COMPARISON OF MULTIDRUG THERAPY TREATMENT RESULTS BETWEEN MULTIBACILLARY LEPROSY PATIENTS IN HYPERENDEMIC AND HYPOENDEMIC AREAS IN GOWA REGENCY, SOUTH SULAWESI, INDONESIA

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Abstract. We studied 88 multibacillary (MB) leprosy patients, who received multidrug therapy (MDT) treatment in hyperendemic (44 persons) and hypoendemic (44 persons) areas in Gowa Regency, South Sulawesi, Indonesia. Bacteriological examinations were carried out (bacteria index and morphology index), immunological examinations (MLPA and TNF-α) and genetic variation in blood and ear lobe slit skin smears of MB leprosy patients, which had been treated by MDT, were performed. The collected data were analyzed with the chi-square test and discriminant analysis. Eight persons (9.1%) had a positive bacteria index and 2 persons (2.4%) had a morphology index. All patients (100%) increased their TNF-α concentration. All the samples (100%) showed an increase in the TNF-α concentration, compared to controls. MLPA positive conversion was found in 4 persons (47.7% samples) and TNF-α gene genetic variation at the position of -308, occurred in 12 persons (13.6% samples). Statistic test results showed a significant difference ($p<0.01$) in the TNF-α concentration between hyperendemic and hypoendemic areas. On the basis of a positive conversion by bacteriological results and its association with immunological results, it can be concluded that multibacillary leprosy patients with MDT treatment did not become a source of leprosy transmission.

INTRODUCTION

People have long known about leprosy. The disease was found in patients in Egypt since 4,000 years B.M, and in India and China about 1,500 years B.M. In 1872, Hansen discovered mycobacterium leprae as the cause of leprosy. Recently, 10-12 million leprosy patients were distributed widely in various continents. Patients of leprosy were primarily founded in Asian countries, Africa and in Latin America. There were 3.1 millions patients in Indonesia in 1993, most of them found in the eastern part of Indonesia.

In early 1997, the WHO estimated the number of leprosy patients throughout the world to be about 1,150,000 cases. Out of this, 888,340 cases were registered as being treated in 28 endemic countries. Seventy percent of these patients were distributed in Indonesia, India, Bangladesh and Myanmar. In Indonesia alone was presumed to have about 50,000 cases, with 33,739 new cases reported in 1996 (WHO, 1997).

The MDT (multidrug therapy) program announced by the WHO in 1994 had the purpose of ridding the world of leprosy by the year 2000.

In Indonesia, the prevalence of leprosy tended to decrease yearly. In 1986 the prevalence was 7.6/10,000, which then declined to 3.8/10,000 in 1994, 1.75/10,000 in 1995, 1.64/10,000 in 1996, and 1.39/10,000 in 1997.

The number of leprosy patients in the South Sulawesi Province followed the national trend of declining prevalence; in 1984 the number of patients were 23,870, in 1998, 17,582; in 1994, 4,450; in 1995, 3,524; in 1996, 3,177; and in 1997, 2,526, with a prevalence of 3.29/10,000.

In the Gowa Regency, there were 1.254 leprosy patients in 2000, which consisted of 759 persons labeled as RFT (Released From Treatment), 245 persons were RFC (Released From Control) and the remainder were being treated with MDT.

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Based on the WHO criteria for leprosy disease types, the multibacillary (MB) type is still increasing, while the paucibacillary type (PB) is decreasing. The prevalence of leprosy decreased from 1983-1987, when MDT was established by the WHO.

The treatment of leprosy is based on two criteria only, PB and MB; where MDT is used for PB over 6-9 months and MB over 1-2 years. When the treatment is completed, the patient is called RFT. Two years after RFT, the PB patient is considered RFC. Five years after RFT, the MB patient is considered RFC.

A factor that affects treatment is where they live, the city is endemic for malaria and many patients live there. In this study bacteriological and immunological examinations on multibacillary leprosy patients were performed in hyperendemic and hypoendemic areas. Also studied were the treatment results of leprosy patients in relation to where they live, an area of hypo or hyperendemicity.

MATERIALS AND METHODS

This study used samples from 88 leprosy patients of the multibacillary type that received MDT treatment in the endemic areas of the Gowa Regency of South Sulawesi, Indonesia. Fourty-four of the samples came from an hypoendemic area (Bontonompo I) and the remaining 44 were taken from an hyperendemic area (Bontonompo II).

Bacteriological, immunological, and slit skin smear examinations of leprosy patients (which had been treated with MDT) were examined for bacteriological and morphological indices. The blood of leprosy patients was taken for MLPA examination, TNF-α, genetic varation, and TNF-α gene position at -308. The bacteriological examination was carried out at the Microbiology Laboratory, Faculty of Medicine, Hasanuddin University, and the MLPA test used serodia leprae (Fujirebio Inc, Tokyo, Japan). The TNF-α test was performed using the ELISA method. The Bacteriological index was examined based on Ridley's logarithmic scale.

