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The Effect of Black Cumin Supplementation on the Level of Interferon-γ in Multibacillary Leprosy Patient under MDT-WHO Therapy

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Research Article

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Abstract

Background: Multibacillary (MB) leprosy patients have cellular immune response defects of M. leprae causing high bacillary load and a high number of skin lesions (≥5), thus causing the untreated MB leprosy patients to be the main transmission source of M. leprae. Elevated Th2 cytokine levels such as IL-4, IL-5, and IL-10 are characteristic of lepromatous or multibacillary patients. While the level of Th1 cytokines is low, including Interferon-γ(IFNγ). IFNγ which is a major macrophage-activating cytokine, has a role in natural defense and cellular immunity against intracellular microbes such as *M. leprae*. Several studies have shown that black cumin can increase IFNγ production, thus having a potential effect on the cellular immune system, by stimulating Th1 and an an inhibitory effect on Th2 cells.

Aim: To determine the effect of black cumin supplementation on the level of interferon-γ in multibacillary leprosy patient under MDT-WHO therapy

Methods: This research is an experimental research with a randomized controlled design. A total of 44 MB leprosy patients were divided into 2 groups:

(i) Placebo group, who received MDT-WHO and placebo

(ii) Black cumin group, who received MDT-WHO and black cumin. The independent variables were the administration of MDT-WHO plus black cumin and MDT-WHO plus placebo, which was given for 2 months. The dependent variable was the IFNγ level.

Results: The mean IFNγ levels after treatment were significantly higher in the black cumin group ($p < 0.0001$) as were the mean delta values of IFNγ ($p < 0.05$) than placebo.

Conclusion: Supplementation with black cumin extract increases IFNγ levels in multibacillary type (MB) leprosy patients.

Keywords: Black cumin extract; Nigella sativa; Multibacillary leprosy; IFNγ

Abbreviations: MDT: Multi-Drug Therapy; MB: Multibacillary; ELISA: Enzyme-Linked Immunosorbent Assay; LD50: Lethal Dose

Introduction

Leprosy is a contagious chronic infection that can lead to deformity, disability, and stigmatization [1,2]. Treatment using Multi-Drug Therapy (MDT) from WHO has reduced the global prevalence of leprosy from 5.2 million in 1980 to 200,000 cases in 2014. Nevertheless, the detection rate of new cases until 2014 was still high, where about 220,000-250,000 new cases of leprosy were diagnosed each year [3]. Indonesia is the third country with the largest number of leprosy cases in the world after India and Brazil. In Indonesia, Central Java is the province with the second highest case-finding rate after East Java. As of June 2010, 507 new cases were recorded [4]. Therefore, in addition to early detection and treatment with MDT WHO which has been a major milestone in leprosy eradication strategies, other appropriate strategies are needed. Currently, a lot of studies are ongoing about micronutrients that can be of adjuvant use in the therapy of leprosy as well as prevention of contact transmission [3,5,6].

Leprosy has a spectrum of clinical manifestations related to the immune response to pathogens. On the one hand, the spectrum of tuberculoid or paucibacillary leprosy is characterized by a strong cellular immune response that limits the growth of pathogens, the small number or zero number of bacilli, and the small number of lesions. Th1 cytokines, such as TNFα and IFN-γ, are more strongly expressed in tuberculoid patients. While on the another spectrum, lepromatous or multibacillary (MB) leprosy shows a low cellular immunity to *M.leprae*. Multibacillary leprosy is characterized by high bacillary load and many skin lesions. Therefore, the untreated multibacillary patients are the main source of leprosy transmission [7-11]. Th2 cytokine levels such as IL-4, IL-5, and IL-10 that are characteristic of lepromatous or multibacillary patients, are elevated in those patients. While the level of Th1 cytokines is low, including IFNγ [8,9,12,13]. Interferon-γ is a major macrophage-activating cytokine and has a role in natural defense and cellular immunity against intracellular microbes such as *M. leprae*. Interferon-γ increases the production of reactive oxygen and nitrogen intermediates by macrophages and stimulates macrophages to kill and limits the growth of intracellular pathogens [2,10,14].

