High rate of *Mycobacterium leprae* infection and nasal colonization among household contacts of previously undiagnosed leprosy patients

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Submitted 29 April 2021; Accepted 14 June 2021

Summary

Objective This study examined the magnitude of *M. leprae* infection and nasal colonization among household contacts (HHCs) of newly diagnosed leprosy patients.

Methods A community-based cross sectional study was conducted from July to October 2019 in Fedis district, eastern Ethiopia. This study involved all healthy HHCs of previously undiagnosed leprosy patients and equal numbers of randomly selected healthy endemic controls. All study participants underwent a standard physical examination. For leprosy-suspected HHCs a bacteriological examination was done. Nasal colonization among HHCs and endemic controls was evaluated for the presence of *M. leprae*-specific 500 bp repetitive elements (RLEP) using PCR. A structured questionnaire was used to obtain demographic, laboratory and leprosy-related clinical history.

Results In this study, 48 HHCs of previously undiagnosed leprosy patients were evaluated for leprosy. Four new leprosy cases (co-prevalent) were confirmed, giving a detection rate of 8.3% (95% CI = 2%, 19%); they were excluded from the HHCs study group. On PCR analysis, 5/44 (11.4%) (95% CI: 3%, 24%) HHCs and 3/15 (20%) (95% CI: 4%, 48%) untreated leprosy cases tested positive. Nasal secretion samples from all 44 healthy endemic controls tested negative by PCR. The positivity rate was significantly different among the study groups ($P = 0.022$) and disability grade is associated with PCR positivity ($P = 0.003$). However, nasal PCR positivity was not associated with the demographic characteristics of the study groups ($p > 0.05$).
Conclusion This study provides evidence that HHCs of untreated leprosy patients have clinical infections and are involved in carriage of bacilli. Therefore, HHCs tracing is important to improve early diagnosis and decipher the transmission chain.

Keywords: *M. leprae* infection, household contact, nasal carriage, Fedis

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Introduction

Leprosy or Hansen's disease is a chronic infectious disease caused by *Mycobacterium leprae*.¹ Disease transmission is ongoing, as indicated by the stable new case detection rate.² Thus, the disease continues to be an important public health challenge in leprosy endemic areas.^{1,3}

Most people residing in a leprosy-endemic setting have been exposed to *M. leprae* but few develop the disease.⁴ Solid evidence exists of an increased risk to individuals living in close contact with leprosy patients. Household contacts (HHCs) of untreated multi-bacillary (MB) leprosy patients are a population at high risk of developing the disease during their lifetime.⁵ These individuals may not only have subclinical infection, but may also be acting as *M. leprae* carriers and thus sources for transmission and infection.^{5,6} However, the identification of the initial point of infection is the most important and difficult problem in the study of *M. leprae* transmission.⁷

The detection of DNA in nasal swabs shows the existence of bacilli at the site of transmission and bacterial entrance.⁶ The nasal epithelium of untreated MB leprosy patients contributes to the shedding of *M. leprae* into the environment.⁸ In addition, the nasal mucosa of asymptomatic HHCs is heavily colonized by *M. leprae*, which has a critical significance in the prevention and control of leprosy.⁹ A method of early detection of those people prone to develop the most infectious form of leprosy would help to break the chain of transmission.¹⁰

In a disease with a long incubation period like leprosy, the sub-clinical infection phase is a potentially important opportunity to cure the disease before the signs and symptoms become apparent.¹¹ However, there is no test available that can easily detect asymptomatic *M. leprae* infection or predict progression of infection to clinical disease.¹² Early detection of leprosy represents a considerable barrier in present leprosy control programs¹³ and in particular, there are challenges in diagnosing early leprosy.¹⁴

The detection of *M. leprae* in asymptomatic HHCs of leprosy patients augments the concept that these individuals can contribute to the existing intensity of transmission.¹⁵ Household contact screening is helpful for early diagnosis,^{12,16} and would optimize leprosy control programs.¹⁷ The presence of bacilli in contacts has the potential to reflect occult leprosy, and must be followed by chemoprophylaxis.⁷ The increasing reliability of quantitative PCR (qPCR) makes it a promising tool for the early diagnosis of leprosy. Hence, qPCR can be used to estimate the risk of progress toward leprosy among HHCs.¹⁸

The Ethiopian national leprosy control strategic interventions recommend tracing close contacts of newly diagnosed leprosy patients to promote early identification of leprosy, thus decreasing its severity, and reducing transmission.¹⁹ However, the magnitude of leprosy from HHC tracing is not well studied in Ethiopia. Therefore, this study examined the magnitude of *M. leprae* infection and nasal colonization among HHCs of newly diagnosed leprosy patients in a leprosy endemic setting.

