DISCUSSION

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CONCLUSION
Discussion

Despite the gradually decreasing prevalence and incidence of leprosy, the incidence rate of atypical cases is increasing, calling for more sensitive and specific methods of \textit{M. leprae} detection in referral settings. In addition to conventional methods of defining leprosy such as hypopigmented or reddish skin lesions with definite loss of sensation, involvement of the peripheral nerves, as demonstrated by definite thickening with loss of sensation and skin smear positive for acid fast bacilli, serological examination and nucleic acid probes have been suggested for leprosy diagnosis. Most leprosy patients are diagnosed based on the results from conventional methods such as clinical examination, skin smear and histopathology. Unfortunately, none of these tests have shown sufficient sensitivity or specificity to serve as routine diagnostic tools for leprosy.

Detection of leprosy cases is difficult as the disease is usually asymptomatic in the early stage. Again due to social stigma, leprosy patients usually report late to the clinicians. More over in this background, traditional parameters like bacteriological index and histopathology needs a lot of pursuance. As there is no method, which is “Gold Standard” for, diagnosis of leprosy and as the organism \textit{M. leprae} is not cultivable in vitro, efforts are being taken worldwide as a constant endeavour in search of an alternative method for the diagnosis of the disease properly.

As stated earlier, the diagnosis by Acid Fast Staining requires at least $10^4$ organisms per gram of tissue for reliable detection. So, the sensitivity of BI is low particularly low in case of tuberculoid spectrum where the bacillary load is low.

The analysis of Histopathological slides requires expertise. Again there remains every chance of inter observer variation in interpretation of the histopathological picture of the tissue section for diagnosis of leprosy.
In developing countries, where most new cases are detected, clinical signs and skin smears are still main tools for detection. The WHO has urged the development of simple diagnostic technologies, because that would help facilitate access to a greater number of leprosy patients. The major advantages of PCR over the other conventional diagnostic tools are that it is a rapid, specific and sensitive approach for the identification of the *M. leprae*. It is possible to utilize crude biological samples for PCR with no need for isolation or growth on culture. This is particularly important when trying to identify organisms that are difficult to be cultured like *M. leprae*. (111)

We initiated the study to develop multiplex polymerase chain reaction (PCR) as a better diagnostic tool for detection of leprosy at an early stage. In this study, (as explained in detail previously under Section 1) out of 340 subjects 250 subjects were included as study subjects and 80 subjects were taken as controls, that are free from any anaesthetic and hypopigmented patches, which is the characteristic of leprosy; the remaining 10 being indeterminate subjects. The study was done in Eastern India to develop and evaluate the efficacy of the newly developed Multiplex PCR. (27)

In our Multiplex PCR, the primer R1 and R2 is very specific for *M. leprae* and was absent in 20 other mycobacterial species and the primers TTCA and TTCB is specific for multibacillary patients only and was absent in paucibacillary and other Mycobacterial species.

This Multiplex PCR is highly sensitive (Sensitivity is about 85.5% in our study). Polymerase Chain Reaction can detect even 1-100 organisms in a tissue specimen. In this study more than 75% of biopsy samples with no detectable AFB by microscopic examination showed amplification of the 372 bp DNA of *M. leprae*. (27) In our study it has been shown that Multiplex PCR can diagnose about 96.4% of MB cases and 75.2% of the PB cases. More
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Percentage case detection for MB cases is attributed to the fact that the bacterial load is higher in these cases. But in case of PB where the bacterial load is low, thus posing a difficulty in diagnosis by traditional methods, the 75.5% case detection rate carries a meaningful result. Thus, it is clear that Multiplex PCR is much advantageous than the traditional methods in the diagnosis of leprosy. More over 30% of the indeterminate cases could be detected by MPCR which could not be detected otherwise by traditional methods.

Though the prevalence of leprosy is declining, new case detection rate is either stable or increasing. New case detection rate is better indicator of the disease, as it is not affected by the changing case definition or duration of the treatment. According to WHO, the success of eradication was based on the hypothesis that a prevalence of <1 case per 10,000 population is inconsistent. The International Leprosy Association's Technical Forum noted that there is little evidence to support this hypothesis as decline in the prevalence rate may not really indicate the elimination of the disease as (i) leprosy has long incubation period ranging from 2 to 20 years (ii) patients who are newly diagnosed with leprosy may have transmitted the disease to others in the family. (113)

Moreover, the true incidence of leprosy is difficult to measure and the rate of infection in a community cannot be measured in contrary to tuberculosis. Using WHO definition South Africa had attained elimination in 1924 but new cases continued to be detected in Northern Transvaal province. (113) The principal means of transmission of \( M. leprae \) is probably by aerosol spread of nasal secretion and uptake through nasal or respiratory mucosa. (1) \( M. leprae \) cannot traverse intact skin and the infection cannot spread by touching. (155) \( M. leprae \) can be also detected in the nasal swabs from up to 5% of healthy individuals of India and Indonesia, which suggest that sub clinical infection occur more frequently in these areas than previously thought. (123) In such circumstances this Multiplex PCR can be a very useful
diagnostic tool to detect the bacteria, where the bacterial load is very low and chances of getting unnoticed can be decreased.

