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CONTENTS (CONTD.)

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Utility of polymerase chain reaction as a diagnostic tool in cutaneous tuberculosis

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ABSTRACT

Background: Differentiation of cutaneous tuberculosis from other infective granulomas of the skin is difficult due to paucity of the organisms in tissue biopsies. Polymerase chain reaction (PCR) is a newer technique to identify the DNA of *Mycobacterium tuberculosis* in the tissues. **Aim:** We examined the utility of PCR as a tool for rapid diagnosis of cutaneous tuberculosis especially in cases negative by ZN staining and culture. **Material and Methods:** Twenty five random skin biopsies from patients with various types of cutaneous tuberculosis were subjected to PCR. **Results:** An overall positivity of 64% was observed, which is comparable to other series. Seventy five percent of lupus vulgaris cases, 62.2% of tuberculosis verrucosa cutis and 50% of scrofuloderma cases showed PCR positivity. **Conclusion:** Though useful, the cost and the technique involved limit the use of PCR in developing countries like ours.

Key Words: Cutaneous tuberculosis, PCR, Rapid diagnosis

INTRODUCTION

Tuberculosis is common in India. Demonstration of organisms in skin biopsy specimens is often difficult due to the minimal bacillary load and also due to the attenuated state of the organisms. A variety of diagnostic tools are available for the study of these cases. Cultures have a higher sensitivity (about 80 to 90%) and may detect as few as 10-100 bacilli/ml. However, the delay of 3 to 6 weeks required for culture results currently limits their use in the diagnosis of tuberculosis. The powers of newer molecular diagnostic techniques like PCR permit detection of as little as one organism in a sample.[1]

DNA amplification by PCR is a rapid and sensitive method for detection of *M. tuberculosis* organisms in samples. Fewer than 10 microorganisms could be detected. Though precise estimation of the sensitivity is difficult, the primer directed amplification system used is both highly sensitive (98%) and specific (100%) in the detection of *M. tuberculosis* complex DNA from clinical specimens.[2-4]

The study was done to find out the utility PCR in the diagnosis of cutaneous tuberculosis among the patients attending the dermatology department of RMMC hospital, located in Chidambaram, a taluka headquarters in South Arcot district, Tamil Nadu, catering to the needs of predominantly a rural population.

MATERIAL AND METHODS

Skin biopsies of 25 randomly selected patients with clinical and histopathological diagnosis of various types of cutaneous tuberculosis, negative for AFB by culture and special stains, were included in the study. The formalin fixed skin biopsy specimens as well as tissues from the paraffin blocks were analyzed using the

standard procedure of polymerase chain reaction.[5,6]

RESULTS

Out of the 25 patients with various types of cutaneous tuberculosis, 75% (6 out of 8 biopsy specimens) of lupus vulgaris, 62.2% (5 out of 9 biopsy specimens) of tuberculosis verrucosa cutis and 50% (3 out of 6 biopsy specimens) of scrofuloderma, showed PCR positivity. One case each of lichen scrofulosorum and erythema induratum submitted for PCR also tested positive, thus confirming their tuberculous etiology.

Cultures for *Mycobacterium tuberculosis* in these patients were negative. None of these patients showed any evidence of immunosuppression and all tested negative for HIV. All these patients responded well to antituberculous therapy and their lesions healed well.

DISCUSSION

Diagnosis of tuberculosis depends on the demonstration of the organisms in the tissue or body fluids. However, the degree of bacillary load essential for this is usually seen in sputum positive cases of advanced tuberculous cavitatary disease. Here the sensitivity of direct sputum examination is about 40 to 75%.

PCR results can be obtained within days, and a PCR based technique may facilitate the diagnosis of cutaneous tuberculosis.[7] Several anecdotal reports of the utility of PCR in the diagnosis of cutaneous tuberculosis are available. An early case of tuberculosis verrucosa cutis of only one-week duration was documented by employing PCR.[1] In another instance, tuberculosis complex DNA was detected in formalin fixed tissue by PCR in a PPD (tuberculin) negative patient with a chronic cutaneous lesion with histological features resembling sarcoidosis.[8] The lesion cleared with antituberculous therapy. *M. tuberculosis* DNA in skin biopsy specimens was demonstrated in another case of lupus vulgaris that had been followed for several years with frequent unrewarding biopsies and cultures.[9] The utility of PCR in the diagnosis of orificial tuberculosis with miliary spread,[10] erythema induratum,[11,12] papulonecrotic tuberculid,[13] and other forms of cutaneous tuberculosis[14-16] has been reported from different parts of the world.

In our series, 75% of lupus vulgaris, 62.2% of tuberculosis verrucosa cutis and 50% of scrofuloderma cases, showed PCR positivity. One case each of lichen scrofulosorum and erythema induratum submitted for PCR also tested positive, thus confirming their tuberculous etiology.

All our patients responded well to standard antituberculous therapy and their lesions healed well. Since culture for AFB was negative in our patients, PCR results were correlated with histopathology and therapeutic response to standard antituberculous therapy. The overall PCR positivity of 64% (16 out of 25 cases tested) in our study is comparable to the results of other workers.

Although specific, the relatively low positivity rate of PCR in the common varieties of tuberculosis is of concern. Thus Tan et al concluded that PCR based detection of *M. tuberculosis* DNA in skin samples may extend and improve the diagnostic panel for cutaneous tuberculosis and may be also used to differentiate atypical mycobacterial infections in an immuno-compromised patient with negative culture, if the technique is prudently and properly used. Moreover, PCR has not been found to be a useful complement to the clinical and histologic diagnosis of "paucibacillary" forms of cutaneous tuberculosis in their experience.[17]

There are other limitations to the use of PCR as well. It is a technically complex method that requires trained hands to perform the test. The inherent sensitivity of PCR amplification can lead to amplification of nonspecific sequences and contaminants. Greater care and meticulous technique are essential for a reliable PCR result.

PCR remains positive for periods far greater than the bacterial cultures. An important limiting factor is the inability of PCR to differentiate between the live and dead organisms which are likely to persist in inactive or treated cases. In view of this, PCR positivity does not necessarily indicate active disease.

The quality control, reproducibility of results and variations from laboratory to laboratory are still important issues to be sorted out. The cost of the PCR technique is another important limiting factor in employing this method in routine diagnosis.

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