Application of Allele-Specific Polymerase Chain Reaction for Rapid Differentiation of *Mycobacterium Leprae* from *Mycobacterium Tuberculosis*

Yu-Ta Yen  Yu-Wen Cheng  Wei-Ming Wu

Leprosy (Hansen’s disease) is a chronic granulomatous infection caused by *Mycobacterium leprae* affecting primarily skin and nerves. Leprosy is an important clinical problem and is also a challenging diagnostic problem for clinicians. Clinically, leprosy might at times be difficult to be distinguished from lupus vulgaris. We report a 70-year-old woman who showed a well-demarcated erythematous enlarging plaque on left peri-ocular region suggesting leprosy resembling lupus vulgaris. Skin biopsy was performed and mycobacterial infection was identified. However, it was uncertain to differentiate between tuberculosis and leprosy. To solve the problem, we design an allele-specific polymerase chain reaction based on two-nucleotide difference between bacilli of TB and leprosy. Our result showed allele-specific polymerase chain reaction can rapidly differentiate the infection caused by *M leprae* or by *M tuberculosis*. (Dermatol Sinica 27: 170-175, 2009)

Key words: Allele-specific PCR, Leprosy, Lupus vulgaris

INTRODUCTION

Leprosy is a chronic granulomatous infection caused by *M leprae* affecting primarily skin and nerves. The dermatological and pathological presentations varied according to the host immune status. On the other hand, lupus vulgaris is a chronic and progressive form of tuberculosis of the skin caused by *M tuberculosis* primarily involving the head and neck in 90 percent of patients. In this article, we report the application of allele-specific polymerase chain reaction (PCR) for rapid differential diagnosis of a 70-year-old woman presenting clinically as lupus vulgaris or leprosy on face as well.

CASE REPORT

A 70-year-old Taiwanese female had an erythematous enlarging infiltrated mild painful non-itching scaly plaque over left peri-orbital region for more than 6 months. She also developed erythematous papules and plaques on trunk and limbs in the later time (Fig. 1). She has been lived in Kaohsiung, Taiwan since childhood without history of traveling abroad. She was diagnosed as herpes zoster at other medical agent and the lesion exacerbated after treatment. She didn’t have symptoms of fever, recent body weight loss, numbness, nor any decreased sensation...
Clinical examination with KOH did not show evidence of superficial fungus infection. A skin biopsy was arranged under the impression of lupus vulgaris, deep mycosis, tinea incognito, or lymphoma. Sections of hematoxylin & eosin stain showed extensive granulomatous inflammation and dense lymphocytic infiltration in dermis. No obvious epidermal change suggested a presumptive diagnosis of lupus vulgaris. Subsequent special stains revealed positive staining in Fite's stain but negative in Ziehl-Neelsen stain (Fig. 2). Tissue culture showed no growth of any mycobacterium.

To reconfirm the diagnosis and establish the rapid differential diagnostic method for the possible forthcoming confusing cases of leprosy and lupus vulgaris in our hospital, we designed the primers specifically amplifying the sequence for succinate dehydrogenase of *M leprae* and *M tuberculosis* respectively. The primers were as follows: for leprosy, AACGCGCATCGCTTCATT and CGCTTTGGTCACCTCTATGTTG; for tuberculosis, AACGCGCACCGCTTCATC and CGCTTTGGTCACCTCAATGCC (Fig. 3).

DNA from paraffin-embedded samples was extracted with Gentra Puregene Tissue Kit (QIAGEN) as directed by manufacturer. PCR was carried out as follows: 95°C for 5 minutes, 36 cycles of 94°C for 20 seconds, 58°C for 60 seconds, and 72°C for 30 seconds.

PCR were conducted with samples of lesions from the patient’s face. For positive control, we used specimens with confirmed
diagnosis of tuberculosis and leprosy. DNA of normal skin was used as negative controls. The results showing positive bands in patient’s DNA reacted with leprosy primers, but negative with TB primers (Fig. 4), confirmed the diagnosis of leprosy.

For the treatment of the multibacillary disease, we use the combination of rifampin 600 mg daily and dapsone 100 mg daily. The patient was then transferred to Lo-Sheng sanatorium, the government-run leprosy-specific institution, for further management. The central area of the plaque became atrophic after treatment for 5 months, while it became flattened with hyperpigmentation and retraction of eyelids after treatment for 1 year and 5 months (Fig. 1).

**DISCUSSION**

Leprosy is caused by *M leprae* and predominantly affects the skin and peripheral nerves. The disease is endemic in many tropical and subtropical countries in the Indian subcontinent, Southeast Asia, sub-Saharan countries in Africa, and Brazil. The mode of transmission of leprosy is unknown, but it is probably transmitted by inhalation of bacilli, implanted from organisms in the soil, or direct person-to-person infection by means of skin contact.

