculty of Medicine, Airlangga University, Indonesia, <sup>3</sup>Associate Professor, Department of Pediatric, Faculty of Medicine, Airlangga University Dr. Soetomo General Academic Hospital, Surabaya, Indonesia, <sup>4</sup>Professor, Department of Dermatology and Venereology, Faculty of Medicine, Airlangga University / Dr. Soetomo General Academic Hospital, Surabaya, Indonesia

## Abstract

**Background:** Leprosy is chronic infectious disease caused by *Mycobacterium leprae*. Endemic areas still existed with stable number of new cases including new cases of pediatric leprosy and female leprosy cases. Close contact and women's role in household increase the risk of transmission of leprosy to their children. Population lived in endemic areas are prone to contract leprosy due to dysregulation of immune system. This study aims to analyze correlation of hematological parameters and immunity ration in maternal and pediatric leprosy in endemic areas.

**Methods:** This is a cross-sectional study in endemic areas in Tuban Regency, East Java Province, Indonesia. The blood sample was taken from the subjects and underwent complete hematological parameter test (hemoglobin, red blood cells, white blood cells, platelets, hematocrit) and measurement of T cells activity ratio (Th1/Th2 ratio and Th17/Treg ratio) by ELISA. Correlation test was done between blood test results and the immunity ratio of maternal and pediatric leprosy in endemic areas.

**Results:** 33 pairs of maternal and pediatric leprosy cases were analyzed. This study found correlation between RBC ( $p$  value= 0.029) and HCT ( $p$  value= 0.038) with Th17/Treg ratio in children population. No significant results observed in mothers population.

**Conclusion:** The result of this study shows that hematological parameters (HB and HCT) related to the Th17/Treg ratio and could play roles in the incidence of dysregulation of immune system in children leprosy.

**Keywords:** Hemoglobin, Hematocrit, Maternal, Pediatric, Leprosy, Endemic

## Corresponding Author:

Prof. Cita Rosita Sigit Prakoeswa, MD, Sp. KK (K), FINS DV, FAADV, Department of Dermatology and Venereology, Faculty of Medicine, Airlangga University / Dr. Soetomo General Academic Hospital, Surabaya, Indonesia. Mayjend Prof. Dr. Moestopo Street, Number 47, Surabaya, Indonesia.  
Email: cita-rosita@fk.unair.ac.id

## Introduction

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* and considered as one of neglected tropical disease.<sup>1</sup> Leprosy remains to be a significant health burden in some endemic countries, namely Brazil, India, or Indonesia that accountable for 80% global leprosy cases.<sup>2</sup> Indonesia is one of the biggest endemic countries located in South East Asia.

Leprosy endemic areas in Indonesia is mainly located in the eastern part of the country. Based on Indonesia health profile on 2019, East Java province has the highest number of leprosy cases in Sumatera and Java Island with 2,940 cases.<sup>3</sup> Tuban Regency is one of leprosy endemic pocket in East Java Province with relatively stable of new cases findings in the last five years with 159 new leprosy cases in 2019.<sup>4</sup> In 2019, Indonesia had reported 19,938 cases, in which 17,439 were new cases consisted of 10,741 female leprosy cases (61.59%) and 2,009 (11.52%) of child leprosy.<sup>3</sup>

Children are believed to be the most vulnerable group to leprosy infection due to their immature immunity.<sup>5</sup> The constant number of new leprosy cases including pediatric leprosy cases in children aged below 15 years old indicates underdiagnosed cases in the community, active infection states and the failure to stop leprosy transmission.<sup>6</sup> Close household contacts with leprosy patient was amongst the contract leprosy and the position of women and their role within family increases the risk of leprosy transmission to their child.<sup>5</sup> The termination of leprosy transmission depends on several aspects such as microbiological aspects, environmental factors, and immunological aspect.<sup>7,8</sup>

