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Research Article

Immune Profile (Th1, Th2, Th17, T-reg) of Maternal-Paediatrics Population in Leprosy Endemic Areas in East Java, Indonesia: A Cross-Sectional Study

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I N F O

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A B S T R A C T

Background: Leprosy is one of the neglected tropical infectious diseases in developing countries caused by Mycobacterium leprae. Various morbidity and stigma associated with leprosy infection which affects women more than men have led to its late diagnosis and treatment. Gender status, the role of a person in the household, and parenting are some of the factors that greatly influence the transmission of Mycobacterium leprae to children. This increase in the number of cases will also affect the number of new cases of leprosy in children.

Aim: To analyse the immune profile in the maternal-children population in leprosy endemic areas in East Java, Indonesia.

Method: We investigated the activities of four subsets of T cells, Th1, Th2, Th17, and Treg by measuring the circulated cytokines (IFN-γ for Th1, IL-4 for Th2, IL-17 for Th17) or marker levels (FOXP3+ for Treg) by using ELISA.

Results: The comparison analysis of this study showed a significant difference in FOXP3+ level of maternal leprosy compared with a healthy maternal population and IL-17 level of children leprosy compared with a healthy children population. A negative correlation was found between maternal FOXP3+ levels and children IL-17 levels.

Conclusion: The immune profile of the maternal-paediatrics population could be beneficial in planning an intervention to eradicate leprosy.

Keywords: Leprosy, Immune, Maternal, Paediatric, Endemic

Introduction

The acid-resistant bacteria Mycobacterium leprae causes leprosy, a chronic infectious disease.[1] Clinical manifestations of leprosy can affect several organs in the body, especially the nerves and skin. Leprosy is still considered a mistreated tropical infectious illness because of the severe stigma associated with it. Due to multiple factors that contribute to leprosy transmission, including immunological factors, the unfinished eradication programme of leprosy has left several endemic locations.[2]

According to the most recent World Health Organization (WHO) report from 2020, there were 177,175 leprosy cases worldwide, with a prevalence rate of 22.7 per million people.[3] India had the highestnumber of new leprosy cases (114,451), followed by Brazil with 27,863 cases and Indonesia with 17,439 cases. The number of new female leprosy cases reached 6,698 (38.41%), with 2,009 (11.52%) cases being paediatric cases in Indonesia.[3] East Java province had the highestprevalence of leprosy cases among Java and Sumatera Island provinces in 2019, with 2,940 patients, and the number of new female leprosy cases having reached 1,150 (39.12%) and new paediatric leprosy cases having reached 202 (6.87%). East Java features nine leprosy-endemic cities/ districts, such as Tuban Regency, where new cases are recorded on a regular basis.[4]

The presence of endemic areas with a consistent number of new cases reported could indicate a failure to stop Mycobacterium leprae transmission.[5] Due to immune system disruption, people living in leprosy-endemic areas become more vulnerable to the diseases. Despite the fact that worldwide and national data show that male patients outnumber females, prior research in Indonesia has shown that female patients are more affected by the stigma and discrimination associated with leprosy sickness.[3,6] Due to the dominant role of women, particularly mothers, in taking care of their families, their health state at reproductive age may have an impact on the health of family and house members. House activities (such as cooking, laundry, and dishwashing) and intimate contact with children and other family members enhance the risk of females with leprosy transmitting the disease to other members of the household, particularly their children. Furthermore, in developing nations, females are more likely to receive late health treatment in health care facilities for any health issues.[7] Because of their immature immune systems, children are considered prone to leprosy infection.[8] As a result, a steady number of paediatric leprosy cases could point to undiagnosed cases in the community, active infection states, and inability to cease transmission.[8]