Methods

STUDY SETTING AND DESIGN

A comparative cross-sectional community based study was conducted in Fedis district of eastern Hararghe zone, Oromiya regional state from July to October 2019. Fedis district was selected because it is one district in East Hararghe Zone with a high leprosy burden. The capital city of the district, Boko, is 534 km east of Addis Ababa and 24 km to the south of Harar town, which is the capital city of the East Hararghe zone. The district contains 19 rural and 2 urban

villages with a total population estimated to be 133,382 persons, of which the estimated urban population is 26,575 and the estimated rural population is 127,877.^{20,21}

In the district, five health centers provide general health services, including tuberculosis and leprosy control activities. According to the Zonal Health Office report, in 2017, 57 new leprosy cases were on treatment in Fedis district, which gives a 4.3/10,000 prevalence rate.

SELECTION OF STUDY PARTICIPANTS

The study was conducted on the basis of a simple random selection of six kebeles (the smallest administrative unit in Ethiopia) in the Fedis district. A comprehensive baseline survey was conducted before this study. In this survey, health extension workers covered 4798 households to identify leprosy suspects. A total of 214 suspects were identified and 11 new leprosy patients were diagnosed. During the survey individuals with no known contact history of leprosy were also identified and used as a control group.

Individuals who had lived in the selected kebeles for more than six months were the source population. This study excludes individuals on treatment (Multi-drug treatment), those disinclined to participate and those with known previous contact history with treated leprosy patients.

For the HHC evaluation study, all HHCs of newly diagnosed leprosy cases, who had been living for more than six months in selected kebeles were included. As a comparisons group, an equal number of non-exposed individuals with no history of contact, were randomly selected as endemic controls (EC).

ETHICAL APPROVAL

The study was conducted according to the Helsinki Declaration and Ethiopian research regulations. The Institutional Health Research Ethics Review Committee (IHRERC) of the College of Health and Medical Sciences, Haramaya University, Ethiopia (ref no: IHRERC/152/2018) and the AHRI Ethics Committee (ref no: P002/18 AHRI/ERC) approved the protocol. To minimize the stigma, privacy was a priority during the examination of study subjects. All individuals confirmed as leprosy cases were started on treatment.

DATA COLLECTION PROCEDURE AND TOOLS CLINICAL EXAMINATION

Each study participant was examined for signs and symptoms of leprosy according to the national guidelines. Briefly, an individual who presents with pale or reddish patches (skin patch with discoloration) on the skin; painless swelling or lumps in the face and earlobes; loss of, or decreased, sensation on the skin; numbness or tingling of the hands and/or the feet; weakness of eyelids, hands or feet; painful and/or tender nerves; burning sensation in the skin; or painless wounds or burns on the hands or feet.¹⁹ The household contacts group was evaluated for clinical skin signs of leprosy by trained leprosy experts and a trained health officer.

BACTERIOLOGICAL EXAMINATION

Bacteriological examination of slit skin smears was done by Zielh Neelsen (ZN) stain for clinically suspect cases to confirm the diagnosis. Slit skin smears (SSS) taken from two sites (earlobe and active lesion) and stained with ZN to test for acid-alcohol-resistant bacilli. In brief, ZN staining was performed using carbol fuchsin for 10 min, discoloration with acid-alcohol for 3 minutes, and coloration of contrast with methylene blue for 2 min.¹⁹ The stained smear was examined under an oil immersion lens.

NASAL SWAB COLLECTION

Nasal samples were obtained from all index cases, HHCs, and randomly selected non-exposed individuals (EC) by gently rubbing a nasal swab (sterile hydraFolk) in the vestibule on each side of the nose. After collection, each swab was immersed in a sterile primeStore molecular transport medium (MTM) and stored at -20°C until processed.²² The samples were collected after the identification of the leprosy case (Index case) through the house to house the survey.

EXTRACTION OF DNA FROM NASAL SWAB

From nasal swab specimens, DNA was extracted and purified using a DNeasy Blood & Tissue Kit (catalog number 69504; QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions.²³

AMPLIFICATION

The *M. leprae*-specific repetitive element (RLEP) was the most frequently used marker.²⁴ Therefore, a PCR assay targeting RLEP was used in this study. Briefly, each specimen was amplified by PCR using a pair of primers to produce a 500 bp fragment of the *M. leprae*. The primers used were 5 μl of 2 μM RLEP 7 (5'-TGA GGC TTC GTG TGC TTT GC-3') and 5 μl of 2 μM RLEP 8 (5'-ATC TGC GCT AGA AGG TTG CC-3'); 25 μl hot start master mix, 10 μl molecular-grade water and 5 μl DNA template added to amplify 500 bp fragment in a final volume of 50 μl . Initial denaturation for 15 min at 95°C by 1 cycle followed by 39 cycles of denaturation for 30 s at 95°C , annealing for 40 s at 58°C , and extension at 72°C for 30 s and a final extension cycle of 10 min at 72°C .

