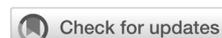## Community Research

Risk factor for *Mycobacterium leprae* detection in household contacts with leprosy patients: a study in Papua, East Indonesia

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ABSTRACT

BACKGROUND In the era of leprosy eradication, Jayapura is still one of the biggest leprosy pockets in Papua, Indonesia. The trend for leprosy case detection rate has remained relatively stable over recent years. This study was aimed to detect *Mycobacterium leprae* in household contacts and to evaluate the associated factors with the detection.

METHODS This cross-sectional study recruited household contacts of leprosy patients who were diagnosed consecutively from March to August 2015 in Hamadi Point of Care, Jayapura. The leprosy patients were diagnosed using polymerase chain reaction (PCR). For each leprosy patient, up to four household contacts that had no symptom were included. Every household contact received screening through DNA detection of *M. leprae* extracted from nasal swab specimens and examined using PCR. Factors for bacteria detection included intensity, time duration and number of contacts living together in the same house, and random blood glucose levels were evaluated. Bivariate analysis was used to associate them with *M. leprae* detection in household contacts.

RESULTS From 107 household contacts of 35 patients who had leprosy, *M. leprae* was detected in 19.6%. Household contacts with leprosy patients for >1 year was a risk factor for detection (OR = 12.45; 95% CI = 1.595–97.20; *p* = 0.002). Blood glucose (*p* = 0.444), ethnic (*p* = 0.456), sleeping proximity to leprosy case (*p* = 0.468) and relatives (*p* = 0.518) give no effect to *M. leprae* detection in household contacts.

CONCLUSIONS Among the various risk factors studied, duration of living together with the patient significantly increased the risk of *M. leprae* transmission.

KEYWORDS households, leprosy, *Mycobacterium leprae*, polymerase chain reaction

According to the World Health Organization (WHO) reports, Indonesia ranked third in world leprosy endemic in 2006, and in 2013 was still among the top 12 endemic countries.[1] Since 2010, intense leprosy controls take place in Papua to ensure complete eradication. Despite these efforts, Papua was still considered as one of the largest leprosy pockets in Indonesia in 2014.[2] The elimination of leprosy from Papua province requires further research directed toward the identification of risk factors involved in the transmission of leprosy.

Factors affecting leprosy include infectious agents, genetic, and environmental factors.[3] In general, the transmission of leprosy does not involve any vectors and is suspected to occur through droplets or fluids arising from the infected patient's body. A study revealed that *Mycobacterium leprae* is transmitted via the respiratory

tract.[4] *M. leprae* can be detected in skin lesions as well as nasal passages of infected individuals.[5] Group of individuals sharing the same household with the leprosy patients are more susceptible to the infection.[6] In this era of leprosy elimination, exposure to *M. leprae* in household contacts in the areas that supply a large number of leprosy cases should be evaluated as per the new models of study.

For various host factors affecting leprosy, a study conducted in Kuwait reported higher incidences of diabetes in leprosy patients as compared to the control group.[7] In other diseases involving mycobacterial infection like tuberculosis, diabetes mellitus has been found to be associated with increased risk of *Mycobacterium tuberculosis* infection.[8] Unfortunately, there is not much information available on the association of blood sugar levels and the risk of *M. leprae* infection. According to the WHO data, the global prevalence of diabetes mellitus is 6.4% which will reach 7.7% by 2030.[6] Thus, it can be hypothesized that blood glucose levels could be one of the important factors associated with leprosy which will be explored in the present study.

M. leprae is a slow replicating bacterium which is generally uncultivable in microbiological culture medium. A study has reported the use of armadillos for the proliferation of this pathogen but this procedure is not feasible for the regular diagnostic.[9] The diagnostic process commonly used in clinics for leprosy detection involves Ziehl-Neelsen staining of slit-skin specimens obtained from leprosy patients. Alternatively, molecular biology approach using polymerase chain reaction (PCR) allows for the detection of *M. leprae* in symptomatic and asymptomatic patients. Detection of *M. leprae* using PCR also helps to check its transmission from patients to asymptomatic household contacts.[10]

Since the transmission of *M. leprae* occurs via respiratory tract, it becomes highly important to check the exposure of *M. leprae* in household contacts in the endemic areas.[4] This study was aimed to identify the risk factors for the transmission, both environmental factors and host factors like blood glucose levels were studied.