A good immune system needs to be nourished from the beginning of life by improving perinatal health status of both mother and child.<sup>5</sup> Previously, four subset of T cells, namely Th1, Th2, Th17, and Treg were known to play roles in human immune response against leprosy.<sup>9</sup> The imbalance activity of these cells, called dysregulation of immune system, was associated to higher risk of leprosy infections.<sup>10-13</sup> Population lived in endemic areas become more vulnerable to contract leprosy due to dysregulation of immune system that makes leprosy transmission easier.<sup>9</sup>

Previous studies used immunity ratio to show immunity status with comparing the T cells ratio, such as Th17/Treg (IL-17/ FOXP3+) or Th1/Th2 (IFN- $\gamma$ /IL-4).<sup>14-16</sup> Hematological parameter is one of the test used to determine individual health status. In our knowledge, there is still no research conducted to study leprosy in immunity ratio, especially in mothers and children population. In this study, we would like analyze the correlation of hematological parameter with immunity

ration in maternal and pediatric leprosy cases in endemic areas.

## <sup>4</sup> Materials and Methods

### Study area and population

This cross-sectional study was conducted from March until June 2020 in 10 sub-districts in Tuban Regency, East Java Province. This regency is considered a leprosy pocket area, with 172 cases in 2018 of which 5.81% cases were cases among children. These 10 areas across 10 sub-districts (Bulu, Jenu, Jetak, Kerek, Palang, Soko, Sumurgung, Tambakboyo, Temandang, Tuban) are considered endemic areas of leprosy, where in the last 5 years there are always new cases every year.

Subjects were selected from the local primary health center's registry data. The inclusion criteria for subject with leprosy was those with confirmed diagnosis of leprosy and aged between 5-18 years old for children; whilst the excluded were those with any leprosy reaction, poor general condition, and diagnosed with inflammatory or autoimmune disorder, allergy, or infection other than leprosy, and pregnancy. All of the subjects were given informed consent. Thereafter, to confirm the diagnosis, the subjects underwent clinical examination done by a dermatologist and then acid-fast staining by trained health and laboratory professional from Dr Soetomo General Hospital and Tropical Disease Centre of Airlangga University.

### Data collection

Blood sample was taken from the subjects and underwent complete blood count including haemoglobin (HB), red blood cells (RBC), white blood cells (WBC), platelets (PLT), haematocrit (HCT). The equipment used to collect data were tourniquet, 3 mL syringe, vacutainer tube, alcohol swab, and adhesive plaster. Samples were collected in EDTA tube for haematological parameter. Haematological parameter were measured using Sysmex XN haematology analyser.

<sup>13</sup> Blood sample also used to measure levels of IFN- $\gamma$ , IL-4, IL-17, FOXP3+ in the blood circulation using the enzyme-linked immunosorbent assay (ELISA) method. For ELISA, blood was collected in sterile test tubes and centrifuged for 15 min at 50g. Serum was

separated and kept at -80°C until used for estimation of IFN-γ, IL-4, IL-17, FOXP3+, by the Human IFN-γ BioAssay ELISA kit, Human IL-4 R&D ELISA Kit, Human IL-17 Quantikine LSbio ELISA Kit and Human FOXP3+ BioAssay ELISA kit manufacture guideline, respectively.

**Data analysis**

Data were analyzed using SPSS® software (IBM Corp., Armonk, New York, USA). Variables were analyzed using correlation test to assess the association between hematological parameter and leprosy in each mother and child populations.

**Ethical considerations**

The study protocol has been approved by the Health

Research Committee of Dr Soetomo General Hospital, Surabaya (Ref. 1664/KEPK/XI/2019). Subjects were only included after written informed consent was obtained and they were reassured that non-participation would not affect their treatment.

**Results and Discussion**

Information was obtained from 33 pairs of maternal-pediatric leprosy cases in endemic areas. The data characteristics in pediatric leprosy group showed mean age of all children participants is 13.70 (SD ± 4.613) and mean age in maternal leprosy group is 42.64 (SD ± 8.721). The result of hematological complete test and correlation analysis are shown in Table 1.

