(e) ISSN Online: 2321-9599
(p) ISSN Print: 2348-6805

ORIGINAL ARTICLE

ASSESSMENT OF VARIOUS LABORATORY DIAGNOSTIC METHODS FOR CUTANEOUS TUBERCULOSIS: A RETROSPECTIVE ANALYSIS

Poonam Singh[1], Manisha Bhargava[2]

[1]Assistant Professor, Career Institute of Medical Sciences Lucknow, U.P., [2]Associate Professor Integral University Lucknow, U.P.

ABSTRACT:
Background: One of the challenges in the field of diagnostic pathology is the search for bacilli in a cutaneous tuberculosis (CTB) lesion are a challenge. For increasing the accuracy of diagnosis of cases of latent and CTB, several laboratory diagnostic methods have been revised with times which have higher sensitivity and specificity. Hence; we evaluated the laboratory results of the skin biopsy specimens, in which final diagnosis of CTB was confirmed. **Materials & Methods:** The present study included 22 skin biopsy specimens which were submitted to the department of general pathology from 2011 to 2014 and were processed with AFB staining, growth cultural in mycobacterium cultural medium and PCR evaluation. PCR technique was used for the assessment sequence and mutations in the core region that are associated with rifampicin resistance. All the results were analyzed by SPSS software. **Results:** 9 out of 22 patients were positive as CTB by the recovery of M. tuberculosis in culture and/or histopathological affirmation. All the 9 patients showed a negative staining pattern for AFB staining. Five out of nine patients showed positive cultural growth characteristics. All the patients showed positive CTB findings compatible with the diagnosis of CTB. Only three patients out of 9 showed positive PCR technique. Tuberculosis verrucosa cutis was the most common biological variant detected. **Conclusion:** A combination of cultural, staining and PCR techniques should be used for increasing the specificity and sensitivity of the diagnostic methods for the diagnosis of CTB.
Key words: Cutaneous, Mycobacterium, Tuberculosis

Corresponding Author: Dr. Poonam Singh, Assistant Professor, Career Institute of Medical Sciences Lucknow, U.P

This article may be cited as: Singh P, Bhargava M. Assessment of various laboratory diagnostic methods for cutaneous tuberculosis: A retrospective analysis. J Adv Med Dent Scie Res 2016;4(5):11-13.

Access this article online	
Quick Response Code	Website: www.jamdsr.com
	DOI: 10.21276/jamdsr.2016.4.5.3

INTRODUCTION

One of the challenges in the field of diagnostic pathology is the search for bacilli in a cutaneous tuberculosis (CTB) lesion are a challenge. All diagnostic methods have lower sensitivity and specificity rates for cutaneous presentations compared to the pulmonary form.[1] Considering that atypical erythema nodosum and nonspecific appearance are not uncommon, and also that histopathology may not be elucidative, physicians must resort to every possible test, so that the sum of positive elements can create the basis for diagnosis, thus reducing empirical treatments or those based solely on the positivity of tuberculin test.[2] Beyond mandatory tuberculin skin test (TST) and chest radiograph for all patients, when confronted with the hypothesis of CTB, it is of paramount importance to collect material for histopathological examination and also separate samples for acid-fast bacilli (AFB) detection through culture and amplification of Mycobacterium tuberculosis (Mtb) DNA by polymerase chain reaction (PCR) both in the sample as well as in the blood. For increasing the accuracy of diagnosis of cases of latent and CTB, several laboratory diagnostic methods have been revised with times which have higher sensitivity and specificity.[3, 4] Hence; we evaluated the laboratory results of the skin biopsy specimens, in which final diagnosis of CTB was confirmed.

MATERIALS & METHODS

The present study included 22 skin biopsy specimens which were submitted to the department of general pathology from 2011 to 2014 and were processed with AFB staining, growth cultural in myobacterium cultural medium and PCR evaluation. Skin punch biopsies were performed from the active part of the lesions clinically

suspected for CTB and divided into two portions, one part processed for histopathological evaluation and the other was used for microscopic examination of AFB and inoculation for the isolation of mycobacteria. One more skin biopsy was performed for PCR technique when available. Specimens were transported to the laboratory under septic conditions and were stained with Acid-fast staining. The Löwenstein–Jensen slants were incubated at 37°C and 25°C and examined for growth every week. If no growth was observed after 8 weeks of incubation, the specimen was reported as culture‑negative. PCR technique was used for the assessment sequence and mutations in the core region that are associated with rifampicin resistance. All the results were analyzed by SPSS software. Chi-square test was used for assessment of level of significance.