METHODS

Study population

This cross-sectional study was carried out from March to November 2015 in the villages of Jayapura district of Papua province that served as the main pocket of leprosy in Indonesia. Subjects of this study were household contacts with leprosy patients. Leprosy patients were identified by well-trained health care workers based on cardinal signs as well as Ziehl-Neelsen staining, living in Jayapura city for more than two years, and completion of the study process, such as interview, house visit, and specimen collection. All the leprosy patients were then confirmed using PCR. For each leprosy patient, up to four household contacts that had no symptom were included in the study. The inclusion criteria of the household contact group were people living with leprosy patients in the same house for more than 8 months and healthy individuals without any symptoms of leprosy. The exclusion criteria for both leprosy cases and household contacts were individuals with severe chronic diseases, influenza, mucus hypersecretion, or undergoing tuberculosis treatment. Household contacts with low quality of the extracted DNA were also excluded. The study was approved by the Ethical Committee of National Institute of Health Research and Development (No: LB.02.01/5.2/KE.155/2015). All the participants approved the research protocol and gave written informed consent.

Sample collection

Slit-skin biopsies and nasal swab specimens were collected from index cases to confirm the diagnosis. Nasal swab specimens were collected from household contacts. Postnasal cleaning was carried out with sterile cotton bud and nasal swab specimens were collected using a sterile cotton swab. Skin biopsies were taken from active lesions or ears using sterile scalpel by trained physicians from leprosy patients During transportation from the field to the laboratory, both nasal swab and biopsies were preserved in cryotubes containing 300 µl phosphate buffer saline. The samples were transported to Microbiology Laboratory in Jayapura and were kept in icebox at 4–8°C before transferring to bio-refrigerator for storage. Assays for DNA extraction, PCR, and blood analysis were performed at Microbiology Laboratory of Institute of Health Research and Development of Papua. Sanger sequencing was carried out at Center of Research and Development of Biomedical and Health Basic Technology, Jakarta.

DNA extraction from nasal swab and slit-skin samples

QIAamp® DNA Mini Kit (cat.51304, QIAGEN, Germany) was used for DNA extraction from nasal swab according to the manufacturer's protocol with slight

modifications which was sonicated at 75% amplitude for 1 min before extraction.[11] In addition to this, 20 µl lysozyme was added to the lysis buffer.[12]

PCR

The primers LP1 (5'-TGCATGTCATGGCCTTGAGG-3') and LP2 (5'-CACCGATACCAGCGGCAGAA-3')[13] were used to amplify a 143 bp segment of *M. leprae* present in the samples from leprosy patients and household contacts. PCR was performed with a reaction cocktail consisting of 4 µl template, 1 µl LP1 primer, 1 µl LP2 primer, 1 µl double distilled water, and 13 µl of PCR Master Mix (GoTaq® Green Mastermix Promega Corporation, USA). C1000 Thermal Cycler (Biorad, USA) was used for PCR with following cycle conditions: initial cycle of 3 min at 95°C, 35 cycles with each cycle consisting of 30 sec at 94°C, 30 sec at 58°C, 60 sec at 72°C, and a final extension of 10 min at 72°C. The resulting PCR products were separated on a 2% agarose gel, run in tris-borate-EDTA buffer at 100 volts for 1 hour. The gels were stained in SYBR safe for visualization of bands. A 100 bp DNA Ladder (Invitrogen Life Technologies, Brazil) was used as molecular marker. Both positive and negative controls were included in each agarose gel. For positive control, 4 µl of DNA extracted from skin lesions of confirmed leprosy patient was used, while 5 µl of nuclease-free water (Promega, USA) served as a negative control. DNA spectrophotometry was then used to examine the extracted DNA. The negative results in electrophoresis with low purity in spectrophotometry (1.7–2.0 on A 260/280) were concluded as low quality of DNA.

