Review Article

Recent Laboratory Advances in Diagnostics and Monitoring Response to Treatment in Leprosy

Abstract

The present review briefly summarizes the highlights of the recent advances in *Mycobacterium leprae*-specific tests for early diagnosis of leprosy. In addition to establishing the diagnosis of clinical cases of leprosy, these tests have also been used to detect subclinical infections in endemic population. Several attempts have been made from 1980 onward for standardization of specific diagnostic assays for early detection of leprosy. Brief account about the development and use of these assays has been described in this review article.

Keywords: *Antigens, leprosy, Mycobacterium leprae, polymerized chain reaction, serology*

Utpal Sengupta

Departemnt of Microbiology and Immunology,
Stanley Browne Laboratory,
TLM Community Hospital,
Nand Nagri, New Delhi, India

Introduction

Leprosy, a chronic infectious disease of skin and neurons, caused by *Mycobacterium leprae* (ML) and *Mycobacterium lepromatosis*, has been known to inflict humans from ancient times. It is still prevalent in many countries including India. India still hosts 63% of the world leprosy population,[1] and more than 70% new cases of leprosy in the world are detected in India every year.[2] Although India attained the elimination figure, a prevalence rate (PR) of less than 1 case per 10,000 population size (<0.9/10,000) in December 2005, but PR is still persisting at 0.74/10,000 (April 2017) indicating no significant decline in PR over the last decade. Furthermore ANCDR (annual new case detection rate) which had almost plateaued earlier, has demonstrated a rising trend recently (from 9.71/100,000 of 2016 to 10.12/100,000 in 2017).[3] All these trends indicate that despite the advent of multidrug therapy (MDT), burden and transmission of leprosy in India is still a matter of major health concern. In order to limit the burden and transmission of leprosy in the community, early detection and treatment is of utmost importance.

Leprosy is clinically diagnosed on the basis of presence of following cardinal signs: (i) hypopigmented or erythematous anesthetic patch on skin, (ii) thickened and/or tender peripheral or cutaneous nerve supplying the affected area, and (iii) acid fast bacilli in the skin smear. The disease manifests as spectrum of different clinical forms determined by the immune status of the host. This spectrum ranges from tuberculoid (TT) and borderline tuberculoid (BT) in patients having a strong cell-mediated immunity (CMI) and weak humoral immunity (HI) to borderline lepromatous (BL) and lepromatous (LL) forms in those with a robust HI and an almost nonexistent CMI to *M. leprae* with mid-borderline (BB) form lying in between. The spectrum of clinical manifestation has also been classified on an immunohistological and bacteriological scale by Ridley and Jopling.[4] In addition, there is a very early form of disease termed as indeterminate (I) leprosy which appears with small hypopigmented macules in skin without any loss of sensation. If it is left untreated, it may progress to other clinical forms or clears on its own owing to upgradation in CMI. Another form of leprosy which has been encountered frequently, remains confined to single or multiple nerves without involving the skin is termed as pure neuritic leprosy. Histopathological features of the involved nerves in pure neuritic leprosy is similar to that of involved skin in other forms of lprosy.[5] Diagnosis of leprosy thus can be made by the clinical signs alone; however, in absence of definitive cardinal features,

How to cite this article: Sengupta U. Recent laboratory advances in diagnostics and monitoring response to treatment in leprosy. Indian Dermatol Online J 2019;10:106-14.

Received: July, 2018. **Accepted:** September, 2018.

Address for correspondence:
Dr. Utpal Sengupta,
Stanley Browne Laboratory,
TLM Community Hospital,
Nand Nagri,
New Delhi - 110 093, India.
E-mail: usengupta2002@yahoo.com

Access this article online

Website: www.idoj.in

DOI: 10.4103/idoj.IDOJ_260_18

Quick Response Code:

confirmation of leprosy can be difficult in some patients especially in a non-endemic country.[6] Histopathology is the usual modality for confirmation of a clinically doubtful case of leprosy. However, other procedures like skin testing with *M. leprae* antigen (lepromin), antibody responses of the host to *M. leprae* and molecular techniques to detect the components of *M. leprae* in the lesions have also been used for diagnosis of leprosy at early stage. The present review discusses in detail the various approaches which have been developed and adopted for clinical diagnosis and monitoring of response in on-treatment leprosy cases.

Use of Lepromin

Lepromin is a saline suspension of whole *M. leprae*, which is inoculated intradermally on the volar surface of forearm to test the status of delayed type of hypersensitivity (DTH) response or CMI of an individual to the organism. While patients at the TT/BT end evoke a strong DTH skin reaction, those at the BL/LL end, fail to develop any skin reaction to lepromin. Later, *M. leprae* soluble antigens were prepared either by disruption or sonication of purified armadillo derived *M. leprae* and these soluble antigens of *M. leprae* also known as "leprosin" showed a background sensitization pattern of the population to mycobacterial antigens with bimodal distribution like lepromin.[7] These antigens have been better utilized for classification of leprosy and for evaluation of CMI of leprosy patients on treatment. As *M. leprae* have common sharing antigens with other environmental mycobacteria which are ubiquitous in the nature.[8] lepromin or leprosin test positivity will only indicate the status of CMI of the individual to *M. leprae* or cross-reactive mycobacterial antigens. As the positivity of these tests are not specific for *M. leprae* infection, lepromin or "leprosin" can't be used for diagnosis of leprosy. However, negative response to lepromin in a subject will indicate deficiency in host CMI to *M. leprae* and the individual may benefit from immunomodulation to boost the CMI to *M. leprae*. Therefore, there is a scope for lepromin to be used for mass survey to identify the prospective lepromin-negative candidates in a population who can benefit from vaccines to *M. leprae*.

Use of M. leprae–Specific Serology

Leprosy-specific serological tests emerged only after identification of *M. leprae*-specific antigens. Specificity and sensitivity of the serological assays have been summarized in Table 1.