The immune system of children must be nurtured gradually from perinatal life onwards by increasing both the mother's and the child's health.[9] The immune system's reaction to leprosy was investigated and shown to include both innate and adaptive immunological responses.[10] Four subsets of T cells have been identified to play roles in the immune response against leprosy in adaptive immunity, including Th1, Th2, Treg, and Th17 cells.[11] Th1 and Th2 have been linked to the immune system's ability to fight leprosy. The discrepancy in leprosy clinical manifestation is due to an imbalance and deficiency in Th1 and Th2 responses. Furthermore, new research has clarified the existence of other T cell subsets, such as Treg and Th17.[12-14]

A robust homeostasis system between effector and regulator T cells is required for a successful immune response against leprosy. Treg cells have long been thought to play a role in balancing Th1 and Th2 immune responses. Treg cells containing FOXP3+ transcription factor in the nucleus play a role in immune response downregulation, and greater expression of this transcription factor leads to severe clinical manifestations such as Borderline (BL)/ Lepromatous Leprosy (LL).[15] Th17 cells, on the other hand, produce inducible Nitric Oxide Synthase (iNOS), which kills Mycobacterium leprae and acts as a pro-inflammatory cell that protects against leprosy infection.[16] Increased Th17 cell numbers have also been linked to improved clinical symptoms such as paucibacillary (PB)/ tuberculoid tuberculoma (TT) type tuberculoma.[13,14]

Although research and reports about immunity against leprosy are available, there hasn't been any earlier study focused on immune profile in maternal and children populations in leprosy endemic populations. A previous study that analysed the maternal and child cytokine relationship stated that the development of immune responses is influenced by environmental and genetic factors interaction.[17] Thus, this study aimed to be the first to analyse the maternal and children cytokine relationship in leprosy endemic and non-endemic populations.

Method

Research Design and Population

A cross-sectional study was undertaken in leprosy endemic villages in Tuban Regency, East Java Province in March 2020. Tuban Regency has a high incidence of leprosy, with 173 cases, or 5.09% of the total cases of leprosy in East Java, and 5.81% of paediatric leprosy patients, according to the 2019 East Province Health Profile Data.[4]

The subjects of this study were selected from the local primary health centre's registry data. The total sample size was 66 (33 mothers and 33 children). The inclusion criteria for the subject with leprosy were those with a confirmed diagnosis of leprosy and aged between 20-49 years for productive age/ child bearing females and 5-18 years old for children; whilst the excluded were those with any leprosy reaction, poor general condition, and diagnosed with an inflammatory or autoimmune disorder,

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allergy, or infection other than leprosy, and pregnancy. We chose children with age > 5 years because we considered the duration of the leprosy incubation period. According to WHO 2020, leprosy is an infectious disease caused by a slow-growing bacillus, Mycobacterium leprae. On average, the incubation period of the disease is 5 years but symptoms can appear within one year. It can also take as long as 20 years or even more to happen. Based on this, among the paediatric populations in this study, paediatric patients aged > 5 years were selected to match the incubation period so as not to produce false-negative data.[3]

Data Collection

Blood samples were taken and the levels of IFN- γ, IL-4, IL-17, and FOXP3+ in the blood circulation were measured using the enzyme-linked immuno sorbent assay (ELISA) method. Blood samples were obtained from both patients and healthy volunteers. Blood was taken in sterile test tubes and centrifuged at 50 gm for 15 minutes for ELISA. The Bioassay Human IFN- γ ELISA Kit, RnD Human IL-4 ELISA Kit, LSBio Human IL-17 Quantikinine ELISA Kit, and Bioassay Human FOXP3+ Elisa Kit manufacture guidelines were utilised to separate serum and store it at -80°C until used to estimate IFN- γ, IL-4, IL-17, and FOXP3+ levels, respectively.

Statistical Analysis

Microsoft Excel spread sheets were used to process the data, while IBM SPSS version 21 software was used to analyse it (IBM Corp, Armonk, NY, USA). The data were presented using descriptive statistics and cross-tabulation. The Manova test was used to make comparisons between groups. The results were also validated using the Pearson correlation test. Statistical significance was defined as a p-value of < 0.05.

