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Effect of Nigella Sativa on IL-10 in MB Leprosy that Received MDT-WHO Therapy

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ABSTRACT

Background: Leprosy, a chronic infectious disease presents a broad clinical spectrum that is correlated with the immunological response of the patient, mainly related to Th1/Th2 cells. IL-10 is a major cytokine produced by Th2 cells inhibits imunostimulatory cytokine produced by Th1 cells. Suppressive effects of IL-10 on monocytes and cytokine synthesis by Th1 cells presumably because IL-10 has a general suppressive effect on immune function. Nigella sativa has a potent potentiating effect on cellular immunity through suppression of Th2 cells and IL-10, resulting in potentiation of Th1 cells.

Method: The study design is a randomized pretest and posttest controlled design involving 40 subjects of Multibassiler leprosy patients. Serum levels of IL-10 were measured by ELISA.

Result: The mean decrease in serum levels of IL-10 (IL-10 delta) in the treatment group (average fell 3.12 pg/ml) is greater than the control group (average rose 0.21 pg/ml), where the difference is statistically significant (p = 0.029). Nigella sativa giving significant correlation with a decrease in IL-10 compared to the control group (p=0.044, OR: 10.23).

Conclusion: Supplementation of Nigella sativa 1000 mg three times daily for 2 months in patients with MB leprosy can reduce levels of IL-10, thus increasing the cellular immune response in patients with MB leprosy.

Keywords: MB Leprosy, Nigella sativa, IL-10.

INTRODUCTION

Leprosy is a spectrum of clinical manifestations associated with immune response to pathogens, leprosy provide exceptional knowledge in immune regulation in humans. On one side of the spectrum, patients with tuberculoid leprosy illustrates the resistance response that limits the growth of pathogens. At the end of the spectrum, there are patients with lepromatous leprosy excessive vulnerability to infection with M. leprae. This paradox can be explained well to the pattern of cytokines in lesions. Th1 cytokines, particularly IL-2 and IFN-γ, more strongly expressed in tuberculoid lesions. In contrast, Th2 cytokines, such as IL-4, IL-5 and IL-10, in lesions typical lepromatous.[1-3]

One of the most important cytokine produced by Th2 is IL-10, that inhibits cytokine synthesis produced by Th1 cells. Suppressive effect of IL-10 in monocytes

and cytokine synthesis by Th1 cells presumably because IL-10 has a general suppressive effect on immune function.[3,4]

Some research suggests that some of the content of Nigella sativa has a potent potentiating effect on cellular immunity through potentiation of Th1 and Th2 cell suppression, while some others have a content of suppressor effects on B cell-mediated immunity (humoral).[5,6] Because IL-10 is a Th2 cytokine which has downregulation effect on Th1 cell and potentiating effect on B cells, IL-10 are not directly affected by the administration of Nigella sativa. Previous studies also mentioned that giving Nigella Sativa for 30 days would increase the levels of IL-4 and IFN-γ, in contrast, decreased levels of IL-10. Explained that the mechanisms that act is imunomodulatorik effect on the balance of Th1 and Th2 cells. Th1 type cells that secrete IL-2, IFN-γ and TNF-α, whereas Th2 cells secrete IL-4, IL-5 and IL-10.[5-7].

MATERIALS AND METHODS

This research is true experimental design with randomized clinical pre and posttest control design, where researchers provide treatment for the provision of Nigella sativa 1000 mg three times daily for 2 months on the type of MB leprosy patients were treated with Multidrug Therapy World Health Organization (MDT-WHO). Research began soon after the ethical clearance issued by the ethics committee University of Diponegoro.

We collected leprosy patient at Donorojo Hospital Jepara, Jawa Tengah from August until November 2015. Before start of the study, the research subjects are given information and explanations in detail about what will be done during research. Subjects who are willing to participate in the study were asked to sign a consent form that has been provided. Blood samples were taken from the mediana cubiti vein as many as 3 cc pretreatment, and post treatment (after 2 months) we take blood samples again as many as 3 cc. The tools is ELISA *Quantikinine* (kit produce from Minneapolis, MN 55413 USA for human IL-10). ELISA *Quantikinine* kit for human IL-10 is an examination for the human IL-10 in serum, plasma, cell culture, supernatans and urine. Researcher use blood serum in this study. The examination using antibodies specific for human IL-10 at a wavelength of 450 nm. Selection of this design is aimed to look at the differences in the dependent variable start of the study, differences in the dependent variable end of the study, the difference between the dependent variable and the beginning of the last study, the difference between a dependent variable of control (giving placebo, contain saccharum lactis that packed in capsules as same as Nigella sativa) and treatment groups (giving seed extract of Nigella sativa packed in capsules), and the difference of delta on dependent variable. With randomization technique, researchers can allocate the sample into two groups based on predetermined criterion then followed forward. Randomization techniques aimed at creating same characteristics of 2 group in the study.