DNA sequencing and analysis

The presence of *M. leprae* in specimens was obtained by sequencing of PCR products. PCR positive products obtained from both leprosy patients that was diagnosed with clinical symptoms as well as asymptomatic household contacts were sequenced using Sanger sequencing machine (Applied Biosystem). The sequencing was performed according to the protocol of BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) with LP1 (upstream) and LP2 (downstream) primers. For analysis, basic local alignment search tool (BLAST) was performed to match the sequence of samples to the GenBank® sequences.

Risk factor analysis

Various risk factor parameters were also evaluated to study the transmission of *M. leprae* in household contacts. This included random blood glucose, ethnic, sleeping proximity to leprosy patient (in 1 bedroom or separate bedroom), relatives, and period of living together with the patients. Interviews using questionnaire tool to observe the demographic data of patients as well as the risk factors habit such as sleeping proximity to leprosy case, relatives, and period of living together with the patients were performed for data collection.

Data analysis

The presence of a single band of PCR product (143 bp) corresponding to *M. leprae* DNA marked the clinical samples positive for *M. leprae* detection, while the absence of amplification band as compared to PCR controls marked them as negative. The sequenced data were analyzed using BLAST to match the sequence of the samples with the GenBank®. The epidemiology data was analyzed using univariate to describe the characteristics as well as bivariate to analyze the association of risk factors with SPSS software, version 21 (IBM Corp, USA).

RESULTS

There were some leprosy patients that did not live together with four persons in one house. Of total 134 household contacts, there were 27 persons who were

Table 1. Characteristics of the leprosy patients

Characteristics	n (%) (N = 35)
Male sex	21 (60.0)
Signs	
Patch	31 (88.6)
Infiltrate	2 (5.7)
Nodule erythema	2 (5.7)
Duration of symptoms	
≤1 year	26 (74.3)
>1 year	9 (25.7)
Classification of leprae	
MB	31 (88.6)
PB	4 (11.4)
Density housing	
High	7 (20.0)
Low	28 (80.0)

MB=multibacillary leprosy; PB=paucibacillary leprosy
Low-density housing was defined as <1 residents per 8 m², otherwise it would be considered as high

Table 2. Risk factors analysis for *M. leprae* detection in household contacts

Risk factors	*M. leprae*, n (%) (N = 107)		OR	95% CI	*p*
	Positive	Negative			
Blood glucose					
Normal	16 (15.0)	55 (51.4)	1.804	0.603–5.399	0.440
High	5 (4.7)	31 (29.0)			
Ethnic					
Papua	20 (18.7)	74 (69.2)	3.243	0.398–26.457	0.456
Non-Papua	1 (0.9)	12 (11.2)			
Period of living together					
≤1 year	1 (0.9)	33 (30.8)	12.45	1.595–97.20	**0.002**
>1 year	20 (18.7)	53 (49.5)			
Sleeping proximity to a leprosy case					
In 1 bedroom	4 (3.7)	10 (9.3)	1.788	0.501–6.388	0.468
Separate bedroom	17 (15.9)	76 (71.0)			
Relatives					
Familial	9 (8.4)	39 (36.4)	0.904	0.345–2.367	0.518
Non-familial*	12 (11.2)	47 (43.9)			

OR=odds ratio; CI=confidence interval
*Non-familial=non-first degree relative such as cousin or other relative such as neighbor. The association analysis was run using Cochran-Mantel-Haenszel statistics to observe the OR of the risk factors

excluded (8 persons declined to participate and 19 persons had low quality of DNA). Hence, there were 107 household contacts from 35 leprosy patients included in this study. The leprosy cases were involved in point of care, in-depth interviews, and sample collection. For the patients who signed informed consent form, house visits were conducted for in-depth interviews and sample collection. Table 1 lists the characteristics of 35

Figure 1. Agarose gel electrophoresis for polymerase chain reaction (PCR) assay. The size of target sequence amplified using LP1 and LP2 primers is 143 bp. Three index cases are represented with codes P, A, and S at the first character of the code above; C at the last character indicates the slit-skin samples and N indicates the nasal swab samples. Code m indicated 100 bp marker. No band was observed for negative control and the presence of 143 bp band corresponding to presence of *Mycobacterium leprae*. bp=base pair

leprosy cases. The results listed in Table 1 reveal that most of the leprosy patients included in the study were male and having main symptom of patch for less than 1 year. Multibacillary patients were higher in number than paucibacillary. Most of these patients lived in houses with a density ≥8 m² floor area, which was still within the standard criteria for a healthy house.

