Profile of IgM Anti PGL-1 and IgM Anti LID1-NDO on Leprosy Patients of Dr. Soetomo General Hospital, Surabaya

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Abstract: Serological tests have been used to assist leprosy disease management. The latest serological test towards anti LID1-NDO ((Leprosy IDRI Diagnostic 1- Natural Disaccharide Octyl) challenges the leprosy antigen already in use, PGL-1, to provide a better, more sensitive leprosy serological test. This research is to study the profile of IgM anti PGL-1 compared to IgM anti LID1-NDO in leprosy patients from Surabaya. Sera sample 91 leprosy patients from Dr. Soetomo Hospital, Surabaya (PB : 30, MB: 61) and tested against IgM anti PGL-1 and IgM anti LID1-NDO using the ELISA technique. The result of anti PGL-1 is stated in u/mL and in optical density (OD) unit for IgM anti LID1-NDO. The average IgM anti PGL-1 titer in PB patients are 1577.1 u/ml, whereas for anti LID1-NDO antibody the average OD is 0.166. In MB patients IgM anti PGL-1 has the average of 3629.2 u/ml. The absorbance of IgM anti LID1-NDO was 0.228 on average. The majority of MB patients presented with positive anti LID1-NDO and anti PGL-I responses (77.05 % and 68.85 %). For PB patients presented with positive anti LID1-NDO and anti PGL-I responses (70% and 60%). strong correlation was found (P=0.003) between IgM anti LID1-NDO and anti PGL-1. PGL-1 originates from the polysaccharide of M. leprae, and LID1-NDO fuses this polysaccharide with another set of protein LID1-NDO is potential to be used in serological tests for leprosy diagnosis and monitoring, as it performs to a similar manner to PGL-1.

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1 INTRODUCTION

Leprosy is a chronic infectious disease caused *Mycobacterium leprae*. Although the disease affects the skin and peripheral nerves, it can present with a wide array of pathologies and clinical manifest to cause leprosy present as a bacteriologic, clinical, immunologic, and pathological spectrum ranging from the extremes observed in paucibacillary (PB) and multibacillary (MB) patients depending upon the patient's immune response. (Bührer-Sékula et al., 2008; Spencer and Brennan, 2011; Amorim et al., 2016) The diagnosis of leprosy is not simple and, not surprisingly, many professionals have neither the experience to recognize the various signs and symptoms of the disease nor the ability to differentiate them from other diseases. (Duthie et al., 2014, 2007; da Conceição Oliveira Coelho Fabri et al., 2015) Thus, leprosy patients often receive incorrect diagnoses and appropriate treatment is delayed. Antibody responses to specific M. leprae antigens can be evaluated by several tests. (Duthie et al., 2016) The detection of IgM antibodies against phenolic glycolipid I (PGL-I) represents the most evaluated serologic assay for leprosy, with levels correlating with bacillary loads such that levels rise across the TT to LL spectrum. Among these are serologic tests that measure the levels of immunoglobulin M (IgM) against phenolic glycolipid-1 (PGL-1) the synthetic mimetope natural disaccharide octyl – leprosy IDRI diagnostic 1 (LID1-NDO). The aim of this study was to evaluate the antibody responses against phenolic glycolipid-1 (PGL-1), Leprosy IDRI Diagnostic 1 Natural Disaccharide Octyl (LID1- NDO) in leprosy patients (Hunter et al., 1982)

2 METHODS

Blood (3cc) from 91 leprosy patients from Dr. Soetomo General Hospital, Surabaya (PB : 30, MB: 61) were collected from the cubituous vena of each patient, to be centrifuged and have the serum tested

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against IgM anti PGL-1 and IgM anti LID1-NDO using the Enzyme Linked Immunosorbent Assay (ELISA) technique. Briefly, polysorp 96-well plates (Nunc Maxisorp) were coated with 0.01 µg/mL natural trisaccharide-phenyl conjugated to bovine serum albumin (NT-P-BSA), the trisaccharide NTP-BSA ride synthetic analog of PGL-I kindly provided by Dr Fujiwara, Nara University, Japan, incubation overnight at 4ºC and blocked with phosphate-buffered saline tween (PBST)-2% skim milk.Incubation for 1 h at 37ºC. Serum samples diluted 1/300 in PBS 1% skim milk were added to duplicate wells of either NT-P-BSA coated plates. After and washing with PBS-Tween, horseradish peroxidase-conjugated to anti- human IgM (Wako, USA) was then added. After incubation for 1 h at 37ºC and further washes, Antibodies against PGL- 1 were detected by indirect ELISA with peroxidase-conjugated anti-human IgM with *0-phenylene diamine* as substrate (Sigma) was added for each well. The colour reactions of the entire plate were stopped with 2.5 N-H_2SO_4. The optical density (OD) was read at 492 nm /620 nm using microplate reader Texan Infinite f50. For final OD value PGL-1 (NTP-BSA) of each serum sample was calculated by Biolise program software (Titer). The Cut-off was defined as Titer > 605 u/ml.