Serological test using phenolic glycolipid-1

Phenolic glycolipid-1 (PGL-1) is one of the first mycobacterial antigens which was identified and isolated from the major glycolipid cell wall antigen of the bacterium.[9] Using this as an antigen, an enzyme-linked immunosorbent assay (ELISA) was developed initially for diagnosis of leprosy.[10-12] Although the sensitivity of this assay was 90-95% in BL/LL cases; the sensitivity was poor for detection of PB cases, (0-40%). Healthy endemic controls tested mostly negative in this assay for PGL-1 antibody and about 26% of household contacts were found to be positive. Later on, trisccharride[3,6-di-*O*-methyl-β-d-glucopyranosyl-(1→4)-2,3-di-*O*-methyl-α-l-rhamnopyranosyl-(1→2)-3-*O*-methyl-α-l-rhamnopyranose][13-15] and the disaccharide components of PGL-1 were found to be the components which react specifically with IgM antibodies in patients' sera.. Hence, these synthetic sugars, natural trisaccharide (NT), and natural disaccharide (ND) were synthesized individually and conjugated with either bovine serum albumin (BSA) or human serum albumin (HSA) using either octyl (O) or phenyl (P) linker arms (ND-O-BSA/HSA or NT-O-BSA/NT-P-BSA) and used in standardization of ELISA for diagnosis of leprosy.[16,17] It was noted that these glycoconjugates had higher affinity for IgM antibody than PGL-1[18] and showed a rising trend in the antibody levels from tuberculoid to lepromatous spectrum associated with increase in bacterial load.[19-22] However, a positive correlation with bacterial load and PGL-1 antibody levels was not always observed.[23] Using this neoglycoconjugate, newer assays known as *M. leprae* dipstick assay[24,25] and particle agglutination assay were developed.[26] In dipstick format, two antigen bands are present, one of which signifies reactivity to ND-O-BSA and the other is used as internal control for human IgM. The agreement of dipstick assay with ND-O-BSA-based assay was found to be 94.9%.[24] The other particle agglutination assay, gelatin particle agglutination test, was developed by the initial activation of colored gelatin particles by tannic acid and finally mixing with NT-P-BSA.[26] This NT-P-BSA-labeled gelatin particles agglutinated with serial two-fold dilutions of patientsera with an average cutoff value for positivity ranging between serum dilutions of 1:64 and 1:128.

In order to make the ML-dipstick assay suitable for field conditions, the neoglycoconjugate-based assay was modified by developeing it on a solid support using immunochromatographic technique in a lateral flow assay, termed as ML-flow test. In this assay, the nitrocellulose (NC) strips are loaded with 1-mm wide parallel lines of human IgM (positive control) and neoglycoconjugate, which react with the IgM antibody present in patientserum. The NC strip is encased in a plastic module with a sample charging slot and is followed by a reagent pad area for serum or whole blood sample with diluents to flow through and to be absorbed in the absorbent pad at the bottom of the case. Samples while flowing through the reagent pad pick up the colloidal gold-labeled antihuman IgM which binds specifically human IgM present on the parallel lines to give positive results for the test and IgM. The test is read generally within 10 min of charging of the samples. The specificity of the ML-flow test was found to be 90.2%. The agreement between ML-flow test and PGL-1 ELISA

Table 1:Comparative efficacies of immunological and molecular markers in diagnosis of leprosy

Name of the test	Types of leprosy		Sensitivity/Specificity (%)
	PB patients	MB patients	
	Percent (%) positivity		
Serological marker (PGL-1)	0-40%[10-12,27]#	70-95%[10-12,27]	91%[27]
Evaluation of dipstick assay using ND-O-BSA-based ELISA	-	-	94.9% concordance with ELISA[24]
ML-flow test	40%[27,28]	97.4%[27,28]	97.4%/90.2%[27,28]
35-kD-based serology	46.7%[39]	98.5%[39]	98.4%/100%[40]
			90%/97.5%[42]
NDO-LID rapid test	15.4-21.2%[58]	83.3-87%[58]	87%/96.1%[58]
PCR using gene targetRLEP	73%[67]	100%[67,68]	73.6%/100%[67]
	83%[70]	96.6%[71]	87.1%[72]
PCR using 16SrRNA gene target	50%[71]	100%[71]	51%/100%[70]
PCR using Ag85B gene target	80%9[72]	100%[72]	56%/100%[70]
PCR using 18kDa gene target	74%[73]	99%[73]	100%/83%[73]
Proline-rich antigen (pra-36 kDa)	36-60%[75-77]	87-100%[75-77]	-
Multiplex PCR	83%[68]	100%[68]	-

#Reference numbers have been super fixed against the values

was found to be 91%.[27] In addition, this test was found to be positive in 97.4% of multibacillary (MB) and 40% of paucibacillary (PB) cases and in 28.6% of household contacts. However, it was noted that in highly endemic countries, about 10% of uninfected individuals may be positive to PGLantibody.[10,27]

Status of PGL-1 antibody level with treatment

As antibody levels have been found to correlate with the bacterial load,[19-22] it is justified to presume that the levels of PGL-1 antibody will decline after adequate chemotherapy. Hence PGL-1 antibody-based serology could provide a method to monitor leprosy patients under treatment.[26] A recent cohort study with 105 leprosy patients on MDT regimen, followed up for 6 months to 2 years with periodic assessment of BI and PGL-1antibody levels, showed that monitoring of antibody levels in leprosy patients while on chemotherapy is useful in determining the efficacy of MDT.[28] Several other earlier studies also showed significant reduction in the levels of PGL-1 antibody after chemotherapy in leprosy patients.[28-33]