DNA DETECTION BY CONVENTIONAL PCR

Each 50 μl amplicon was mixed with 10 μl loading dye and 10 μl of it loaded on 1% agarose gel prepared in a Tris-Acetate EDTA ($1 \times$ TAE buffer). The results were ascertained according to the presence or absence of specific bands visualized after agarose gel (1%), ethidium bromide staining, and UV transillumination. Positive and negative controls were included in each PCR run. PCR reaction without DNA (DNA free/molecular grade water) was used as the negative control (reagent control) and a known *M. leprae*-positive sample was used as the positive control.

QUESTIONNAIRE

Structured questionnaires were administered to household contacts and endemic controls to obtain data on demographic characteristics, laboratory and clinical history. Information related to leprosy diagnosis was documented, including WHO classification of leprosy, disability grade, contact history of leprosy, and previous history of leprosy. Data for index cases were collected from a previous active case finding study.

DEFINITIONS

Household contact: a family member or any person who lived under the same roof with the index case for more than six months.⁵

Endemic control: an individual without known contact with leprosy patients and clinical signs and symptoms² and living in the same endemic area with HHCs.

Leprosy case: a person with one of the cardinal signs of leprosy requiring chemotherapy: Definite loss of sensation in a pale (hypo-pigmented) or reddish skin lesion, thickened or enlarged peripheral nerve with or without tenderness; acid-fast bacilli in a slit skin smear.¹⁹

Paucibacillary (PB) case: a case of leprosy with 1 to 5 skin lesions, without the demonstration of bacilli in a skin smear.

Multibacillary (MB) case: a case of leprosy with more than five skin lesions; or with nerve involvement (pure neuritis, or any number of skin lesions and neuritis); or with the demonstrated the presence of bacilli in a slit-skin smear, irrespective of the number of skin lesions.¹⁹

STUDY VARIABLES

The outcome variables were prevalence leprosy and nasal DNA carriage. The independent variables include contact duration, age, sex, occupation, residence, educational status, and marital status of HHCs, index clinical form of leprosy, and bacteriological index.

DATA ANALYSIS

The Shapiro-Wilk test was used to test data normality. Comparisons between groups were carried out by Kruskal-Wallis and Chi-square tests. Statistical significance was considered at $p < 0.05$ in all tests.

Results

SOCIODEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

Prior to this study we identified, 11 previously undiagnosed leprosy patients. Among the new leprosy cases, 91% of them were MB and two of them presented with grade II disability (not shown in the table).

In this study, a total of 51 HHCs of previously undiagnosed leprosy patients were identified. While three of them declined to participate in the study, four of them were diagnosed with leprosy at initial screening and excluded from the nasal colonization study. Finally, 44 HHCs were included in this study. Of these, 65.9% of them were female and 58.3% were children age less than 15 years. As a negative control, equal numbers ($n = 44$) of healthy endemic controls with no known contact history of leprosy were included (Table 1).

All ECs had no known contact history with leprosy patients and were negative clinically during the screening. During the first contact surveillance, four household contacts were diagnosed with leprosy clinically giving a co-prevalence rate of 8.3%. Among the positive HHC cases, two of them were females, two of them were younger than 15 years, and all are MB patients and presented with no visible deformity. The majority, 41 (93.2%), of HHCs were contacts of MB patients. Cohabitation for more than 96 months was reported by 27% of contacts. 33.3% of the contacts were siblings and 20.8% were offspring of the index case.

Household contacts of the newly diagnosed leprosy cases were tested for exposure by analyzing nasal secretions using a 500 bp RLEP target (Figure 1). On PCR analysis, 5/44 (11.4%) (95% CI: 3%, 24%) HHCs tested positive. These HHCS who were colonized by *M. leprae* bacilli were all contacts of MB patients. Three (20%) (95% CI: 4%, 48%) of the 15 newly diagnosed MB leprosy patients showed positivity in their nasal secretion by PCR. Nasal secretion samples from all 44 healthy endemic controls (EC) tested negative by PCR.