In the present case, the patient had an erythematous well-demarcated mild-tender plaque on left peri-orbital region with gradual enlargement, and certain smaller erythematous papules and plaques on trunk and limbs. The histopathology showed dense granulomatous inflammation and dense lymphocytic infiltration in dermis, and absence of pseudoepitheliomatous hyperplasia suggesting mycobacterial infection. The acid fast bacilli were found on Fite stain but not in Ziehl-Neelsen stain, so borderline lepromatous leprosy was a favorable diagnosis. Cutaneous leprosy lesions must be distinguished from lesions of sarcoidosis, lymphocytic lymphoma, lymphocytoma cutis, lupus erythematosus, secondary syphilis, blastomycosis, leishmaniasis and other deep mycotic infections. Sarcoidosis could be excluded by the presence of bacilli on acid fast stain, detection of mycobacterium DNA in the skin specimen by PCR, as well as negative pulmonary picture of sarcoidosis. Certain other mycobacterioses in immunosuppressed pa-

---

**Fig. 3**

Alignment of nucleotide sequences of succinate dehydrogenase protein subunit in *M tuberculosis*, *M avium*, *M marinum*, *M abscessus*, *M ulcerans*, and *M laprae*, respectively. The dash represents identical nucleotide within the corresponding allele, and the star indicates missed nucleotide. The dashed arrows represent primers of *M tuberculosis*, and the arrows represent primers of *M laprae*. By using the designed primers, *M tuberculosis* and *M laprae* can be differentiated.
patients, such as *M avium-intracellulare*, may produce histoid multibacillary lesions without nerve involvement, necessitate further study for their molecular differentiation.

For differential diagnosis of *M leprae* and *M tuberculosis*, we used allele-specific PCR by designing primers selectively amplifying 147bp sequences at the locus for succinate dehydrogenase of *M leprae* and *M tuberculosis*, respectively. Succinate dehydrogenase is an iron-containing flavoprotein enzyme that catalyzes the dehydrogenation of succinic acid to fumaric acid in the Krebs cycle, and it is widely distributed in animal tissues, bacteria, and yeast. Three different allele-specific oligonucleotides were located in the 3’ end primers to prevent mismatch, and the specificity was confirmed in our control samples (Fig. 4). An allele-specific oligonucleotide will only anneal to sequences that match it perfectly, and a single mismatch is sufficient to prevent hybridization. Taq polymerase is used in allele-specific PCR for the discrimination of two alleles that differ by a single nucleotide because it lacks a 3’-5’ exonuclease activity. The robustness of the method was strongly improved by the introduction a mismatch adjacent to the 3’ end of the allele-specific PCR primer, which was supported from other studies. Fig. 3 shows the alignment of nucleotide sequences of *M tuberculosis*, *M avium*, *M marinum*, *M abscessus*, *M ulcerans*, and *M laprae*, respectively. The identities of them ranged from 100% to 87% when compared with the sequences of *M tuberculosis* in BLAST (Basic Local Alignment Search Tool, NCBI).

Leprosy is sometimes difficult in diagnosis due to the varied clinical and pathological presentation and limitation of conventional diagnostic methods. PCR is a useful, rapid method that has become available in recent years in the diagnosis of leprosy. Katoch AM et al. reported 40% to 50% of cases missed on histologic evaluation can be detected. PCR had been found to be sensitive to 1 to 10 organisms and is positive in almost all borderline lepromatous/lepromatous cases.

In spite of high sensitivity and specificity, PCR has several limitations including low sensitivity for smear-negative specimens or paucibacillary samples. This may be speculated by the loss of DNA during extraction, failure to sample target DNA on sectioning, or the existence of inhibitory substances. Fixative has also been reported to diminish the PCR signal, particularly when the fixation time is prolonged. Efforts had been made to increase the sensitivity and specificity of PCR in diagnosing paucibacillary leprosy by one-tube nested PCR, by real-time PCR or by PCR using a shorter amplicon. PCR also has a pitfall that it detects the DNA from the dead bacilli. However, this can be resolved.
by using reverse transcriptase PCR detecting 16SrRNA, for this molecule degrades soon after the death of bacilli.

The worldwide incidence of leprosy had declined continuously, and the declining trend is expected to continue. Initial diagnosis of leprosy could be difficult as still no culture system exists for \textit{M. leprae}. By allelespecific PCR, we rapidly differentiated \textit{M. leprae} from \textit{M. tuberculosis}. This method may add in the diagnosis when conventional methods fail to identify these diseases in selected clinical situations.

REFERENCES
使用對偶基因特異性聚合酶連鎖反應快速辨別麻風分枝桿菌及結核分枝桿菌感染

顏育達  鄭裕文  吳唯銘
高雄長庚紀念醫院皮膚科  長庚大學醫學院

麻風（漢生病）是一種由麻風分枝桿菌所引起之慢性肉芽性感染，主要侵犯皮膚及神經。麻風是一種重要的臨床課題，對於臨床醫師而言此疾病的診斷也是一項挑戰。有時麻風與結核菌感染之尋常性狼瘡不易做鑑別診斷，特別當感染發生於顱部時。我們報告一位70歲女性在左側眼部週圍有界限明顯逐漸增大的紅色斑塊，疑似麻風或尋常性狼瘡。在皮膚切片及施行對偶基因特異性聚合酶連鎖反應後確認麻風的診斷。使用對偶基因特異性聚合酶連鎖反應可以快速正確鑑別診斷麻風及結核感染。（中華皮誌：27: 170-175, 2009）