35-kD-based serology

Using immuno-biochemical technique, the evidence for the presence of 35kD protein in the membrane of *M. leprae* was established and the monoclonal antibody, MLO3-A1, reacted specifically with the epitope on 35kD antigen of ML.[34] After identification of the gene encoding 35kD of ML,[35] it could be cloned in *Mycobacterium smegmatis* and was available in sufficient quantities in pure form as recombinant 35kD (r35kD). It was revealed later that 82% and 90% DNA and amino acids, respectively, of ML 35kD are shared with another mycobacterial species *Mycobacterium avium*.[36] Another series of monoclonal antibody (MLO4) having specificity for the same 35kD was also utilized for serological studies. The assay was initially developed as a radioimmunoassay based on competitive inhibition between patient's serum and I^{125} labeled MLO4[37] and later was standardized as ELISA using horse radish peroxidase labeledMLO4.[38] Screening of a large number of blood samples of MB and PB patients with 35kD ELISA demonstrated a sensitivity of 98.5% and 46.7%, respectively.[39,40] A filter paper-based sample collection of blood from remote field area was standardized to perform field-based studies.[41] Inspite of 35kD antigen's sharing of some genes with *M. avium*, *Mycobacterium kansasii*, and *Mycobacterium paratuberculosis*, the standardized serodiagnostic assay was found to be 97.5% specific and 90% sensitive[42] in the diagnosis of leprosy. Another study which compared PGL-1-based ELISA with 35kDa-based serology, found both the assays to be reproducible and comparable.[43] Roche *et al*. compared PGL-1-based ELISA and 35-kD inhibition-based ELISA for their accuracy in diagnosis of leprosy with different levels of antibodies. It was noted that while PGL-1-based ELISA was suitable for diagnosis of cases with all the levels of antibodies, 35-kD inhibition-based ELISA did not perform well for diagnosis of patients having antibody levels near the cutoff value.[44] Later, r35kD was used directly for assessment of specificity and sensitivity. It was noted that while the specificity of the assay was 94.3%, the sensitivity for diagnosis of MB and PB cases were 83.0% and 17.0%, respectively.[44] The reason for low sensitivity of the assay with r35kD antigen may be due to the presence of cross-reactive mycobacterial proteins of *M. smegmatis* in the cloned purified recombinant protein or due to the presence of subclinical infection in the exposed contacts. Further, using both PGL-1 and r35kD, a dipstick ELISA was developed and compared with the conventional ELISA, and it was noted that there was a good concordance between the dipstick and conventional ELISA. A 35kD- test card identified 59% of untreated PB cases compared to that of 27% detection by PGL-1; however, the

sensitivity was found to be 90% by the r35kDtest card and 100% by PGL-1 dipstick.[44]

Status of 35kD antibody level with treatment

The number of anesthetic patches in patients has been shown to positively correlate with the level of antibody.[40,45] Later, the antibody levels were also found to correlate positively with the number of nerves involved in primary neuritic leprosy.[46] Attempts were also made to find out the presence of antibody in urine, cerebrospinal fluid and skin scraping samples of patients.[47-49] However, these samples were not superior to blood samples in diagnosing a case of leprosy. Antibody level against 35kD was found to decline following effective chemotherapy of patients.[50]

Search for new antibody reactive M. leprae recombinant proteins and development of LID-1 and NDO-LID rapid test

Considering a low level of false positivity with PGL-1 antigen, a large panel of expressed recombinant proteins was analyzed in a protein array format for their reactivity with categorized leprosy sera. Antigens, which reacted strongly with patients' sera and minimally with control sera, were selected for further analysis. The proteins selected were ML0405 and ML2331, which were found to be suitable for diagnosis of MB leprosy. These two proteins have been made as a fusion construct and have been named as LID-1 [Leprosy Infectious Disease Research Institute Diagnostic-1].[51] LID-1 has been shown to detect particularly MB cases in Brazil, China, Japan, and Philippines.[52-55] As PGL-1 or ND-O-BSA/HSA conjugate assay demonstrated positive results sometimes in uninfected controls as well,[27,55-57] LID-1 assay has been preferred[51] for diagnosis of MB leprosy. Both of these antigens LID-1 and ND-O-BSA have been synthetically conjugated to work in one platform and a rapid test based on NDO-LID has been developed and has been named as NDO-LID rapid test (Orange Life®, Rio de Janeiro, Brazil). NDO-LID kit is a ready-to-use kit for testing in field. Serum sample (10 µl) and running buffer (100 µl) are charged in the sample well causing the migration of sample and colloidal gold beads loaded with anti-IgG and anti-IgM through the membrane across the detection window. The reaction of the test and control yields a red color. Readings are recorded within 20 min of charging of samples. A clear development of the control line validates the test. A positive result is established when both the lines of control and test are developed. Visual reading scores are graded as 1+, 1.5+, and 2+ and development of a faint color or no color is considered as negative. For field application, a "point-of-care" assay was developed using a smart phone reader to record the density of color development.[58] These rapid tests detected higher proportion of leprosy cases compared to that of laboratory-based PGL-ELISA. Using this NDO-LID rapid test for MB cases, the positivity of PGL-ELISA was enhanced from 83.3% to 87% and for PB cases from 15.4% to 21.2%. The sensitivity and specificity of NDO-LID test were found to be 87% and 96.1%, respectively, in detection of MB cases.[58] Recently, while screening a Venezuelan MB population, no difference has been noted between the percentage of serological positivity using NDO-HSA, LID-1, and NDO-LID, although small sample size in the study could have the reason of the above observation.[59] Screening of household contact endemic normal population revealed that the frequency of anti-NDO-LID and anti-NDO-HSA positivity was much higher in general population than that of household contacts indicating subclinical infection or exposure of the community to the infection.[60]

Status of LID-1/NDO-LID antibody levels with treatment

It was noted that antibody level to LID-1 declined more rapidly after MDT regimen compared to that of PGL-1-antibody level.[61] Recently, in a study which detected antibody levels using all the three antigens PGL-1, LID-1, and NDO-LID, found that the antibody levels declined significantly after 6 months of uniform MDT (UMDT) or 12 months of full course of MDT. This reduction in antibody levels also correlated with reduction in bacillary load. Further, this group suggested that UMDT was noted to be similar to full course MDT in reduction of both the antibody levels and bacillary burden.[62]