PCR assay on nasal swab specimens of household contacts further showed that 19.62% (n = 21) of household contacts were exposed to *M. leprae*. The presence of 143 bp of target sequence in PCR products was confirmed using agarose gel electrophoresis (Figure 1). To confirm these results, sequencing was performed on randomly selected PCR positive samples. For both index cases as well as household contacts, eight samples were randomly selected and sequenced on Sanger cycle sequencing machine. The results from BLAST studies showed a similarity of ≥90% between PCR positive samples and *M. leprae*.

Table 2 lists the characteristics and transmission risk factors associated with household contacts included in the study. Field observations and in-depth interviews indicated that most of the participants recruited as household contacts were females. Distant family members dominated the list as compared to first-degree relatives. Most of these household contacts were living
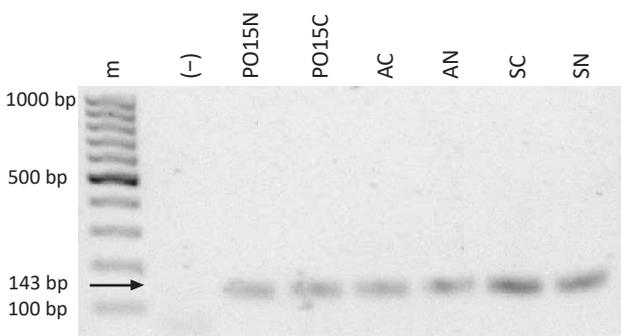
with the patients in different bedrooms for more than a year. Most of them showed normal blood glucose levels as indicated by blood glucose examination results. The household contacts that live more than 1 year with the leprosy patient showed that the risk of having *M. leprae* were 12 times higher than those living away from the patient. Other factors such as blood glucose, ethnic, sleeping proximity to leprosy patient, and relatives give no effect to the *M. leprae* detection in household contacts.

DISCUSSION

We are interested in elucidating the characteristics of leprosy cases living in Jayapura and the transmission of *M. leprae* from these patients to household contacts. A molecular biology approach was used to detect the presence of *M. leprae* in asymptomatic household contacts. More than one nuclear family living together under the same roof is a common cultural practice in Papua.

PCR studies showed that 19.62% of household contacts were positive for *M. leprae* presence. This technique has been previously used for different types of samples including urine,[14] blood,[15] saliva,[16] archeological skeletal,[17] and nasal swab.[18,19] Nasal swab specimens from leprosy patients and household contacts were collected for PCR assay. In 1993, a study conducted in Makassar reported 7.8% of 1,228 cases, including both household contacts and residents having no interaction with patients in endemic areas, to be PCR positive.[20] In another study by Klatser et al,[21] PCR was applied for *M. leprae* detection in nasal swab specimens which were collected during a total population survey conducted in two villages located in South Sulawesi, another leprosy endemic area in Indonesia. The results of that study confirmed the nasal carriage of *M. leprae* among the general population in an area endemic for leprosy and this feature was not restricted to few patients.[21]

The percentage of household contacts with positive for *M. leprae* detection in the present study is similar to the study conducted in a hyper-endemic leprosy area in Brazil. The authors reported the presence of bacillary DNA in 23.89% of asymptomatic household contacts using qPCR.[22] Another study in Colombia, combined three methods of detection to evaluate *M. leprae* detection in household contacts. For 402 household contacts linked to 104 index patients included in the

study, 13.4% showed IgM seropositivity, 9.4% were Mitsuda-negative, and 5.5% were found positive using PCR of nasal swab specimens.[23] In another study conducted in Columbia post-elimination, 16% (18 of 113) household contacts were found PCR positive for the presence of *M. leprae* in the nasal swab samples. Genotyping of the *M. leprae* strain present in the index cases and household contacts was performed and similarities was reported between the two strains in both groups.[24]

In Brazil, da Costa Martins et al[18] used a different approach for PCR where serological examination of the phenolic glycolipid 1 antigen was used to detect the transmission of *M. leprae* from index case to contact case. Although, there was sera positive in household contacts, the clinical form was not appeared as in the index cases; nevertheless, a tendency higher rate of multibacillary for index cases was reported. The clinical form of index case was unrelated to serum positivity of contact as this index case was not the primary case or the source of transmission for the secondary case.[18] In a study by Pattyn et al,[19] 23 nose swab specimens obtained from eight multibacillary patients were examined and found three cases of leprosy-positive. This study also used PCR based method for detection of *M. leprae* in nasal swab samples.