RESULTS

Table 1 shows the results for laboratory test of patients diagnosed with CTB. 9 out of 22 patients were positive as CTB by the recovery of M. tuberculosis in culture and/or histopathological affirmation. All the 9 patients showed a negative staining pattern for AFB staining. Five out of nine patients showed positive cultural growth characteristics. **Table 2** highlights the PCR analysis of patients diagnosed with CTB. All the patients showed positive CTB findings compatible with the diagnosis of CTB. Only three patients out of 9 showed positive PCR technique. **Table 3** shows the morphological variants of patients diagnosed with CTB. Tuberculosis verrucosa cutis was the most common biological variant detected.

Table 1: Laboratory data of patients diagnosed with CTB

Patient No.	AFB staining	Cultural growth
1	Negative	Negative
2	Negative	Negative
3	Negative	Positive
4	Negative	Positive
5	Negative	Positive
6	Negative	Negative
7	Negative	Positive
8	Negative	Negative
9	Negative	Positive

Table 2: PCR analysis of patients diagnosed with CTB

Patient No.	PCR	Pathologic evaluation
1	Not done	Compatible with CTB
2	Negative	Compatible with CTB
3	Negative	Compatible with CTB
4	Negative	Compatible with CTB
5	Not done	Compatible with CTB
6	Negative	Compatible with CTB
7	Positive	Compatible with CTB
8	Not done	Compatible with CTB
9	Positive	Compatible with CTB

Table 3: Morphological variants of patients diagnosed with CTB

Patient No.	Variant morphological
1	Lupus vulgaris
2	Tuberculosis verrucosa cutis
3	Tuberculosis verrucosa cutis
4	Scrofuloderma
5	Lupus vulgaris
6	Primary inoculation tuberculosis
7	Tuberculosis verrucosa cutis
8	Tuberculosis verrucosa cutis
9	Periorificial tuberculosis

DISCUSSION

One of the infectious micro-organism which is a worldwide, problematic, communicable pathogen that has increasingly been regarded as a notable, serious infection in the United States is the Mycobacterium tuberculosis. Factors such as the association of tuberculosis (TB) with the human immunodeficiency virus (HIV) epidemic, increased immigration from endemic countries, and the transmission of TB in crowded settings, such as healthcare facilities, prisons, and homeless shelters are responsible for the underlying basis of this recent epidemic.[5- 8] Most often TB is an airborne transmissible disease with skin manifestations presenting as a result of hematogenous spread or direct extension from a latent or active foci of infection. However, primary inoculation may occur as a direct introduction of the mycobacterium into the skin or mucosa of a susceptible individual by trauma or injury. Increased risk of acquiring disease occurs with HIV infection, intravenous drug abuse, diabetes mellitus, immunosuppressive therapy, malignancies, end-stage renal disease, and infancy. Cutaneous tuberculosis (CTB) is frequently elusive as it mimics a wide differential diagnosis and also evades microbiological confirmation despite recent advances in sophisticated techniques.[9] It is important for the pathologist to have knowledge of the biological and morphological variant of CTB so that correct diagnosis can be made as soon as possible.[10] Hence; we evaluated the laboratory results of the skin biopsy specimens, in which final diagnosis of CTB was confirmed.

Confirmation of the diagnosis of TB is done by the detection o f AFB and/or isolation of M. tuberculosis from biopsy specimens.[11] AFB staining was negative in the 9 patients with positive diagnosis of CTB in the present study (**Table 1**). Egg-based media (Löwenstein–Jensen) and semisynthetic media with agar are the most commonly used culture media used for isolation of M. Tuberculosis.[12] PCR techniques results were positive in two to three cases. Tuberculosis verrucosa cutis was the most commonly identified morphologic variant of CTB (**Table 2, Table 3**). Afsar et al evaluated the results of diagnostic laboratory tests available for CTB. They analyzed Twenty-six skin biopsy specimens belonging to clinically suspected cases of CTB were studied