Use of M. leprae-specific molecule employing polymerized chain reaction

M. leprae -specific polymerized chain reaction (PCR) can be routinely performed with a variety of biological specimens like skin biopsies, skin sections, skin smears, nerve sections, biological fluids such as blood, pleural effusions, ascetic fluid, cerebrospinal fluid, saliva, nasal swabs, etc. PCR is able to detect even 10–30 fg of *M. leprae* component which is equivalent to 2.8–8.3 bacilli.[63] Several stretches of *M. leprae* genome are specific for ML and therefore ML-specific PCRs were developed using genes like *RLEP*, *hsp65*, *18kDa*, *36kDa*, *16SrRNA*, *sodA*.[64-66] Most of these genes have been used singly for diagnosis of leprosy. A quantitative PCR (qPCR) was used in clinical samples using *RLEP*,[67-69] *16SrRNA*,[70,71] *Ag85B*,[72] *18kDa*,[73] *36kDa*,[74-77] gene targets and it was noted that *RLEP*-PCR was most sensitive out of all these specified gene-based PCRs.[70] *RLEP*-PCR was also used by several other workers and was found to be most sensitive and specific of all the other gene targets.[78,79]

Status of M. leprae specific PCR with treatment

ML-specific PCR can also be used for determination of the outcome after chemotherapy. As early as in 1993, a method employing–ML PCR was developed and it was noted that after 3,6,12, and 24 months of chemotherapy although there was no significant change in BI, the number of genomes detected by PCR reduced sharply which correlated with

the reduction in the morphological index of the bacilli.[80] In the recent past, a quantitative real-time (RT)-PCR based on *hsp18*mRNA, demonstrated that after 2 years of MDT treatment, no viable ML could be detected in 47 leprosy cases; however, considerable amount of DNA could be detected in many of these samples suggesting that RT-PCR could be used effectively in monitoring patients under chemotherapy.[81] The method using RT-PCR was not further developed because of its complexity to perform under field conditions. However, with the emergence of drug-resistant ML, this technique is presently being employed in reference laboratories in samples collected from the remote areas.[81,82] Specificity and sensitivity of the PCR-based assays have been listed in Table 1.

Use of molecular-based technology for drug resistance in leprosy

Emergence of drug resistance in leprosy has been recently reported from several countries including India.[81,82] As mouse foot pad technique for the detection of drug resistance takes a minimum of 6 months, molecular-based techniques in finding mutation in drug-resistant determining region (DRDR) of ML are being used in patients who are not responding to MDT. Many of the mutations responsible for drug resistance in *folP1 r*egion for DDS, *GyrA* region for Oflaxacin, and *rpoB* region for Rifampicin have been shown to be responsible for resistance in ML. Therefore, slit skin smears or biopsies preserved in 70% ethanol from patients not responding to treatment could be sent to reference laboratories for finding out mutations by gene sequencing in respective DRDR regions of drugs responsible for drug resistance.[82]

M. leprae-specific Antibody or PCR Positivity in the Context of Normal Household Contacts and Endemic Population

From the discussion above, it may be concluded that he above mentioned ML-specific antibody and PCR tests are valuable tools in the diagnosis of a doubtful or a definite case of leprosy. However, clear guidelines in case of positive results of any of these assays in household contacts or an individual from endemic population are lacking. It is known that many normal household contacts of cases turn out to be leprosy cases in future, and household contacts of MB cases have been shown to have 3.8–10-fold more chance of getting leprosy than the general population.[83-85] Several studies in Indonesia, India, and Brazil have indicated that in an endemic community as population are exposed to infection, the biological samples such as blood, nasal swabs, saliva, and slit-skin smears of contacts of patients remain positive either for ML-specific antibody or for specific component of ML.[86-91] How many of these biomarker-positive contacts of the population will transform into cases is generally uncertain and depends on the immune status of the individual having subclinical infection. These diagnostic tests are performed only once in individuals who pass through a dynamic state of the immune system, and therefore every individual who test positive to these assays do not develop leprosy in future. Rather, it has been noted in a 2-year follow-up study that large number of cases appear from the ML-specific test negative group from the community which outnumbers the cases that appear from the small cohort population of household contact group.[86] Therefore, these tests performed only at a single point of time may not be useful for prediction of a future case. However, these tests could be applied in a cohort population at risk under surveillance but will not prove to be a cost-effective proposition for the leprosy control program.

Conclusion

The above discussion has briefly described the recent progresses that has been made in the area of specific diagnostic tests for leprosy. Despite the attempts to develop a definitive early diagnostic test for leprosy especially for patients in whom cardinal signs of leprosy are not fulfilled, the objective of an ideal diagnostic test is still to be attained. Rather, these assays fail to detect almost 60% cases of PB leprosy patients demonstrating one of the cardinal signs. Another major concern with these tests is the positive results in significant number of contacts not showing any clinical signs of leprosy. These contacts have been found to have the same level of antibodies or markers of ML in their biological samples like early cases of PB leprosy. However, these antibody-based assays using any of the antigens like PGL-1 or LID-1 or NDO-LID may prove to be useful in cases of early diffuse lepromatous or MB leprosy having no major nerve deficit or thickening which may be missed by leprosy experts. In spite of the above advancement in technology, there is still a need for development in early diagnosis of leprosy. Future efforts could be directed to search for new and novel antigens or host biomarkers which will be mainly expressed only in subclinical, preclinical, and in early leprosy cases and at the same time will also be able to discriminate these cases from uninfected endemic contacts.

Acknowledgments

The author acknowledges the help provided by Dr. Ravindra P. Turankar, Dr. Mallika Lavania, and Dr. Itu Singh for scrutinizing the manuscript. Authors also acknowledge the Leprosy Mission Trust India for providing opportunity to write this review.