The rate of DNA detection in nasal swabs obtained from contacts varies from 1 to 10%, which sometimes depends on the clinical form of the index cases. However, the high rate of *M. leprae* detection among healthy populations using PCR test questions the feasibility of this method as a diagnostic tool for leprosy risk evaluation, particularly for this area of sample collection. A separate study showed that the household contacts with positive anti-phenolic glycolipid-I test[3] from blood samples and PCR assay showed a higher risk of leprosy development.[25] Thus, combining PCR and serological test for leprosy detection can increase the accuracy of the disease prediction. For future studies, accurate prediction of risk for developing leprosy in household contacts might be a useful tool for deciding priority for chemoprophylaxis blanket area and providing early treatment before any spread out of leprosy manifestation.[26] The transmission of leprosy can be greatly curbed by providing necessary treatment at early stages.

Various risk factors affecting leprosy exposure in household contacts were also evaluated in the present study (Table 2). These included blood glucose levels,

ethnicity, period of living together with the patients, sleeping proximity with the leprosy case, and relatives. Among these, period of living together with the patient for more than a year increased the risk of *M. leprae* detection by 12.45 times (*p* = 0.002). Some studies have shown that *M. leprae* is transmitted via inhalation,[19,25] which might explain the correlation observed between the period of living together and exposure to *M. leprae*. The results of this study also revealed that most of the index cases were multibacillary that have a higher risk of transmission than paucibacillary because multibacillary patients carry the bacteria in their nasal mucosa.[25]

In Papua, living together with more than one nuclear family is a common cultural. Most of the houses included in the study has 8 m^2 or more floor area which meets the criteria of a healthy house. However, the surrounding environment observed during this study reported the study site to be one of the dirty and crowded areas of Jayapura. Association between house conditions and risk for leprosy development has been discussed in previous studies. According to Pescarini et al,[27] socio-economic status including house conditions strongly affects leprosy transmission. The results from another study conducted in a different population indicated that socio-economic conditions such as sanitation, house conditions, and economic status increase the risk of leprosy development.[28]

Household contact is the group at highest risk for leprosy transmission. This idea is supported by the findings of a cohort study conducted in Rio de Janeiro from 1987–2010 which revealed that 4.9% of household contacts developed leprosy.[14] In the present study, positive results obtained from PCR of nasal swab specimens of household contacts indicated the presence of active bacterial transmission via inhalation in Papua. Based on these findings, we can predict that about 19.6% of household contacts found positive in PCR studies are at higher risk of developing leprosy in the area of study. Since Indonesia aims to reach leprosy elimination by 2020, intensive and breakthrough approaches such as chemoprophylaxis blanket, intensive case finding, and integrated family health movement should be applied in Papua to cut this transmission chain and endorse the health quality in this leprosy pocket.

The limitations of this study are only four household members were recruited from one confirmed case; a single detection method based on PCR, and a small sample size. Furthermore, PCR positive samples were randomly selected for Sanger sequencing for cases as well as household samples. However, this study shows that PCR can effectively detect *M. leprae* in nasal swab of household contacts and it can be utilized as an important tool for contact survey in combination with another assay for detection. In this leprosy elimination era, household contact surveys are very important especially in areas like Papua, one of the biggest leprosy pockets in Indonesia. The identification of new and efficient diagnostic tools for *M. leprae* detection in asymptomatic household contacts will support the efforts aimed at breaking the chain of transmission in endemic areas.

In conclusions, *M. leprae* was detected in 19.6% of asymptomatic household contacts. Among the various risk factors studied for leprosy transmission, duration of living together with the patient significantly increased the risk of *M. leprae* transmission.

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