Data analysis

The data, after going through the screening process was entered into computer, where we used the Epiinfo version 6.0 program (CDC, 1994). The data analysis was performed using the SPSS (Statistical Package for Social Sciences) program. (Norusis, 1990).

Type of analysis used was univariate analysis, bivariate (chi-square, independent *t*-test, correlation test), and multivariate (discriminant analysis). The level considered significant was set at $\alpha = 0.05$.

RESULTS

General description

This study was performed on 88 samples of multibacillary (MB) leprosy patients, where 44 persons came from hyperendemic areas and 44 persons came from hypoendemic areas. General descriptions in terms of age, time after treatment with MDT, and illness time are shown in Table 1.

As shown in Table 1, the average age of the patient in hypoendemic areas that was used for this study was 44 years and for the hypoendemic areas was 39.3 years. This difference is not statistically significant ($p>0.05$). In terms of the status after treatment, this showed no statistical difference ($p>0.05$). The average status time for the hyperendemic area (61.8 months) was higher than the status time for the hypoendemic areas (58.0 months).

In terms of the sex, the percentage of men (65.9%) is higher than the percentage of woman (34.1%), however the comparison between the hyperendemic and hypoendemic areas ($p>0.05$) was the same (Table 2).

Bacteriological examination

The bacteria index examination results are shown in Table 3. Of 8 persons (9.1%), 5 persons (11.4%) came from hypoendemic areas, and 3 persons (6.8%) came from hyperendemic areas. The difference was not significant statistically ($p>0.05$).

According to the morphology index examination, only two samples (2.3%) were positive. They came from an hyperendemic and an hypoendemic area.

Immunological examination results

The concentration of TNF-α in the hyperendemic area was 27.1±7.27, from the hypoendemic

Table 1
Comparison of the age, status time, illness time, and TNF-α between hyperendemic and hypoendemic areas in the Gowa Regency, South Sulawesi, Indonesia, 2002.

	Area	N	Mean	SD	p
Age (years)	Hyperendemic	44	39.3	14.79	0.148
	Hypoendemic	44	44.4	17.67	
Status time (month)	Hyperendemic	44	61.8	30.57	0.599
	Hypoendemic	44	58.0	35.81	
Illness time (month)	Hyperendemic	44	9.8	15.37	0.087
	Hypoendemic	44	15.9	17.40	
TNF-α	Hyperendemic	44	27.1	7.27	0.000
	Hypoendemic	44	85.5	38.13	

Table 2
Sex distribution in hyperendemic and hypoendemic areas in the Gowa regency, South Sulawesi, Indonesia, 2002.

Sex	Area		Total N (%)
	Hyperendemic N (%)	Hypoendemic N (%)	
Male	28 (63.6)	30 (68.2)	58 (65.9)
Female	16 (36.4)	14 (31.8)	30 (34.1)
Total	44 (100)	44 (100)	88 (100)

Table 3
Distribution of the bacteria index in hyperendemic and hypoendemic areas in the Gowa Regency, South Sulawesi, Indonesia, 2002.

Bacteria index	Area		Total N (%)
	Hyperendemic N (%)	Hypoendemic N (%)	
Positive	3 (6.8)	5 (11.4)	8 (9.1)
Negative	41 (93.2)	39 (88.6)	80 (90.9)
Total	44 (100)	44 (100)	88 (100)

area was 85.5 ± 38.13, and the control group was 10.35 ± 2.76. The results show that the sample from the hypoendemic area has a higher TNF-α concentration compared with the hyperendemic area ($p<0.01$) (Table 1).

Based on the MLPA test, there was positive conversion in 42 samples (47.7%). Half (N=21) were from an hyperendemic area and the other half were from an hypoendemic area (Table 5).

The genetic variation examination showed that 12 persons (13.6%) had the presence of a genetic mutation. Of this amount, 8 persons (18.2%) came from an hyperendemic area and 4 persons (9.1%) came from an hypoendemic area. This difference was not significant statistically ($p>0.05$) (Table 6).

Table 4
Distribution morphology index in hyperendemic and hypoendemic areas in the Gowa Regency, South Sulawesi, Indonesia, 2002.

Morphology indexs	Area		Total N (%)
	Hyperendemic N (%)	Hypoendemic N (%)	
Positive	1 (2.3)	1 (2.3)	2 (2.3)
Negative	43 (97.7)	43 (97.7)	86 (97.7)
Total	44 (100)	44 (100)	88 (100)

Table 5
Distribution of the MLPA test in hyperendemic and hypoendemic areas in the Gowa Regency, South Sulawesi, Indonesia, 2002.