A brief research background of Dr. Utpal Sengupta

Dr. Utpal Sengupta completed his B.V.Sc. in 1963 from Bengal Veterinary College, Calcutta University. Later, in 1966, he obtained M.V.Sc. (Pathology) from Indian Veterinary Research Institute, Izatnagar. Further, in 1973,

he did PhD (Pathology) from Post Graduate Institute of Medical Education and Research, Chandigarh. He then specialized in Imuunopathology, Immunology, and Molecular Biological aspects and remained engaged in leprosy research from 1974 till today. He worked as a postdoctoral fellow under Dr. R.J.W. Rees at the National Institute for Medical Research, Mill Hill, London, UK. Later in 1976, he joined at the National JALMA Institute of Leprosy and Other Mycobacterial Diseases (NJIL and OMD) as Sr. Research Officer under ICMR and retired as Director of the Institute in 2001. After retirement, he worked as an Emeritus Scientist of ICMR for 5 years at NJIL and OMD. Following this, he worked as Laboratory Manager in a Bill Gates Funded project at ICMR for HIV prevalence in long distant truck drivers of Indian National Highways from 2006 to 2011. Later, in 2011, he joined as a Consultant to the Leprosy Mission Trust India (TLMTI) for overseeing the laboratory research activities of the Stanley Browne Laboratory of TLMTI. During his tenure of research in leprosy, he had published 215 research papers in leprosy journals (*International Journal of Leprosy*, *Indian Journal of Leprosy*, *Leprosy Review*) and in other journals with high impact factors like *Nature Genetics*, *Journal of Infectious Diseases*, *Journal of Clinical Microbiology*, *Clinical and Experimental Immunology*, *Transactions of The Royal Society of Tropical Medicine and Hygiene*, *Microbial Pathogens*, *Immunology Letters*, *Immunology Today*, *Infection and Immunity*, *American Journal of Infectious Diseases*, *CMI*, *Human Pathogen*, *AIDS*, *Microbes and Infection*, *Frontiers in Immunol*, etc.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. WHO. Weekly epidemiological record, September 1,2017;92:501-20. http://www.who.int/wer.
2. NLEP Annual Report, 2015-2016; WHO. Available from: http://www.who.int/lep/en/. [Last accessed on 2017 Sep 12].
3. NLEP Annual Report, 2016-2017; Available from: http://www.nlep.nic.in/. [Last accessed on 2018 Feb 22].
4. Ridley DS, Jopling WH. A classification of leprosy for research purposes. Lepr Rev1962;33:119-28.
5. Ridley DS, Ridley MJ. Classification of nerves is modified by the delayed recognition of *M. leprae*. Int J Lepr Oth Myc Dis 1986;54:596-600.
6. Forno C, Hausermann P, Hatz C, Itin P, Blum J. The difficulty in diagnosis and treatment of leprosy. J Travel Med 2010;17:281-3.
7. Gupte MD, Anantharaman DS, Nagaraju B, Kannan S, Vallishayee RS. Experiences with Mycobacterium leprae soluble antigens in a leprosy endemic population. Lepr Rev 1990;61:132-44.
8. Sengupta U. Experience and lessons from the use of lepromin and Mycobacterium leprae-specific serology. Workshop Proceedings. Lepr Rev 2000;71:S63-6.
9. Brennan PJ, Barrow WW. Evidence for species-specific lipid antigens in Mycobacterium leprae. Int J Lepr Other Mycobct Dis 1980;48:382-7.
10. Cho SN, Yanagihara DL, Hunter SW, Gelber RH, Brennan PJ. Serological specificity of phenolic glycolipid I from Mycobacterium leprae and use in serodiagnosis of leprosy. Infect Immun 1983;41:1077-83.
11. Young DB, Buchanan TM. A serological test for leprosy with a glycolipid specific for Mycobacterium leprae. Science 1983;221:1057-9.
12. Brett SJ, Draper P, Payne SN, Rees RJ. Serological activity of a characteristic phenolic glycolipid from Mycobacterium leprae in sera from patients with leprosy and tuberculosis. Clin Exp Immunol 1983;52:271-9.
13. Hunter SW, Fujiwara T, Brennan PJ. Structure and antigenicity of the major specific glycolipid antigen of Mycobacterium leprae. J Biol Chem 1982;257:15072-8.
14. Brett SJ, Payne SN, Draper P, Gigg R. Analysis of the major antigenic determinants of the characteristic phenolic glycolipid from Mycobacterium leprae. Clin Exp Immunol 1984;56:89-96.
15. Fujiwara T, Hunter SW, Cho S-N, Aspinall GO, Brennan PJ. Chemical synthesis and serology of disaccharides and trisacchaides of phenolic glycolipid antigens from the leprosy bacillus and preparation of a disaccharide protein conjugate for serodiagnosis of leprosy. Infect Immun 1984;43:245-52.
16. Chatterjee D, Cho SN, Brennan PJ, Aspinall GO. Chemical synthesis and seroreactivity of O-(3,6-di-O-methyl-beta-D-glucopyranosyl)-(1----4)-O-(2,3-di-O-methyl-alpha-L rhamnopyranosyl)-(1----9)-oxynonanoyl-bovine serum albumin--the leprosy-specific, natural disaccharide-octyl-neoglycoprotein. Carbohydr Res 1986;156:39-56.
17. Fujiwara T, Aspinall GO, Hunter SW, Brennan PJ. Chemical synthesis of the trisaccharide unit of the species-specific phenolic glycolipid from *Mycobacterium leprae*. Carbohydr Res 1987;163:41-52.
18. Gigg J, Gigg R, Payne S, Conant R. The allyl group for protection in carbohydrate chemistry. 17. Synthesis of propyl O-(3,6-di-O-methyl-beta-D-glucopyranosyl)-(1----4)-O-(2,3- di-O-methyl-alpha-L-rhamnopyranosyl)-(1----2)-3-O-methyl-alpha- L-rhamnopyranoside: The oligosaccharide portion of the major serologically active glycolipid from Mycobacterium leprae. Chem Phys Lipids 1985;38:299-307.
19. Schwerer B, Meeker HC, Sersen G, Levis WR. IgM antibodies against phenolic glycolipid I from Mycobacterium leprae in leprosy sera: Relationship to bacterial index and erythema nodosum leprosum. Acta Leprologica 1984;2:394-402.
20. Bach MA, Wallach D, Flageul B, Hoffenbach A, Cottenot F. Antibodies to phenolic glycolipid-1 and to whole Mycobacterium leprae in leprosy patients: Evolution during therapy. Int JLeprOther Mycobact Dis 1986;54:256-67.
21. Sinha S, McEntegart A, Girdhar BK, Bhatia AS, Sengupta U. Appraisal of two Mycobacterium leprae-specific serological assays for monitoring chemotherapy in lepromatous (LL/BL) leprosy patients. Int J Lepr Other Mycobact Dis 1989;57:24-32.
22. Roche PW, Britton WJ, Failbus SS, Williams D, Pradhan HM, Theuvenet WJ. Operational value of serological measurements in multibacillary leprosy patients: Clinical and bacteriological correlates of antibody responses. Int J Lepr Other Mycobact Dis 1990;58:480-90.
23. Lyons NF, Shannon EJ, Ellis BP, Naafs B. Association of IgG and IgM antibodies to phenolic glycolipid-1 antigen of Mycobacterium leprae with disease parameters in multibacillary