retrospectively. They observed that out of the 26 biopsy specimens, 11 were confirmed as CTB by identification culture characteristics and/or histopathology affirmation. From the results, they concluded that the recovery rate of Mycobacterium tuberculosis complex from biopsy specimens was found to be satisfactory for CTB with histopathological correlation, but the combination of culture with a rapid method, PCR, may improve the diagnostic rate.[13] Tan et al evaluated the role of the polymerase chain reaction (PCR) for the detection of Mycobacterium tuberculosis DNA as a diagnostic aid in cutaneous tuberculosis using routinely processed skin biopsy specimens. From the result, they concluded that the role of PCR in clinical dermatologic practice, at this stage, may be in differentiating between cutaneous tuberculosis and atypical mycobacterial infections in the context of an immunocompromised patient where AFB can be demonstrated on biopsy and cultures may be negative.[14] Hsiao et al evaluated the incidence of cutaneous tuberculosis and atypical mycobacterial infection in unspecified granulomatous inflammation and negative results for AFB, and analyzed the pattern of cutaneous tuberculosis in this group of patients. They concluded that in specimens with granulomatous inflammation, cutaneous tuberculosis represents a significant proportion.[15]

CONCLUSION

From the above results, it can be concluded that a combination of cultural, staining and PCR techniques should be used for increasing the specificity and sensitivity of the diagnostic methods for the diagnosis of CTB.

REFERENCES:

1. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica . Manual de Recomendações para o Controle da Tuberculose no Brasil. Ministério da Saúde; Brasília: 2011. 284 p. (Série A. Normas e Manuais Técnicos - Series A. Standards and Technical Manuals).
2. Ramam M, Malhotra A, Tejasvi T, Manchanda Y, Sharma S, Mittal R, et al. How useful is the Mantoux test in the diagnosis of doubtful cases of cutaneous tuberculosis? Int J Dermatol. 2011;50:1379–1382.
3. Barbagallo J, Tager P, Ingleton R, Hirsch RJ, Weinberg JM. Cutaneous tuberculosis: diagnosis and treatment. Am J Clin Dermatol. 2002;3:319–328.
4. Marcoval J, Servitje O, Moreno A, Jucglà A, Peyrí J. Lupus vulgaris. Clinical, histopathologic, and bacteriologic study of 10 cases. J Am Acad Dermatol. 1992;26:404–407.
5. Bravo FG, Gotuzzo E. Cutaneous tuberculosis. Clin Dermatol. 2007;25(2):173–180.
6. Fariña MC, Gegundez MI, Piqué E, et al. Cutaneous tuberculosis: a clinical, histopathologic, and bacteriologic study. J Am Acad Dermatol. 1995;33(3):433–440.
7. MacGregor RR. Cutaneous tuberculosis. Clin Dermatol. 1995;13(3):245–255.
8. Bhutto AM, Solangi A, Khaskhely NM, et al. Clinical and epidemiological observations of cutaneous tuberculosis in Larkana, Pakistan. Int J Dermatol. 2002;41(3):159–165.
9. Barbagallo J, Tager P, Ingleton R, et al. Cutaneous tuberculosis: diagnosis and treatment. Am J Clin Dermatol. 2002;3(5):319–328.
10. Handog EB, Gabriel TG, Pineda RT. Management of cutaneous tuberculosis. Dermatol Ther.2008;21(3):154–161.
11. Caminero Luna JA, Casal Román M, Ausina Ruiz V, Pina Gutiérrez JM, Sauret Valet J. Tuberculosis diagnosis. Arch Bronconeumol 1996;32:85‑99.
12. Quirós E, Maroto MC, Bettinardi A, González I, Piédrola G. Diagnosis of cutaneous tuberculosis in biopsy specimens by PCR and southern blotting. J Clin Pathol 1996;49:889‑91.
13. Afsar I, Afsar FS. Evaluation of laboratory diagnosis for cutaneous tuberculosis. Indian J Pathol Microbiol. 2016 Jul-Sep;59(3):274-8.
14. Tan SH, Tan BH, Goh CL, Tan KC, Tan MF, Ng WC, Tan WC. Detection of Mycobacterium tuberculosis DNA using polymerase chain reaction in cutaneous tuberculosis and tuberculids. Int J Dermatol. 1999; 38(2): 122-7.
15. Hsiao PF, Tzen CY, Chen HC, Su HY. Polymerase chain reaction based detection of Mycobacterium tuberculosis in tissues showing granulomatous inflammation without demonstrable acid-fast bacilli. Int J Dermatol. 2003 Apr;42(4):281-6.

Source of support: Nil **Conflict of interest:** None declared