This technique can also be applied even after release from treatment (RFT) to prevent the relapse and resistant cases as, *M. leprae* DNA has been detected even after 8 years after completion of Anti leprosy therapy, and overlooking of cases can be reduced where leprosy is not endemic.

Being encouraged by the result of the Multiplex PCR, the study was extended to Bankura District, of West Bengal, India, an endemic zone of leprosy within the State for contact tracing. As we know leprosy is a disease with long incubation period and the symptoms are difficult to perceive at the early stages of infection. Self-healing does occur in a large number of infected cases. Those do have symptoms may not like to consider them as leprosy cases. Thus clinical diagnosis is often delayed which makes it difficult to identify the immediate causes and factors that contribute to the onset of this disease. Clinical diagnosis is possible only when the patient is symptomatic, exhibiting lesions and damage to the target tissue, which is long after acquiring the infection. A sizable proportion of new cases are among children (WHO 2004) as they often remain in close contact with the infected family members sharing same dwelling units that facilitate infection in them. Similarly as stated earlier an infected child could pass this infection to other children while they are in contact for longer duration such as playing in a group or in a school, which often goes un-noticed during initial phase because of its silent nature of rarely transmission.

Early detection of cases followed by effective chemotherapy appears to be the single most effective strategy for reducing incidence of leprosy cases as well as to prevent transmission. Existing methods of contact tracing and detection of cases are not very effective and sensitive.
Leprosy is a curable disease with well-defined etiology, but better diagnostic tools and therapeutic strategies are lacking, which, together with the socio-cultural prejudice, become important obstacles to overcome for early detection and protection of the susceptible population. Household contacts of leprosy patients (who should be the priority of disease control programmes as they are the group with the highest risk of developing the disease) are not well defined or followed up in leprosy monitoring programs, frequently characterized by a late diagnosis and maintenance of the disease transmission chain. Several groups recently used post genomic approaches to discover new antigen for leprosy diagnosis. (155)

Multiplex-PCR technique employed in our study has shown a better way of community based detection of early cases of leprosy. Paucibacillary status is known to be relatively non infectious. Hence, the main stress should be extended to trace the contacts of multibacillary cases that have higher chance of transmission. Out of 110 multibacillary contacts, Multiplex PCR has detected 12 cases (10.9 %) that did not have any clinical symptoms of leprosy. Of these 12 Multiplex PCR positive cases 2 developed clinical leprosy within the 2 year follow up period. Though the rest 10 Multiplex PCR positive cases did not develop any signs and symptoms of leprosy but they need to be regularly followed up as leprosy has an incubation period between infection and overt disease that varies widely from months to years, and person can also eliminate the bacteria by their own immunity. This study has proved that Multiplex PCR that could be used as an efficient tool for early detection among leprosy contacts which needs further in depth study with adequate population size and control.

Though this Multiplex PCR can only detect M. leprae DNA, hence a contact with Multiplex PCR positive subject should be followed up regularly for detection of development of disease. Multiplex PCR of skin biopsy samples from ear lobules of contact person should also
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be continued in further studies as our previous work experience suggests identification of more cases with the same.

The variable number of TTC repeats in noncoding region of *M leprae* strains is very useful to distinguish between relapse and reinfection which can be used as a genetic marker for the epidemiological investigation. (69) Our sequencing report reveals that different strains of 11, 12, 16, 17, 18 TTC repeats exist in the Eastern Region of India.

Rifampicin, and Dapsone being the components and the backbone of MDT, so development of rifampicin and dapsone (DDS) resistance could be a threat to future of leprosy control. (140,145) So the detection of 3 mutant cases in *rpoB* gene out of 50 patient(6%), though small in number but if a large or the total affected population is concerned this can be an alarming ration. So close and careful monitoring is required.

Conclusion:

Leprosy, one of the oldest diseases known to mankind from very early days of civilization, still remains an important health problem worldwide. Despite significant advances in the treatment procedures and the WHO sponsored efforts to eliminate leprosy as a public health problem; there remain hyper endemic areas in many countries including India, which have shown no substantial decrease in the new case detection rates. India alone contributes to about 64% of the global leprosy case load including fresh infection and reinfection. So, there is an urgent demand for establishment of a sensitive, specific molecular based test for early confirmation of the diagnosis of leprosy among patients and their close contacts. Moreover, the strain differentiation of *M leprae* is required for distinguishing relapse and reinfection of a particular patient and for epidemiological investigation.
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The Multiplex PCR developed in our laboratory as detailed in the study report presented herein not only would help early diagnosis of leprosy (untreated, treated, relapsed, reinfection, treatment failure) cases thereby preventing the cross infection among the family members and to the locality and at the same time bringing down the huge cost of treatment which will be an effective step forward towards eventual elimination of the disease.

Lastly drug resistance poses a serious impediment at a stage when there is a dramatic decline in prevalence due to intensive and concerted chemotherapeutic approach of multidrug therapy (MDT) intervention made by the World Health Organization. Drug resistance surveillance is essential to keep a vigil on the drug resistance scenario at many vulnerable settings in spite of the declining leprosy trend and thereby attaining the success of the final goal of leprosy elimination as a public health problem.