leprosy patients. Lepr Rev 1988;59:45-52.
24. Bührer-Sékula S, Cunha MGS, Ferreira WA, Klatser PR. The use of whole blood in a dipstick assay for detection of antibodies to Mycobacterium leprae: A field evaluation. FEMS Immunol Med Microbiol 1998;21:197-201.
25. Bührer-Sékula S, Cunha MGS, Foss NT, Oskam L, Faber WR, Klatser PR. Dipstick assay to identify leprosy patients who have an increased risk of relapse. Trop Med Int Health 2001;6:317-23.
26. Izumi S, Fujiwara T, Ikeda M, Nishimura Y, Sugiyama K, Kawatsu K. Novel gelatin particle agglutination test for serodiagnosis of leprosy in thefield. J Clin Microbiol 1990;28:525-9.
27. Bührer-Sékula S, Smits HL, Gussenhoven GC, van Leeuwen J, Amador S, Fujiwara T, *et al.* Simple and fast lateral flow test for classification of leprosy patients and identification of contacts with high risk of developing leprosy. J Clin Microbiol 2003;41:1991-5.
28. Zenha EMR, Ferreira, MAN, Foss NT. Use of anti PGL-1 antibodies to monitor therapy regimens in leprosy patients. Braz J Med Biol Res 2009;42:968-72.
29. Bach MA, Wallach D, Flageul B, Hoffenbach A, Cottenot F. Antibodies to phenolic glycolipid-1 and to whole Mycobacterium leprae in leprosy patients: Evolution during therapy. Int J Lepr Other Mycobact Dis 1986;54:256-67.
30. Roche PW, Britton WJ, Failbus SS, Neupane KD, Theuvenet WJ. Serological monitoring of the response to chemotherapy in leprosy patients. Int J Lepr Other Mycobact Dis 1993;61:35-43.
31. Chaturvedi V, Sinha S, Girdhar BK, Sengupta U. On the value of sequential serology with a Mycobacterium leprae-specific antibody competition ELISA in monitoring leprosy chemotherapy. Int J Lepr Other Mycobact Dis1991;59:32-40.
32. Drowart A, Chanteau S, Huygen K, Cock MD, Cartel JL, Bruyn JD, *et al.* Effects of chemotherapy on antibody levels directed against PGL-I and 85A and 85B protein antigens in lepromatous patients. Int J Lepr Other Mycobact Dis 1993;61:29-34.
33. Douglus JT, Steven LM, Fajardo T, Cellona RV, Madarang MG, Abalos RM, *et al.* The effects of chemotherapy on antibody levels in lepromatous patients. Lepr Rev 1988;59:127-35.
34. Mohagheghpour N, Munn MW, Gelber RH, Engleman EG. Identification of an immunostimulating protein from Mycobacterium leprae. Infect Immun 1990;58:703-10.
35. Winter N, Triccas JA, Revoire B, Pessolani MC, Eiglmeier K, Lim EM, *et al.* Characterisation of the gene encoding the immunodominant 35kDa protein of Mycobacterium leprae. Mol Microbiol 1995;16:865-76.
36. Triccas JA, Winter N, Roche PW, Gilpin A, Kendrick KE, Britton WJ. Molecular and immunological analysis of the Mycobacterium avium homolog of the immunodominant Mycobacterium leprae 35-kilodalton protein. Infect Immun 1998;66:2684-90.
37. Sinha S, Sengupta U, Ramu G, Ivanyi J. A serological test for leprosy based on competitive inhibition of monoclonal antibody binding to the MY2a determinant of *Mycobacterium leprae*. Transof Roy Soc Trop Med Hyg 1983;77:869-71.
38. Mwatha J, Moreno C, Sengupta U, Sinha S, Ivanyi J. A comparative evaluation of serological assays for lepromatous leprosy. Lepr Rev 1988;59:195-9.
39. Sinha S, Sengupta U, Ramu G, Ivanyi J. Serological survey of leprosy and control subjects by a monoclonal antibody based immunoassay. Int J Lepr Other Mycobact Dis 1985;53:33-8.
40. Sinha S, McEntergart A, Girdhar BK, Bhatia AS, Sengupta U. Appraisal of two Mycobacterium leprae-specific serological assays for monitoring chemotherapy in lepromatous (LL/BL) patients. Int J Lepr Other Mycobact Dis 1989;57:24-32.
41. Patil SA, Ramu G, Sinha S, Sengupta U. Screening of anti M. leprae antibodies in the blood samples eluted from filter paper blood blots. Int J Lepr Other Mycobact Dis 1990;58:123-6.
42. Triccas JA, Roche PW, Britton WJ. Specific serological diagnosis of leprosy with a recombinant Mycobacterium leprae protein purified from a rapidly growing mycobacterial host. J Clin Microbiol 1998;36:2363-5.
43. Parkash O. A study on the reproducibility of two serological assays for detection of Mycobacterium leprae infection. Int J Lepr Other Mycobact Dis 2001;69:46-8.
44. Roche PW, Failbus SS, Britton WJ, Cole R. Rapid method for diagnosis of leprosy by measurement of antibodies to the M. leprae 35kDa protein: Comparison with PGL-1 antibodies detected by ELISA and `Dip stick' methods. Int J Lepr Other Mycobact Dis 1999;67:279-86.
45. Chaturvedi V, Sinha S, Girdhar BK, Katoch K, Bhatia AS, Sengupta U. Association of mycobacterial-specific and Mycobacterium leprae specific antibody levels with clinical activity in tuberculoid leprosy: A comparative study of three serological enzyme-immunoassays. Lepr Rev1991;62:122-33.
46. Roche PW, Britton WJ, Failbus SS, Theuvenet WJ, Lavender M, Adiga RB. Serological responses in primary neuritic leprosy. Trans Royal Soc Trop Med Hyg 1991;85:299-302.
47. Patil S, Dwivedi P, Kiran K, Singh KP, Katoch K, Sengupta U. Detection of antibodies to 35kDa determinant of M.leprae in urine and serum of leprosy patients. Acta Leprol 1990;7:139-43.
48. Patil SA, Katoch K, Ramu G, Sengupta U. Detection of antibodies against phenolic glycolipid-1 (PGL-1), 35kDa and 30-40kDa components of Mycobacterium leprae in the cerebrospinal fluid of leprosy patients. J Med Microbiol 1995;43:115-9.
49. Parkash O, Beuria MK, Girdhar BK, Katoch K, Sengupta U. Efforts in diagnosing early leprosy using serological techniques. J Biosci 1997;22:111-6.
50. Chaturvedi V, Sinha S, Girdhar BK, Sengupta U. On the value of sequential serology with a Mycobacterium leprae-specific antibody competition ELISA in monitoring leprosy chemotherapy. Int J Lepr Other Mycobact Dis 1991;59:32-40.
51. Duthie MS, Ireton GC, Kanaujia GV, Goto W, Liang H, Bhatia A, *et al.* Selection of antigens and development of prototype tests for point-of-care leprosy diagnosis. Clin Vaccine Immunol 2008;15:1590-7.
52. Duthie MS, Goto W, Ireton GC, Reece ST, Cardoso LP, Martelli CM, *et al.* Use of protein antigens for early serological diagnosis of leprosy. Clin Vaccine Immunol 2007;14:1400-8.
53. Spencer JS, Duthie MS, Geluk A, Balagon MF, Kim HJ, Wheat WH, *et al.* Identification of serological biomarkers of infection, disease progression and treatment efficacy for leprosy. Mem Inst Oswaldo Cruz 2012;107:79-89.
54. Hungria EM, de Oliveira RM, Souza AL, Costa MB, Souza VN, Silva EA, *et al.* Seroreactivity to new Mycobacterium leprae protein antigens in different leprosy-endemic regions in Brazil. Mem Inst Oswaldo Cruz 2012;107:104-11.
55. Qiong-Hua P, Zhong-Yi Z, Jun Y, Yan W, Lian-Chao Y, Huan-Ying L, *et al.* Early revelation of leprosy in China by sequential antibody analyses with LID-1 and PGL-I. J Trop Med 2013;2013.doi: 10.1155/2013/352689.
56. Oskam L, Slim E, Bührer-Se´kula S. Serology: Recent developments, strengths, limitations and prospects: A state of the art overview. Lepr Rev 2003;74:196-205.
57. Cho SN, Cellona RV, Villahermosa LG, Fajardo TT Jr, Balagon MV, Abalos RM, *et al*. Detection of phenolic glycolipid