Ethical Considerations

The Health Research Committee of Dr Soetomo General Hospital in Surabaya granted permission and accepted the study protocol (Ref. 1664/KEPK/XI/2019). Participants were only included after they gave written informed consent and were assured that their treatment would not be affected if they did not participate.

Results

The study was undertaken in Tuban Regency, East Java Province in March 2020. In total, 33 pairs of maternal-paediatric leprosy cases in endemic locations were collected. The data characteristics in paediatric group showed the average age of all children participants was 13.70 years (SD ± 4.613) and in maternal group, it was 42.64 (SD ± 8.721). The results of cytokines level and comparison analysis are shown in Table 1 and the results of correlation analysis are shown in Table 2.

Table 1.Cytokines Level and Comparison Analysis Results

Cytokine Levels (Mean ± SD)									
	n (%)	IFN-γ (ng/mL)	P	IL-4 (ng/mL)	P	FOXP3 + (ng/mL)	P	IL-17 (pg/mL)	P
Children Leprosy in Endemic Areas	12 (36)	35.93 ±25.56	0.983	102.65 ± 61.27	0.674	5.34 ± 2.71	0.936	15.98 ± 2.86	0.031
Healthy Children in Endemic Areas	21 (64)	36.15 ± 20.71		92.55 ± 53.52		5.44 ± 2.60		18.15 ± 2.60	
Maternal Leprosy in Endemic Areas	12 (36)	31.50 ± 13.31	0.123	89.71 ± 44.32	0.271	4.28 ± 2.87	0.43	16.86 ± 3.47	0.734
Healthy Maternal in Endemic Areas	21 (64)	47.66 ± 40.13		116.23± 81.70		6.74 ± 4.01		16.52 ± 2.57	

Table 2.Correlation Analysis Results

		IFN-γ (ng/mL) Children	IL-4 (ng/mL) Children	IL-17 (pg/mL) Children	FOXP3+ (ng/mL) Children
IFN-γ Maternal	R	0.022	0.029	-0.068	-0.042
	P	0.894	0.857	0.673	0.794
IL-4 Maternal	R	0.043	0.042	-0.021	-0.051
	P	0.788	0.793	0.895	0.752
IL-17 Maternal	R	0.156	0.161	0.230	-0.303
	P	0.330	0.316	0.149	0.054
FOXP3+ Maternal	R	0.162	0.145	-0.416	0.213
	P	0.313	0.367	0.007	0.180

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Discussion

According to the results of this study, we found a significant difference in IL-17 levels between children leprosy in endemic areas and healthy children in endemic areas (p = 0.031) where higher IL-17 levels were observed in the healthy children populations. These findings are associated with the protective properties of Th17 cells which have been reported to have better clinical outcomes for leprosy.[18] Th17 response improved the barrier function of mucosa during infection and produced antimicrobial peptides and chemokines to increase neutrophil performance.[19]

In maternal populations, a significant difference is shown in FOXP3+ levels between infected and healthy mothers in endemic areas (p = 0.043). Based on our study, maternal leprosy group showed lower FOXP3+ than healthy mothers in endemic areas. These results are associated with a previous study that stated reciprocal relation between Th17 and Treg FOXP3+.[14,20] In this study, a higher IL-17 level with lower FOXP3+ level was shown in the maternal leprosy group. Higher IL-17 level is associated with inflammatory activities that occur during infection in the maternal leprosy group. IL-17 induced various proinflammatory cytokines such as TNF-α and IL-6 to produce reactive oxygen species to eliminate Mycobacterium leprae.[16] T-reg has been known to be responsible for host immune suppression by inhibiting the activation of Th1 and Th17 as effector T cells to prevent further damage to the tissue injury and activating, proliferating, and recruiting more Treg cells at the injury site.[16] Meanwhile higher level of FOXP3+ observed in the healthy maternal group could indicate the dysregulation of immune system and susceptibility to leprosy infection due to immune suppression performed by Treg FOXP3+ (Figure 1).