Table 1. Characteristics of the Study Subjects

Variables		Treatment group			Control group			p
		N(%)	Mean±SD	Median (Min-max)	N(%)	Mean±SD	Median (Min-max)	
Gender	Man	14(70.0)			15(75.0)			0.723[a]
	Woman	6(30.0)			5(25.0)			
Age (years)			36±13.0	35(16-62)		45±12.0	47(25-64)	0.037[b]
Education	No school	3(15.0)			3(15.0)			0.158[c]
	Primary school	11(55.0)			12(60.0)			
	Junior high school	4(20.0)			2(10.0)			
	Senior high school	2(10.0)			3(15.0)			
BMI			19.8±3.60			19.8±2.33		0.982[b]
	Underweight	6(30.0)			6(30.0)			0.158[c]
	Normal weight	10(50.0)			12(60.0)			
	Overweight	4(20.0)			1(5.0)			
	obese	0(0.0)			1(5.0)			
Family History	Leprae	18(90.0)			17(85.0)			1.000[e]
	No history	2(10.0)			3(15.0)			

Note: *a. Chi Square; b. t Independent; c. Kolmogorov Smirnov; d. Mann Whitney; e. Fischer Exact*

Then, this design also allows the researchers conducted a double-blind, where the researcher nor the respondent does not know the status of the respondent if included in the intervention or non-intervention group. The strength of this design can minimize confounding factors that can lead to bias in the results.

RESULT

This study managed 40 subjects who were diagnosed as leprosy MB type, consisting of 20 patients with leprosy MB type treated with MDT-WHO and Nigella sativa as supplement 3000 mg/day and 20 patients with leprosy MB type treated with MDT-WHO alone. Blood samples were taken pretreatment and 2 months post treatment in both groups. The sample analyzed and titer determination of the cytokine IL- 10 using ELISA technique. In the treatment group (Nigella sativa) majority sex is male (70 %), with a mean age of 36 years old, the majority has been graduated to primary school (55 %), a normal BMI (50 %), the majority have a family history leprosy 90 %. While in the control group (placebo) majority sex is male (75 %), with a mean age of 45 years old, the majority has been graduated to primary school (60 %), a normal BMI (60 %), the majority have a family history of leprosy 85 %. (Table 1).

Table 2. Correlation between Age with Serum Level of IL-10

		Changes in Levels of Serum IL-10
Age	*Correlation Coefficient*	-0,262
	p	0,103[a]
	N	40

Korelasi *Spearman*

From Table 1 shows that the mean age between the two different groups, so it needs to be further analyzed whether the variable age is also associated with changes in serum levels of IL-10.

From table 2 above can be seen that age is negatively correlated with changes in serum levels of IL-10, which means that when you get older the levels of serum IL-10 will be increased so that the changes fewer. However, this correlation was not statistically significant (p=0,103).

The average levels of pretreatment IL-10 in Nigella sativa group was 5.73 ± 2.57 pg/ml with a median of 5.24 (2.84 to 13.61) pg/ml and in the control group (by placebo), the average was 5.35 ± 1.94 pg/ml with a median of 5.24 (2.84 to 10.10) pg/ml, was not significantly different (p = 0.709) between two groups (Table 3). .

Table 3. The Average Levels of Pre and Post Treatment IL-10

Group		N	Mean±SD (pg/ml)	Median	Min-Max	p
NigellaSativa	Pre-treatment IL-10	20	5.73±2.57	5.24	2.84-13.61	<0.0001
	Post-treatment IL10	20	2.61±2.20	1.96	0.45-10.10	
Control	Pre-treatment IL-10	20	5.35±1.94	5.24	2.84-10.10	0.709
	Post-treatment IL10	20	5.56±4.70	3.77	0.45-14.83	

Note: *Wilcoxon, p < 0.05 is significant*

The mean levels of serum IL-10 before treatment and after giving Nigella sativa was significantly different (p < 0.0001), where the average before treatment was 5.73 pg/ml greater than after treatment 3.62 pg/ml. While in the control group before treatment, mean was 5.35 pg/ml less than after treatment was 5.56 pg/ml, where this difference was not statistically significant (p=0.709). The mean reduction in serum levels of IL - 10 in Nigella sativa group (average fell 3.12 pg/ml) is greater than the control group (average rose 0.21 pg/ml), where the difference was statistically significant (p=0.029). (Table 4).