The positivity rate in index cases was significantly higher than that in household contacts and endemic controls ($X^2 = 7.559$, $p = 0.022$) and was associated with their disability grade

Figure 1. Gel electrophoreses showing detection of *M. leprae* DNA from nasal swabs targeting the RLEP (500pb) region in nasal secretions collected from study participants in Fedis District, 2019; lane1:100bp ladder, lane 2 and 3: PCR positive sample from leprosy patients, lane 4&5: PCR positive from HHCs, lane 6 and 7: PCR negative healthy HHC, lane 8: PCR negative leprosy patients, lane 9&10: PCR negative healthy endemic controls, lane 11: blank, lane12: negative control, lane13: positive control.

Table 1. Sociodemographic characteristics of study participants included in the study for household contact evaluation in Fedis District, eastern Ethiopia between July 2019 and October 2019 ($n = 88$)

Variables	Variables category	Study participants	
		HHCs, n (%)	EC, n (%)
Sex	Male	15 (34.10)	19 (43.18)
	Female	29 (65.90)	25 (56.82)
Age category (in years)	Less than 15	25 (56.82)	8 (18.18)
	15 to 30	7 (15.91)	26 (59.09)
	31 to 45	6 (13.64)	7 (15.91)
	Greater than 45	6 (13.64)	3 (6.82)
Educational status	Illiterate	21 (47.73)	13 (29.55)
	primary	21 (47.73)	26 (59.09)
	Secondary	2 (4.55)	3 (6.82)
	Higher education	0	2 (4.55)
Marital status	Never married	29 (65.91)	15 (34.09)
	Married	15 (34.09)	29 (65.91)
Residence	Rural	21 (47.73)	28 (63.64)
	Urban	23 (52.30)	16 (36.36)

($\chi^2 = 8.615$, $P = 0.003$) (Table 2). There was no significant difference in PCR positivity between the sexes ($p = 0.622$), age groups ($P = 0.609$) and contact duration ($p = 0.712$).

Table 2. *M. leprae* DNA in nasal swabs detected by PCR from study participants in Fedis district, 2019

Nasal swab PCR	Index cases, <i>n</i> (%)	HHCs, <i>n</i> (%)	ECs, <i>n</i> (%)	χ^2 , <i>p</i> -value
Negative	8 (72.73%)	39 (88.6)	44 (100.00)	$\chi^2 = 7.559$, $P = 0.022$
Positive	3 (27.27)	5 (11.4)	0	

Discussion

Early detection of leprosy presents a considerable hurdle for leprosy control programs. In this HHC evaluation study, four new MB patients were already known of at the start. Eleven HHCs were diagnosed with leprosy during the HHCs screening at intake. These newly diagnosed co-prevalent untreated MB leprosy patients represented an infection source for other contacts.^{8,25} This finding supports the evidence that HHCs of patients with MB leprosy may have an increased risk of leprosy²⁶ which might be associated with biological and social factors among co-prevalent cases.²⁷ It is also an indication of the feasibility and contribution of active case-finding programs to promote early case detection by tracking HHCs.^{28,29} Therefore, leprosy interventions should target the screening of HHCs in order to further reduce the spread of leprosy within endemic areas.²⁶

The prevalence of leprosy among HHCs in this study is higher than in a study done in Brazil, where only 2% to 4% of HHCs were clinically diagnosed with leprosy at intake.^{16,30} Studies in Colombia also showed a lower leprosy detection rate among HHCs ranges from 0.4% to 4.5%.^{5,31} The difference in the detection rate among the HHCs could be due to variations in socioeconomic, epidemiological and genetic characteristics among the study participants.²⁵ *M. leprae* transmission may vary with environmental conditions.³²

In the present study, half of the newly diagnosed cases were children age less than 15 years old. The presence of childhood leprosy in this study is an indication of poor operational efficacy of the ongoing leprosy control program.^{33,34} This could be due to poor implementation of active case findings and HHC contact tracing in Ethiopia.³⁵ The presence of childhood leprosy among new cases is an indicator of recent transmission from an active case, with high endemicity in that area³⁶ and suggests ongoing transmission with an active infection focus in the community.^{26,37} The proportion of childhood cases among newly diagnosed cases in this study is higher than the national (11.7%) and Oromia regional state (13.3%).³⁸

In this study none of the HHCs diagnosed with leprosy had developed any visible deformity or disability at initial diagnosis, while 2 of 11 original index cases had Grade II disability at diagnosis. This is considerably higher than an earlier review in Ethiopia, which reported 9.8% grade II disabilities^{39,40} and the national report of 13.6% in 2016 among new patients.⁴¹ This could be evidence for the well-known fact that early screening of HHCs is helpful to promote early diagnosis before the development of visible deformities and important to stop transmission^{16,42} and would optimize the leprosy control program.^{16,17} This provides evidence for the need of early screening of HHCs for early diagnosis before the development of visible deformities.¹⁶