Black cumin (Latin: *Nigella sativa*, English: black cumin / black seed; Arabic: el-habat-el-sauda; Indian: kalonji) belongs to the Ranunculaceae family, which is popular as a medicinal plant [15,16]. One important benefit of black cumin is immunomodulation. Several studies have shown that black cumin can increase IFN-γ levels, has a potential stimulating effect on cellular immunity as well as a potential suppressive effect on humoral immunity.

The purpose of this study was to determine the difference in the meanof IFN-γ levels before and after treatment between groups receiving MDT-WHO therapy with black cumin supplementation and groups receiving MDT-WHO therapy alone.

Methods

This is a double-blind randomized controlled trial. In this study, black cumin capsules were administered at a dose of 3000 mg/day for 8 weeks with MDT-WHO in the treatment group. While in the control group, WHO MDT was given with placebo capsules. 44 subjects with age between 18-60 years were divided into two groups, the treatment group, and the control group. The subjects were MB type leprosy patients who went to Donorejo Hospital Jepara. The subjects were selected by consecutive sampling and double blind. The black cumin was given in the form of powder in capsules. The treatment group was given a 1000 mg capsule taken three times a day.

The inclusion criteria were male or female patients, aged 14-60 years, MB leprosy patients (WHO classification), either new patients who will start treatment or who have undergone no more than 3 months of treatment, willing to take part in the study by signing an informed consent form, not pregnant / breastfeeding, not having other infections, not having autoimmune or metabolic disease, not taking immunosuppressant or immunomodulatory drugs. Exclusion criteria were MB leprosy patients with leprosy reaction. Blood samples were taken from the vena medianacubiti before and after treatment, then IFN-γ was calculated using the ELISA (Enzyme-Linked Immunosorbent Assay) method particularly the Quanticine kit.

The data were analyzed using the chi-square test and independent t-test for gender, age, education, BMI, family history, and Wilcoxon test to determine the mean difference of IFNγ serum levels before and after treatment in the control and treatment group.

The ethics committee of Medical Faculty Diponegoro University / Dr Kariadi Hospital has approved this study.

Results

This study showed that the majority subjects in the treatment group were men (68.2%), with an average age of 37 years. Most of them went to elementary school (50%), had a normal BMI (72.7%) and a family history of leprosy (90.9%). In the control group, the majority subject was also male (68.2%), with an average age of 45 years. Most of them went to elementary school (68.2%), had a normal BMI (54.5%), and had family history of leprosy (86, 4%). Gender (p = 1,000) and age were not significantly different (p = 0.182) between the two groups, there was also no significant difference in education, BMI, and family history of leprosy, respectively (p = 0.987, 0,656, 1,000) (Table 1).

		Treatment			Control			p
		N(%)	Mean±SD	Median (Min-max)	N(%)	Mean±SD	Median (Min-max)	
Gender	male	15(68,2)			15(68,2)			1,00[a]
	female	7(31,8)			7(31,8)			
Age			37±14,0	35(18-64)		43±12,0	45(18-60)	0,182[b]
Education	uneducated	4(18,2)			3(13,6)			0,661[a]
	Elementary school	11(50,0)			15(68,2)			
	Junior high school	5(22,7)			3(13,6)			
	Senior high school	2(9,1)			1(4,5)			
BMI			19.9±3,5	20,2(10,9-24,4)		19.5±2,4	19,2(16,6-26,1)	0,656[b]
	Underweight	6(27,3)			9(40,9)			
	normal	16(72,7)			12(54,5)			
	overweight	0(0,0)			1(4,5)			
	Obesity	0(0,0)			0(0,0)			
Family history	Leprosy	20(90,9)			19(86,4)			1,000[a]
	No Leprosy	2(9,1)			3(13,6)			

a.chisquare test; **b**. independent t-test
Table 1: The characteristics of the subject.