**Table 1. Hematological complete test and correlation analysis results.**

Parameters	Mean ± SD		p value			
			Mothers		Children	
	Mothers	Children	Th1/Th2	Th17/Treg	Th1/Th2	Th17/Treg
Hemoglobin (Hb) (g/dl)	13.01 ± 1.24	13.23 ± 2.01	0.846	0.296	0.268	0.064
Red Blood Cells (RBC) (x 1012/µl)	4.63 ± 0.51	4.98 ± 0.62	0.873	0.473	0.147	0.029
White Blood Cells (WBC) (x 109/µl)	8.32 ± 2.58	7.78 ± 2.01	0.871	0.778	0.104	0.521
Platelets (PLT) (x 109/µl)	313.88 ± 72.02	343.73 ± 88.65	0.758	0.614	0.596	0.083
Hematocrit (HCT) (%)	39.21 ± 3.75	39.72 ± 5.54	0.697	0.239	0.132	0.038

From the hematological results, all parameters showed normal value in all participants. The correlation analysis amongst maternal leprosy cases showed no significant result meanwhile significant results observed in RBC and HCT parameters with Th17/Treg ratio amongst pediatric leprosy cases. Th17 cells is one of the recently identified effector T cells and act as pro-inflammatory cells. Th17 cells has protective roles against leprosy due to antibacterial activity and produced inducible Nitric Oxide Synthase (iNOS) to kill *M.leprae* with the help

of reactive oxygen species.<sup>13</sup> Meanwhile, Treg cells has been known to keep the homeostatic between Th1/Th2 cells.<sup>13</sup> Treg cells regulates inflammation activity by inhibiting the activation of effector T cells such as Th17. Recent study shows Treg cells with transcription factor FOXP3+ in the nucleus involved in downregulation of immune response.<sup>17</sup>

The significant results in RBC (p value= 0.029) parameters in children population is in accordance

with previous study that stated the decrease in the RBC amongst leprosy patient could be caused by the high levels of proinflammatory cytokines such as IFN- $\gamma$  and TNF- $\alpha$ . These proinflammatory cytokines have the capability to inhibit erythropoiesis by inhibits erythropoietin receptors leads to proliferation inhibition of erythroid progenitors or induce nitric oxide production that lead to apoptosis of erythroid cell.<sup>18</sup> This findings also correlates with previous study stated that RBC has been known as reservoir of 10 pro-inflammatory cytokines such as, IL-17, IFN- $\gamma$ , IL-1 and play roles in chemokine signaling and inflammation.<sup>19</sup>

The significant results observed in HCT ( $p$  value= 0.038) and Th17/Treg ratio in children population associated with previous study by Dani et al. that reported an increased in HCT after blood transfusion (RBC) in neonates followed by increased level of several cytokines, such as IL - 1 $\beta$ , IL - 8, IFN -  $\gamma$ , IL - 17, MCP - 1, IP - 10, dan ICAM - 1.<sup>20</sup> The insignificant results showed in mother population could be caused by the susceptibility of pediatric leprosy subjects in endemic areas to develop immune dysregulation since the beginning of life. Endemic areas are associated with poor living standard, nutrient deficiency, and poor environmental conditions.<sup>21</sup> In addition, cytokine level of child is affected by their mother cytokine level during pregnancy and breastfeeding time and environmental factors.<sup>22</sup>

The present study has limitation in terms of the type of study, the number of subjects within group, and the methods use for measurement of the parameters. Further studied need to be conducted to confirmed our findings and analyze other factors that correlates with immunity ration in leprosy cases.

### Conclusion

In conclusion, the results of this study showed the correlation of hematological parameters (RBC and HCT) with ) related to Th17/Treg ratio and could play roles in the incidence of dysregulation of immune system in children leprosy. No significant results observed in mothers population. Further studies need to be done to confirmed these findings.

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**Ethical Clearance:** <sup>23</sup> The study protocol has been approved by the Health Research Committee of Dr Soetomo General Hospital, Surabaya (Ref. 1664/KEPK/XI/2019).

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**Conflict of Interest Statement:** Nil.

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