

PHYLLANTHUS NIRURI EXTRACT COULD IMPROVE IMMUNOGLOBULIN-M ANTI PHENOLIC GLYCOLIPID-1 LEVEL IN SEROPOSITIVE CONTACT OF HANSEN'S DISEASE PATIENTS

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ABSTRACT: Number of Hansen's disease (HD) in Indonesia is classified as number three in the world after India and Brazil. New HD patients tend to increase since there is a possibility that seropositive HD is turning into manifest HD. *Phyllanthus niruri* Linn is an herb that stimulating human immunity to protect from diseases. The aim of this study was to analyze the influence of *P. niruri* extract to the IgM anti PGL - 1 level of seropositive contact of HD patients. Thirty two subjects aged 12-25 year were recruited for this study. The *P. niruri* group received 50 mg *P. niruri* extract/day orally for one month. Seropositive HD was determined by examining IgM anti PGL- 1 level. Daily intake was determined by recall method. There was no significant difference daily intake between two groups ($P > 0.05$). The IgM anti PGL - 1 level in subject with *P. niruri* showed lower decrement than the control group, but it was of insignificant difference ($P = 0.171$). There was no significant change of IgM anti PGL - 1 level between both groups. However, IgM anti PGL - 1 level in the subject in the *Phyllanthus niruri* Linn showed lower decrement.

Keywords: *Phyllanthus niruri* Linn, IgM anti Phenolic Glycolipid-1, seropositive HD

INTRODUCTION

HD is still a major public health problem in Indonesia as well as in the world. The number of HD cases in Indonesia could be classified as number three in the world after India and Brazil [1]. Eradication of HD Multy Drug Therapy (MDT) has begun in Indonesia since the 1980s and managed to reduce the prevalence of HD, but the number of new patients per year (incidence rate of HD) is still stable, there is a possibility that seropositive HD is turning into manifest HD. Seropositive HD is a condition when someone is infected by *Mycobacterium leprae*, however, they have not shown any clinical symptoms. More than half of healthy inhabitants in endemic HD area are anti-micro bacterial antibody positive. Therefore seropositive HD should get more attention in the effort to eliminate HD. Detection of seropositive HD subjects can be done by using various methods; one of them is by examining Immunoglobulin M (IgM) anti *Phenolic Glycolipid* (PGL)-1 level [2, 3]. Cellular immune response level will determine HD spectrum. For HD paucibacillary type (PB), the cellular immunity is still good; however for HD multibacillary type (MB), the cellular immunity is decreased [4]. Seropositive contact of HD patient (IgM anti PGL - 1 level more than 600 unit/ml), has a tendency to change into HD MB. Cellular immunity of HD MB is decreased or even reaches energy and it turns into humoral immunity (Th2), which makes patient's condition worse.

Up to now herbs have been widely used to overcome some of the communicable diseases. One of these herbs which grow in Indonesia is *Phyllanthus niruri* Linn (family Phyllanthaceae). *P. niruri* contains some active phytochemicals such as flavonoid, alkaloids, coumarins and saponins [5]. Some research indicates that *P. niruri* has hepatoprotective effect, [6] and HIV replication inhibition [7]. *M. leprae* is one of the intracellular living bacteria. Previous research has proved that *P. niruri* can be used as anti-HIV and anti-hepatitis B agent whereas this research tries to examine whether PnL could improve IgM anti PGL-1 level in seropositive contacts of HD patients.

MATERIALS AND METHODS

This study was conducted on people who live in the same house in Semarang, Central Java, Indonesia in 2007. The sample size was predetermined, i.e. 32 subjects who were randomly chosen from those who met the inclusion criteria. The ethical clearance for this study was obtained from the Medical Ethics Committee of the Medical Faculty, Diponegoro University/dr. Kariadi Hospital, Semarang, Indonesia.

Inclusion criteria

The inclusion criteria were individuals of 12 to 25 years old (both male and female) whom were proven to be HD serology positive (IgM anti PGL-1 level more than 0.200 OD or 600 unit/ml) [8]. In addition, they did not clinically show any HD symptoms and did not take anti-HD medications. They also did not suffer from tuberculosis and did not take any anti-tuberculosis drugs. They did not

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take any anti-immunosuppressant in the last three months before blood samples were taken. Their body mass index was more than 18 kg/m². Prior to study, subjects were willing to join the study by signing an informed consent form.

The extract of *Phyllanthus niruri* Linn

Those 32 subjects were randomly assigned in pairs to receive either the extract of *P. niruri* (treatment group) or placebo (placebo group). Each day for one month, subjects in the treatment group received 1 capsule of *P. niruri* extract ((from Dexa Pharmacist Company in brand of Stimuno®. 1 capsule = 50 mg *P. niruri* extract), while subject in the placebo group received placebo capsules containing flour in the same manner. Compliance with taking the capsules was monitored by the researchers and local health staff.

Procedures

Determination of seropositive HD was done by examining IgM anti PGL-1 level using enzyme-linked immunosorbent assay (*Polyclonal rabbit anti human IgM/HRP* (Dako®) in the Institute Tropical Disease Laboratory, Airlangga University, Surabaya. In addition, daily intake was measured using 24-hr recall method in order to assess the Zn, Cu and protein intake allowances.

Data analysis

Data on characteristic, daily intake and IgM anti PGL-1 were collected. All data were noted and tabulated. Normality of the data was determined by the Kolmogorov-Smirnov test. Statistical analysis was done using statistical software in order to compare IgM anti PGL-1 level, age and daily intake by independent *t*-test.