In the present study, *M. leprae* DNA was present in 11.4% nasal secretions of HHCs of untreated MB leprosy cases. This suggests that *M. leprae* is widespread among the healthy population²² and asymptomatic HHCs are heavily colonized by the causative agent of leprosy.^{8,9} Moreover, another study revealed that contacts of untreated MB cases are at risk for contact with *M. leprae* through the nasal mucosa.⁸ Our finding is also consistent with reports

in the literature showing the population from highly endemic pockets have exposure to *M. leprae* irrespective of the season.⁴³

The present results are in harmony with the previous study conducted by Patrocínio, *et al.* which showed PCR detection of *M. leprae* DNA in 10.1% of the contacts of untreated leprosy patients.⁷ Marcela Romero-Montoya also reported that 16% of nasal swabs taken from HHCs tested positive by RLEP PCR for *M. leprae* DNA.⁵ Also, a Bangladesh study by Tio-Coma *et al.* reported that *M. leprae* DNA was detected in 18.0% of HHCs.¹⁵ The finding is much lower than the study conducted by Ampel M and Araujo S, which revealed that *M. leprae* DNA was found in the nasal passages of about half of the HHCs of leprosy patients.⁴⁴ However, the finding is higher than the study done by using PCR analysis which revealed about 4% to 4.7% of contacts nasal mucosal had exposure before instituting treatment of their index cases.^{6,8}

In this study, all endemic controls were negative for nasal RLEP *M. leprae* DNA. This is contrary to the study by Carvalho *et al.* in a hyperendemic region of Brazil which showed 6% of healthy individuals (negative control group) were positive for qPCR in the nasal mucosa.⁹ A higher (28.1%) prevalence of RLEP positivity for the healthy student (external group) in a hyperendemic region of northeastern Brazil was also reported.⁴³ The variation in nasal colonization could be due to differences in meteorological factors such as temperature and humidity in the study area that can influence the dynamics of *M. leprae*.⁴⁵

Our data showed a statistical difference in the nasal carriage (colonization) among index cases, HHCs, and endemic controls. This finding is not in harmony with the study by Frota *et al.* in a hyperendemic region of northeastern Brazil which showed the presence of bacilli in the nasal secretion of leprosy cases and controls (who were not diagnosed with leprosy) was not statistically different.²²

Strengths and limitations of the study

Our results add to the basic understanding that *M. leprae* is shed from untreated MB patients and household contacts and defines the risk of exposure to their contacts. However, the study did not follow DNA tested positive HHCs to establish whether nasal bacilli are potential for initiating infection. Therefore, further research that requires follow-up of DNA-positive healthy HHCs for monitoring definitive evidence of early infection is needed.

Conclusion

This study presented evidence that HHCs of leprosy patients have clinical infections and are actively involved in bacilli carriage. Therefore, an active tracing of HHC is important to diagnose leprosy and decipher the transmission chain. Furthermore, HHCs of untreated leprosy patients should be a primary target of chemoprophylaxis and immunoprophylaxis to prevent leprosy.

Acknowledgements

The authors would like to acknowledge Fedis district health office, data collectors, Haramaya University and Armauer Hansen Research Institute (Ethiopia) for their support. Our great appreciation also goes to project supervisors and all study participants.

Authors' contributions

Mr. Kedir Bofe was involved in study conceptualization, investigation, obtaining permission for conducting the study, data collection, validation, data analysis and interpretation, and manuscript writing.

Dr. Kidist Bobosha, was involved in study conceptualization, investigation, project administration, validation, data curtain, supervision of the study and commenting on the manuscript.

Dr. Berhanu Seyoum, was involved in conceptualization, data analysis and interpretation, project administration, supervision, drafting and finalizing the manuscript for submission.

Mr. Fitsum Weledegbreale, was involved in project administration, data analysis, project supervision and drafting and finalizing the manuscript for submission

Dr. Biftu Geda, was involved in data analysis, data curtain, project supervision and drafting and finalizing the manuscript for submission

Dr. Adane Mihret, was involved in project administration, supervision and drafting and finalizing the manuscript for submission

Dr. Abraham Aseffa, was involved in was involved in study conceptualization, project administration, method validation, data curtain, visualization, supervision of the study and commenting on the manuscript.

Competing interests

The authors report no conflicts of interest in this work.

Funding

No funding was paid for this research.

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