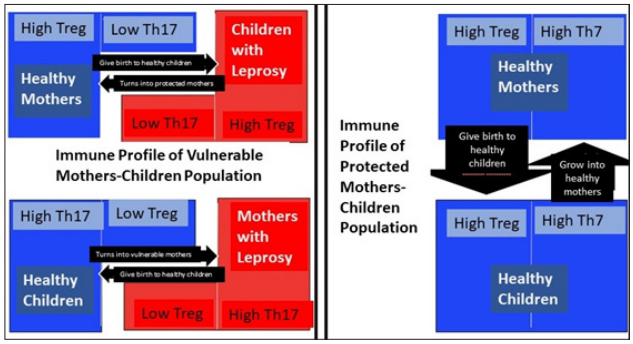

Figure 1.Reciprocal Relation of Treg and Th17 in Maternal and Paediatrics Population

Correlation test showed negative correlation between maternal FOXP3+ levels and children IL-17 levels (p = 0.007, R = -0.416). This finding strengthens the results of a previous study done by Djuardi et al. that stated that maternal cytokine, especially during pregnancy, could act as an environmental factor that influences a child's immune development.[17] This study reported that the ability of an infant's immune cells to produce cytokine is influenced by maternal cytokines and how the maternal-child cytokine relationships become weaker over time. The similarity of an immune profile is only reported in the first 2 months of a child's life.[17] In this study, Treg immune defects in the maternal leprosy group caused an increase in Th17 levels which would actually have a protective effect on the health status of their children. In other words, reciprocal relation between Th17 and Treg could be observed in the maternal-child immune profile that leads to protective effects against leprosy from mothers with leprosy to their children (Figures 2 and 3). This dysregulation of Treg and Th17 with their reciprocal relationship indicates the need of balance and protection in populations to eradicate leprosy. Thus, a different intervention is needed in the healthy maternal group in endemic areas since high FOXP3+ could cause lower IL-17 in their children and may inhibit protective activities against leprosy infection in their children. Further research is needed to confirm and analyse the cytokine patterns in other areas.

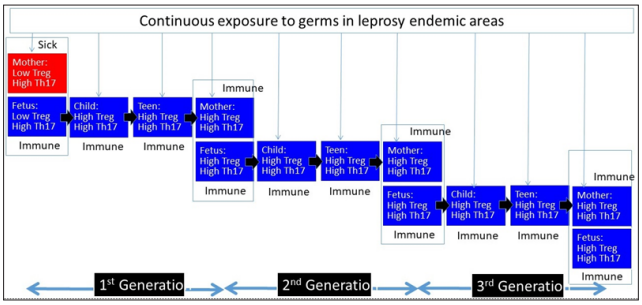

Figure 2.Dysregulation of Treg in Maternal Population and the Effect on their Children

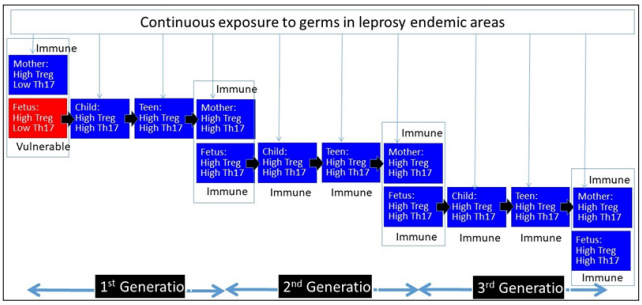

Figure 3.Dysregulation of Th17 in Maternal Population and the Effect ontheir Children

Conclusion

Healthy maternal women in endemic areas showed higher levels of FOXP3+. Moreover, children with leprosy in endemic areas showed higher levels of IL-17, which indicates an active inflammatory process. A reciprocal relationship between the immune profile in maternal FOXP3+ levels and children's IL-17 levels was observed in leprosy endemic areas. This immune profile could be used

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to determine intervention for the maternal and children population. Further research is needed to study the pattern of maternal-child immune profile in other endemic areas.

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Data Availability Statement

The Microsoft Excel data used for this study are available with the corresponding author and can be obtained upon request.

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Conflict of Interest Statement: None

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