RESULTS

In order to know whether someone was HD seropositive, a serology test (IgM anti PGL1) was conducted on 69 subjects who live in the same house with a HD patient. The result showed that 51 subjects (73.9 %) were HD seropositive in the level of > 0.200 OD or 600 unit/ml. However, those who fulfilled the inclusion criteria were only 32 subjects. The mean age of subjects was 16 years old (± 2.9). There was no significant difference between group ($P=0.727$). About 81.3% of subject was female. The comparative result showed that there was no significant difference of daily intake at both groups (Table 1). However, the mean of Zn, Cu and protein intake of subjects were very low.

There was no a significant difference between the treatment and control group in IgM anti PGL-1 level at baseline ($P= 0.180$). After one month, there was a decrement of IgM anti PGL – 1 level on both groups. The mean decrement in IgM anti PGL – 1 in treatment group was 649.1 unit/ml (± 458.2), while the placebo group was 438.7 unit/ml (± 386.4). There was no significant change of IgM

Table 1 Average of difference daily nutrient intake

Nutrient	Groups		P
	Treatment (n = 16)	Placebo (n = 16)	
Energy (Kkal)	1450 \pm 277	1408 \pm 278	0,678
Protein (g)	38,6 \pm 8,13	38,3 \pm 10,2	0,913
Zinc (mg)	4,89 \pm 0,86	4,92 \pm 1,11	0,938
Copper (mg)	1,02 \pm 0,18	1,04 \pm 0,30	0,752

Table 2 Average of difference of IgM anti PGL – 1 level before and after the study

	IgM anti PGL – 1 level (unit/ml)		
	Before	After	Δ
Treatment (n = 16)	1473,4 \pm 645,2	824,3 \pm 429,6	649.1 \pm 458.2
Placebo (n = 16)	1209,8 \pm 411,5	771,1 \pm 399,7	438.7 \pm 386.4
P	0.180	0.719	0.171

anti PGL – 1 level between both groups ($P = 0.171$). However, IgM anti PGL – 1 level in the subject in the *P. niruri* had the lower decrement (Table 2).

DISCUSSION

The development of serologic methods for specific antibodies against *M. leprae* had noticed that this method could detect the seropositive persons among those who were in close contact with HD patients. Many research studies showed that those who have specific antibody against *M leprae* (IgM anti PGL – 1 more than 0.200 OD or 600 unit/ml) without clinical signs of HD, can be identified as seropositive subjects [8].

The intake of protein and micro mineral such as Zn and Cu is played an important role in immune system. Among subject with protein and energy deficiency will impaired the antibody formation, reduce immunoglobulin concentration and lower T cell and interleukin 2 receptor [9]. Zn gives influence the B and T cell lymphocyte and cytokine production such as IFN- γ , interleukin 2 and TNF- α [10]. Similar with Zinc, Cu is also having role in the immune respon. Review in the role of Cu and immunity showed that among subject with Cu deficiency, the level of interleukin 2 is reduce [11].

The balance between TH1 and TH2 cell activation determines the outcome of intracellular infections of HD in humans [12]. If the IgM anti PGL – 1 level is too high, the balance shifts into dominant TH2. Therefore, seropositive subjects could becomelepromatous HD. In our study, the mean decrement of IgM anti PGL – 1 level in treatment group was greater than that in the placebo group. This result showed that *P. niruri* could balance TH1 and TH2 and consequently, the decrease of IgM anti PGL – 1 level in the treatment group was higher than in the placebo group.

The results are consistent with other research which

shows that giving *P. niruri* extract could enhance phagocytosis and lysosomal enzymes activity of murine bone marrow-derived macrophages [13]. Macrophage phagocytosis of *M. leprae* engulfed within phagosomes and exposed to lysosomal enzymes and phagolysosome formation may lead to complete bacillary lysis [14].

P. niruri can also increase the expression of major histocompatibility complex-II (MHC class II) and various makers for dendritic cells maturation (CD40), activation (CD83) and costimulation (CD86) [15]. Dendritic cells have been found to be very effective presenters of *M. leprae* antigen. Dendritic cells stimulated with *M. leprae* membrane antigens unregulated MHC class II and CD40 ligand-associated IL-12 production [4]. After that, cell-mediated immune responses may result and Th1 lymphocyte stimulation induces interferon gamma (IFN- γ) release which promotes and accelerates phagolysosome processing within newly infected macrophages [14]. All the process can reduce level of IgM anti PGL – 1, because IgM anti PGL – 1 correlated positively with bacterial index.

The insignificant difference on IgM anti PGL – 1 decrement level in both groups was probably caused by some factors such as the structure of lipoarabinomannan on membrane cell *M. leprae* that could hamper the activation of T cell and IFN- γ [16], another factor could have been that *M. leprae* blocked the hosts' immune response by decreasing expression of co-stimulator (CD86) [17], and a final factor could have been insufficient doses and treatment period of *P. niruri*. This final factor could probably be ruled out by conducting a similar study for a longer duration and with higher doses of *P. niruri*.

ACKNOWLEDGEMENTS

Our grateful thank to Prof. Shinzo Izumi, MD, PhD, and Prof. Dr. Indropo Agusni, MD, DV, Leprosy Study Group of Tropical Disease Center, Airlangga University, Surabaya for the permission of IgM anti PGL – 1 assay.

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