I of Mycobacterium leprae in sera from leprosy patients before and after start of multidrug therapy. Clin Diagn Lab Immunol 2001;8:138-42.
58. Cardoso LPV, Dias RF, Freitas AA, Hungria EM, Oliveira RM, Collovati M, *et al.* Development of a quantitative rapid diagnostic test for multibacillary leprosy using smart phone technology. BMC Inf Dis 2013;13:497.
59. Rada EM, Duthie MS, Bellorin D, Morales S, Crespo L. Clinical presentation and serum antibody reactivity of leprosy patients attending a dermatology clinic in Caracas, Venezuela. Lepr Rev 2017;88:131-41.
60. Fabri ACOC, Carvalho APM, Araujo S, Goulart LR, Goulart IMB, Mattos AMM, *et al.* Antigen-specific assessment of the immunological status of various groups in a leprosy endemic region. BMC Inf Dis 2015;15:218.
61. Spencer JS, Duthie MS, Geluk A, Balagon M, Kim HJ, Wheat WH, *et al.* Identification nof serological biomarkers of infection, disease progression and treatment efficacy for leprosy. Mem Inst Oswaldo Cruz 2012;107:79-89.
62. Hungria EM, Bührer-Sékula S, Oliveira RM, Aderaldo LC, Pontes MAA, Cruz R, *et al. Mycobacterium leprae*- specific antibodies in multibacillary patients decrease during and after treatment with either regular 12 doses MDT or the uniform 6 doses MDT. Front Immunol 2018;9:915.
63. Bang PD, Suzuki K, Phuong LT, Chu TM, Ishii N, Khang TH. Evaluation of polymerase chain-reaction based detection of Mycobacterium leprae for the diagnosis of leprosy. J Dermatol 2009;36:269-76.
64. Gillis TP, Williams DL. Polymerase chain reaction and leprosy. Int J Lepr Other Mycobact Dis 1991;59:311-6.
65. Martinez AN, Lahiri R, Pitman TL, Scollard D, Truman R, Moraes MO, *et al.* Molecular determination Micobacterium leprae viability by use of real-time PCR. J Clin Microbiol 2009;47:2124-30.
66. Donoghue HD, Holton J, Spigelman M. PCR primers that can detect low levels of *Mycobacterium leprae* DNA. J Med Microbiol 2001;50:177-82.
67. Goulart IM, Cardoso AM, Santos MS, Goncalves MA, Pereira JE, Goulart LR. Detection of Mycobacterium leprae DNA in skin lesions of leprosy patients by PCR may be affected by amplicon size. Arch Dermatol Res 2007;299:267-71.
68. Banerjee S, Sarkar K, Gupta S, Mahapatra PS, Gupta S, Guha S, *et al.* Multiplex PCR technique could be an alternative approach for early detection of leprosy among close contacts–a pilot study from India. BMC Infect Dis 2010;10:252.
69. Yoon KH, Cho SN, Lee MK, Abalos RM, Cellona RV, Fajardo TT, *et al.* Evaluation of polymerase chain reaction amplification of Mycobacterium leprae-specific repetitive sequence in biopsy specimens from leprosy patients. J Clin Microbiol 1993;31:895-9.
70. Martinez AN, Ribeiro-Alves M, Sarno EN, Moraes MO Evaluation of qPCR-based assays for leprosy diagnosis directly in clinical specimens. PLOS Negl Trop Dis 2011;5:e1354.
71. Rudeeaneksin J, Srisungngam S, Sawanpanyalert P, Sittiwakin T, Likanonsakul S, Pasadom S, *et al.* Light cycler real-time PCR for rapid detection and quantitation of Mycobacterium leprae in skin specimens. FEMS Immunol Med Microbiol 2008;54:263-70.
72. Martinez AN, Britto CF, Nery JA, Sampaio EP, Jardim MR, Sarno EN, *et al.* Evaluation of real-time and conventional PCR targeting complex 85 genes for detection of Mycobacterium leprae DNA in skin biopsy samples from patients diagnosed with leprosy. J Clin Microbiol 2006;44:3154-9.
73. Williams DL, Gillis TP, Fiallo P, Job CK, Gelber RH, Hill C, *et al.* Detection of Mycobacterium leprae and the potential for monitoring antileprosy drug therapy directly from skin biopsies by PCR. Mol Cell Probes 1992;6:401-10.
74. Scollard DM, Gillis TP, Williams DL. Polymerase chain reaction assay for the detection and identification of Mycobacterium leprae in patients in the United States. Am J Clin Pathol 1998;109:642-6.