Bakreri Index	Area		Total N (%)
	Hyperendemic N (%)	Hypoendemic N (%)	
Positive	21 (47.8)	21 (68.2)	42 (47.7)
Negative	23 (52.3)	43 (52.3)	86 (52.3)
Total	44 (100)	44 (100)	88 (100)

The multivariate analysis using discriminant analysis for each of the different independent variables (age, status time, illness time, sex, IB, IM, TNF-α, MLPA, and genetic variation) between hyperendemic and hypoendemic areas showed a difference in the TNF-α concentrations between the two areas ($p<0.01$). The ability of the TNF-α variable to distinguish between the hyperendemic and hypoendemic areas was 73.2% (canonical correlation = 0.732).

DISCUSSION

In general, it was found that sample characteristic between the hyperendemic and hypoendemic areas were quite homogeneous. The samples from the hyperendemic and hypoendemic areas were not different in terms of age, status time, illness time and sex distribution ($p>0.05$). Thus, the comparison results of bacteriological and immunological examinations were not affected by these variables.

The result of the bacteriological examinations show the treatment effectiveness for removing the *M. leprae* bacillus. At the beginning of MDT treatment the bacteria index is expected to be elevated, due to the presence of the baillus in the body. This is expected to decrease with treatment. A positive bacteria index was found in the hyperendemic area only (prevalence of active leprosy patients is higher) (Hastings and Opromolla, 1994; Barrera *et al*, 2001).

The morphology index shows no difference between the hyperendemic and hypoendemic areas. This shows MDT treatment efficacy, where the quantity of bacillus found in the post-treatment patients was very small (Jamet *et al*, 1995; WHO, 1995; Hatta, 1997; Chen *et al*, 1999).

The TNF-α result was higher for the hyperendemic area than the hypoendemic area ($p<0.01$), and the study samples were significantly higher than the controls ($p<0.01$). This shows the long standing inflammation caused by the *M. leprae* bacillus and the resultant antibodies (Boyd and Hoerl, 1986; Subowo, 1993; Vries, 1994).

These results are consistent with previous research, in which the TNF-α concentration was

Table 6
Distribution of genetic variation in hyperendemic and hypoendemic areas in the Gowa Regency, South Sulawesi, Indonesia, 2002.

Genetic variation	Area		Total N (%)
	Hyperendemic N (%)	Hypoendemic N (%)	
Positive	8 (18,2)	4 (9,1)	12 (13,6)
Negative	36 (81,8)	40 (90,9)	76 (86,4)
Total	44 (100,0)	44 (100,0)	88 (100,0)

higher in all leprosy types than controls. The high level of TNF-α compared with controls is an indication of macrophage activity. In innate immunity, macrophage activity is triggered directly by the *M. leprae* antigen, and in adaptive immunity, INFγ, a cytotoxin from the T cell triggers macrophage activity, resulting in TNF-α, which results in the cellular immune response (Stites and Terr, 1991).

In our research, the high TNF-α concentration believed not to be triggered by *M. leprae* antigen, because in most of the cases there was not enough antigen. Although 1.1% of the cases showed a positive bacteria index, it is probably triggered by the effect of IFNγ on the macrophage. This belief is supported by the fact that TNF-α is significantly higher in the hypoendemic areas (sample's illness time is longer than the hyperendemic area). The PB group already shows a good cellular immune response, which means the TNF-α was predominantly affected by IFNγ, not by the *M. leprae* antigen.

The MLPA result was positive in 42 patients (47.7%). The result was equal for hyperendemic and hypoendemic areas.

The MLPA results were lower than in untreated patients, which were 71.7%. This shows the existence of the non-acute inflammation process, as a consequence of infection with the *M. leprae*, which has been long standing and has already received treatment (Scollard *et al*, 1994; Spierings *et al*, 2000).

In terms of the existence of the TNF-α genetic variation in 12 samples (13.6%), no significant difference was noted between the hyperendemic (18.2%) and hypoendemic (9.1%) areas. It is difficult to assess whether the genetic variation is related to susceptibility or resistance to *M. leprae*, but there is a significant difference in the TNF-α level in this group compared with the group without genetic variation. TNF-α genetic variation is found less in the PB group, so genetic variation has a role in leprosy pathogenesis. The same results were seen in previous research, which showed that TNF-α genetic variation at the -308 position is more of a type L distribution (Roy *et al*, 1997). The lack of a difference in the genetic variation between the hyperendemic and hypoendemic areas is caused by the genetic variation itself which has a role to play in the susceptibility or resistance to infection (Hajeer and Hutchinson *et al*, 2001).

ACKNOWLEDGEMENTS

I thank Dr Hasanuddin Ishak of the Department of Environmental Health, Faculty of Public Health, Hasanuddin University for reading the manuscript.

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