Serum IgM antibodies to the di-fusion protein LID1-NDO were detected by serology ELISA. LID1-NDO provided by Dr Malcom S Duthi, from Infectious Disease Research Institute (IDRI) Seatle , USA. Polysorp 96-well plates (Nunc Maxisorp) were coated with 2 µg/mL of LID1-NDO at 4ºC overnight and blocked with PBST with 2% skim milk for 1 h at 37 ºC. Serum samples diluted 1/300 in 1% PBS-skim milk were added in duplicates and incubated for 1h at 37 C. Plates were washed and incubated with added 50 µL of peroxidase-conjugated with anti-human IgM (Sigma) diluted to 1/6,000 in PBS, 0.1% skim milk. After washings, reactions were developed with anti-human IgM with *0-phenylene diamine* as substrate (Sigma) was added for each well. The colour reactions of the entire plate were stopped with 2.5 N-H_2SO_4. The optical density (OD) was read at 492 nm /620 nm using microplate reader. The corrected OD of each well at 492 nm / 620 nm was read using a microplate reader. The results were expressed as mean absorbance of the duplicates. The final OD value LID1-NDO of each serum sample was calculated by subtracting the OD value. The cut-off was defined as OD > 0.053.

3 RESULTS

The study group was composed by 91 leprosy patients (MB : 61, PB:30) with ages ranging from 18-64 years (median = 41 years) of whom the majority was male (Table). The IgM anti PGL-I ELISA positive rate among the MB leprosy patients tested was determined to be 68,85 % (42/61), the titers average is 3629,2 u/ml. Among these same MB leprosy patients, the seropositive rate in IgM anti LID1- NDO test was found to be slightly higher at 77,05% (47/61), the average is OD 0.228. Cluster aread on the scatter diagram for MB patients are OD 0.5 (anti LID1-NDO) and 1500u/ml (anti PGL-1).

The IgM anti PGL-I ELISA positive rate among the PB leprosy patients tested was determined to be 60 % (18/30), the titers average of 1577,1 u/ml. The seropositive rate in IgM anti LID1- NDO test was found to be slightly higher at 70% (21/30), the average OD is 0.166. For PB patients, cluster area are OD=0.25 and titer 350 u/mL for their respective antigens. Statistical analysis a strong correlation (p=003) between IgM anti LID1-NDO and IgM anti PGL-1.

Table 1: Proportion of Seropositivity for antibody against LID1-NDO and PGL-1 according to Group

Leprosy Type	No of sample	Gender (M/F)	No (%) positive LID1-NDO	PGL-1
MB	61	46/21	47 (77.05)	42 (68.85)
PB	30	20/10	21 (70.00)	18 (60.00)

MB : Multibacillary, PB: Pausibacillary, M : Male, F : Female

4 DISCUSSION

Leprosy serology has been studied frequently and many of the factors determining seropositivity are well known, as has been reviewed by Oskam et al. We examined the presence of antibodies against particular M. leprae antigens among MB and PB groups, Patients were classified based on the WHO classification system, all were undergoing treatment for leprosy treatment MDT-WHO. PGL-1 originates from the polysaccharide of M. leprae, and LID1-NDO fuses this polysaccharide with another set of protein, ML0405 and ML2331.The majority of MB patients

presented with positive anti LID1-NDO and anti PGL-I responses (77.05 % and 68.85 %, respectively). For PB patients presented with positive anti LID1-NDO and anti PGL-I responses (70% and 60%). In comparative analyses of anti LID1-NDO and anti PGL-I responses in the statistically strong correlation. Although serological tests appear to have a limited ability to aid the diagnosis of PB patients, our data also identified that a greater number of PB patients were seropositive for antibodies against PGL-1 and LID1-NDO. Our data indicate that tests detecting antibodies to PGL-I and/or LID1-NDO represent effective tools for the detection of MB and PB patients.

5 CONCLUSIONS

LID1-NDO is potential to be used in serological tests for leprosy diagnosis and monitoring, as it performs to a similar manner to PGL-1.

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