The mean serum IFNɣ levels before and after black cumin administration were statistically significant different (p <0.0001): the mean level after treatment (7.57 pg / ml) were higher than before the treatment (4.24 pg / ml) (Table 2).

	N	Mean±SD	Median	Min-Max	P
IFNɣ before treatment	22	4.24±4,93	3,50	0,31-25,16	<0,0001[a]
IFNɣ after treatment	22	7,57±5,57	6,51	0,45-28,68	

a. Wilcoxon test
Table 2: Mean difference test of IFNɣ serum levels before and after treatment in the Black Coumin treatment group.

The mean serum IFNɣ level after treatment (7.49 pg / ml) was higher than before treatment (6.59 pg / ml), also in the placebo group, however, this difference was not statistically significant (p = 0.639) (Table 3).

	N	Mean±SD	Median	Min-Max	p
IFNγ before treatment	20	6,59±3,75	5,41	3,18-19,12	0,639[a]
IFNγ after treatment	20	7,49±6,96	3,98	1,27-22,15	

a. Wilcoxon test

Table 3: Mean difference test of IFNɣ serum level before and after treatment in control group.

In the black cumin group, the mean increase of IFN ɣ level was 3.33 pg / ml, while the increase in the control group was 0.91 pg / ml. This difference was statistically significant (p = 0.005) (Table 4).

	N	Mean±SD	Mean difference	Median	Min-Max	p
Black cumin	22	3,33±2,16	2,41	3,5	(-1,27)-8,28	0,005[a]
Placebo	22	0,91±7,36		-0,96	-11,14-17,42	

a. Mann-Whitney test

Table 4: Mean difference of serum IFNɣ levels before and after treatment with black cumin or placebo.

Discussion

The characteristics of patients such as gender, age, education, BMI, and family history of leprosy were not statistically different in between the study groups. We found an increase in IFN γ in both the treatment group and the control group. However, the increase was not significant in the control group, which received MDT-WHO and placebo. There was a significant difference in the mean change of IFN γ levels between the two groups which were higher in the treatment group, who was given black cumin 3x1000 mg and MDT-WHO for 60 days, compared to the control group. Various studies have proven that black cumin extract has an immunomodulatory effect. Black cumin has potential effects on cellular immunity, as well as having a suppressive effect on B cell-mediated humoral immunity. A study by Boskabady *et al* (2011) on the immunomodulating effect of black cumin on albumin sensitized guinea pigs showed an increased IFNγ and decreased IL-10, which indicates that black cumin has an inhibitory effect on Th2 cell and its cytokines and an stimulating effect on Th1 cell and its cytokines [15].

Mean serum IFNγ levels in paucibacillary leprosy patients is significantly higher than in multibacillary (MB) patients, whereas serum IL-10 level of MB patients were significantly higher than in PB patients with lower Th1 cytokine levels, including IFNγ. The IFN / IL-10 ratio may provide information on potential disease progression or response to treatment. Hopefully, black cumin supplementation in MB patients who receive standard MDT-WHO therapy will improve treatment by increasing their cellular immunity activity.

The study of black cumin toxicity in mice demonstrated a median lethal dose (LD50) of 470 mg / kg BW. Symptoms of toxicity were in the form of decreased locomotor activity and sensitivity to touch stimuli. In other toxicity studies, black cumin had a wide safe therapeutic dose range up to 1 g / kg for 28 days [16]. In this study, the author used a dose of 3x1000 mg, which is considered to be well in a safe dose range.

Conclusion

There was a significant difference in the mean serum IFNγ level change before and after treatment between the MDT-WHO and black cumin group compared to the MDT-WHO and placebo group in MB type leprosy patients. The clinical significance of this finding has to be determined.

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