Table 4. The Average Difference of Pre and Post Treatment IL-10 in Control and Nigella Sativa Group

	N	Mean±SD	Average difference	Median	Min-Max	p
Nigella Sativa	20	3.12±2.22	3.34	2.96	-1.98-7.26	0.029[a]
Control	20	-0.21±4.94		1.33	-11.06-5.20	

Note: *Mann Whitney, p < 0.05 is significant*

Statistical analysis obtain significant correlation giving Nigella sativa with a decrease in IL-10 in which the treatment group had a decreased likelihood 10 times greater than the control group (p=0.044, OR: 10.23). (Table 5)

Table 5. Effect of nigella sativa to decreased level of IL-10

		difference					
		increase	decrease	Total	p	OR	Confidence interval 95%
Group	Treatment	19	1	20	0.044	10.23	1.12-93.34
		95.0%	5.0%	100.0%			
	Control	13	7	20			
		65.0%	35.0%	100.0%			
Total		32	8	40			
		80.0%	20.0%	100.0%			

Note: *Chi square*

DISCUSSION

The study characteristics includes gender, age, level of education, BMI, and family history, only variables of age shows significant differences between the two groups. However, after further testing, the age variable is found not to contribute to reduced levels of IL-10 . So it can be said this study had good internal validity. This study has been controlled using study methodology design through randomization, restriction through inclusion and exclusion criteria, and data analysis process, so it was assumed to be distributed evenly in each.

This study prove that the administration of Nigella sativa 1000 mg three times daily for 2 months in patients with leprosy MB type can reduce levels of IL-10, the mean serum levels of IL-10 early and late in treatment group was statistically significant ($p < 0.0001$), where the average before treatment of 5.73 pg/ml greater than after treatment of 3.62 pg/ml. The mean decrease in serum levels of IL-10 (IL-10 delta) in the treatment group (average fell 3.12 pg / ml) is greater than the control group (average rose 0.21 pg/ ml), where the difference is statistically significant ($p = 0.029$). The treatment group had a decreased likelihood 10 times greater than the control group ($p = 0.044$, OR : 10.23).

IL-10 is a Th2 cytokine which has the effect of down regulation of the Th1 cell response. Nigella sativa supplementation would decrease the production of IL-10 by Th2. So that is expected to increase cellular immunity in patients with leprosy who receive MDT treatment that will ultimately improve the effectiveness of treatment.

Several study had noted that the administration of Nigella Sativa would increase the levels of IFN-γ, in contrast, decreased levels of IL-10.[5-7] Indicate that nigella sativa has inhibitory effects on Th2 cells and its cytokine as well as the effect of stimulation of the Th1 and its cytokine.

Explained that the mechanisms that act is immunomodulatory effect on the balance of Th1 and Th2 cells. Th1 type cells that secrete IL-2, IFN-γ and TNF-α, whereas Th2 cells secrete IL-4 , IL-5 and IL-10. The balance between Th1 and Th2 cytokines play an important role to determine the direction of the inflammatory response towards a cellular response form or humoral.[8-11]

Nigella sativa extract at a dose of 1000 mg three times daily can still be applied. Because humans still can tolerate Nigella sativa powder 2 g/day for 28 days. Nigella sativa in humans did not show toxic to liver cells, so that Nigella sativa is safe for consumption.[12,13]

RESEARCH LIMITATIONS

Limitations of this study is Nigella Sativa only as a supplement for the treatment of leprosy MB type with a dose of 1000 mg three times daily, could not be concluded as leprosy MB type of new drugs. This research has not been able to do the initial assessment of the relationship between the administration of Nigella Sativa with clinical improvement, because there are no parameters for clinical improvement of leprosy.

CONCLUSION

Supplementation of Nigella sativa 1000 mg three times daily for 2 months in patients with leprosy MB type who receive MDT-WHO treatment can reduce levels of IL-10.

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