75. Kampirapap K, Singtham N, Klatser PR, Wiriyawipart S. DNA amplification for detection of leprosy and assessment of efficacy of leprosy chemotherapy. Int J Lepr Other Mycobact Dis 1998;66:16-21.
76. Wichitwechkarn J, Karnjan S, Shuntawuttisettee S, Sornprasit C, Kampirapap K, Peerapacom S, *et al.* Detection of Mycobacterium leprae infection by PCR. J Clin Microbiol 1995;33:45-9.
77. Turankar RP, Pandey S, Lavania M, Singh I, Nigam A, Darlong J, *et al.* Comparative evaluation of PCR amplification of RLEP, 16SrRNA, rpoT and SodA gene targets for detection of M. leprae DNA from clinical and environmental samples. Int J Mycbacteriol 2015;4:54-9.
78. Kamal R, Dayal R, Gaidhankar K, Biswas S, Gupta SB, Kumar N, *et al.* RLEP PCR as a definitive diagnostic test for leprosy from skin smear samples in childhood and adolescent leprosy. Int J Lepr Other Mycobact Dis 2016;88:193-7.
79. Jamil S, Keer JT, Dockrell HM, Stoker MG, Lucas SB, Chiang TJ, *et al.* Use of polymerase chain reaction to assess efficacy of leprosy chemotherapy. Lancet 1993;342:264-8.
80. Lini N, Shankarnarayan PN, Dharmalingam K. Quantitative real-time PCR analysis oif Mycobacterium leprae DNA and mRNA in human biopsy material from leprosy and reactional cases. J Med Microbiol 2009;58:753-9.
81. Lavania M, Jadhav RS, Chaitanya VS, Turankar R, Abraham S, Das L, *et al.* Drug resistance patterns in Mycobacterium leprae isolates from relapsed leprosy patients attending The Leprosy Mission (TLM) hospitals in India. Lepr Rev 2014;85:177-85.
82. Cambau E, Saunderson P, Matsuoka M, Cole ST, Kai M, Suffys P, *et al.* Antimicrobial resistance in leprosy: Results of the first prospective open survey conducted by a WHO surveillance network for the period 2009-2015. Clin Microbiol Infec 2018. doi: 10.1016/j.cmi. 2018.02.022.
83. Fine PE, Sterne JA, Ponninghaus JM, Bliss L, Saul J, Chihana A, *et al.* Household and dwelling contact as risk factors for leprosy in northern Malawi. Am J Epidemiol 1997;146:91-102.
84. Van Beers S, Hatta M, Klatser PR. Seroprevalence rates of antibodies to phenolic glycolipid-I among school children as an indicator of leprosy endemicity. Int J Lepr 1999;67:243-9.
85. Goulart IMB, Souza DOB, Marques CR, Pimenta VL, Goncalves MA, Goulart LR. Risk and protective factors for leprosy development determined by epidemiological surveillance of household contacts. Clin Vaccine Immunol 2008;15:101-5.
86. Sinha S, Kannan S, Nagarju B, Sengupta U, Gupte MD. Utility of serodiagnostic tests for leprosy: A study in an endemic population in South India. Lepr Rev 2004;75:266-73.
87. Turankar R, Pandey S, Lavania M, Singh I, Nigam A, Darlong J, *et al.* Comparative evaluation of PCR amplification of RLEP, 16SrRNA, rpoT and SodA gene targets for detection of *M. leprae* DNA from clinical and environmental samples. Int J Mycobact 2015;4:54-9.
88. Lavania M, Turankar RP, Karri S, Chaitanya VS, Sengupta U, Jadav RS. Cohort study of the nasal of the nasal carriage and the presence of *Mycobacterium leprae* in an endemic area in the general population. Clin Microbiol Infect 2013;19:970-4.
89. de Macedo AC, Cunha JE Jr, Yaochete JNU, Taveres CM, Nagao-Dias AT. Salivary anti-PGL-1 IgM may indicate active

transmission of Mycobacyerium leprae among young people under 16 years of age. Braz J Infect Dis 2017;21:557-61.
90. Siwakoti S, Rai K, Bhattarai NR, Agarwal S, Khanal B. Evaluation of polymerase chain reaction (PCR) with slit-skin-smear (SSS) examination to confirm clinical diagnosis of leprosy in eastern Nepal. PloS Negl Trop Dis 2016;10:e0005220.
91. Van Beers S, Hatta M, Klatser PR. Seroprevalence rates of antibodies to phenolic glycolipid-I among school children as an indicator of leprosy endemicity. Int J Lepr1999;67:243-9.