

Skin biopsy: a pillar in the identification of cutaneous *Mycobacterium tuberculosis* infection

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Abstract

Introduction: The present study aimed to establish the frequency and clinical characteristics of cutaneous tuberculosis among Mexican adult patients.

Methodology: Ninety-five patients with clinically compatible lesions to cutaneous tuberculosis participated in the study. All patients were HIV negative and none of them had previous anti-TB treatment. A skin biopsy was taken from every patient suspected of having tuberculosis, and a histopathologic examination was performed as follows: Ziehl-Neelsen staining; culturing of mycobacteria by Löwenstein-Jensen (L-J) medium; Mycobacteria Growth Indicator Tube detection via BACTEC (MGIT-360); and polymerase chain reaction (PCR) with the sequence of insertion IS6110 for *Mycobacterium tuberculosis* complex.

Results: Tuberculosis was confirmed in 65 out of 95 cases (68.4%). Identified lesions were scrofuloderma (42 cases, 64.6%); lupus vulgaris (12 cases, 18.4%); warty tuberculosis (six cases, 9.2%); and papulonecrotic tuberculoid (five cases; 7.7%). The Ziehl-Neelsen staining was positive for acid fast bacilli in nine cases (13.8%) and 48 patients were positive for the PCR amplification (73.8%). All skin biopsies resulted positive for tuberculosis. A positive clinical response to the specific treatment was considered a confirmation for tuberculosis. The noninfectious etiology corresponded to 30 cases (31.6%).

Conclusions: Tuberculosis in developing countries is still an important cause of skin lesions which must be studied via histopathological examination and culture due to their low bacillary load. A PCR test is necessary to obtain faster confirmation of the disease and to establish an early, specific and effective treatment.

Key words: cutaneous tuberculosis; polymerase chain reaction (PCR); *Mycobacterium tuberculosis*

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Introduction

A recent report by the World Health Organization (WHO 2010) reveals that one third of the world population is currently infected with tuberculosis. In Mexico, 15,000 new cases of tuberculosis and 2,000 deaths caused by this illness are identified every year. This evident increase is particularly notable for its common association with the human immunodeficiency virus (HIV) and chronic degenerative diseases such as diabetes mellitus. The diagnosis of TB in the early stage of the disease is extremely important and rapid detection and identification of mycobacteria from clinical samples is mandatory.

In addition to the lung TB cases, extrapulmonary lesions have also been increasing

considerably in our country, accounting for 17% to 20% of all TB cases. Cutaneous tuberculosis is a chronic disease caused when *Mycobacterium tuberculosis* directly infects previously non-exposed skin. Its incidence is around 2% among all clinical forms of tuberculosis [1]. The clinical course depends on the pathogenicity of the bacilli and the host-immune response. The disease is characterized by a painless ulcer with a granulomatous base and painless regional lymphadenopathy evident at the infection site. Tuberculous chancre and the affected lymph node constitute the primary tuberculous complex [2].

Cutaneous tuberculosis represents a real diagnostic challenge because the cutaneous tissue is slightly favors reproduction of bacilli. The challenge increases because of the pathogenic status and minor

damage to the affected skin compared to other extrapulmonary tuberculosis forms. The histopathologic examination is not really specific since other infectious agents such as tertiary syphilis, tuberculoid leprosy, carcinoma epidermoid, granuloma caused by insect stings and sporotrichosis may cause similar histological changes. However, tuberculosis must be considered a cause of cutaneous infections in developing countries due to the high prevalence of the disease in those regions [3].

The results obtained from Papanicolaou's stain and a histopathological examination of the skin biopsy may suggest tuberculosis [4]. The conclusive results generally cannot be achieved with bacteriological methods; however, molecular diagnostic methods are considered more sensitive [5]. Treatments using strictly supervised (DOTS) anti-TB drugs must be immediately instituted. The general prognosis is favorable but depends on the patient's age, the course the disease, the patient's immunological status, and the virulence of the *Mycobacterium* strain [6,7].

This study aimed to establish the frequency and importance of *Mycobacterium tuberculosis* in cutaneous lesions among a population of adult patients who reside in the Mexico City metropolitan area.

Methodology

The study subjects were patients with cutaneous lesions clinically compatible with tuberculosis attending the hospital from January 2008 to January 2010. Patients aged 15 and above who were clinical suspects of having cutaneous tuberculosis, who had not any precedent of having received previous treatment with anti-TB drugs, and who were HIV negative were included in the study. Informed consent was not necessary for this study since paraffin biopsies already in existence were used. The final diagnosis of tuberculosis was based on the presence of histopathologic lesions compatible with cutaneous tuberculosis in skin biopsy and confirmed by the satisfactory clinical response to therapy with specific treatments after one year.

A clinical record was integrated for each patient and routine laboratory examinations were added, with attention to the cutaneous test with PPD-RT23 protein (Statens Serminstitut, Copenhagen, Denmark) and chest X rays.

A biopsy was performed on cutaneous tissue of the skin for histopathologic, microbiological and molecular biology analysis. A treatment was

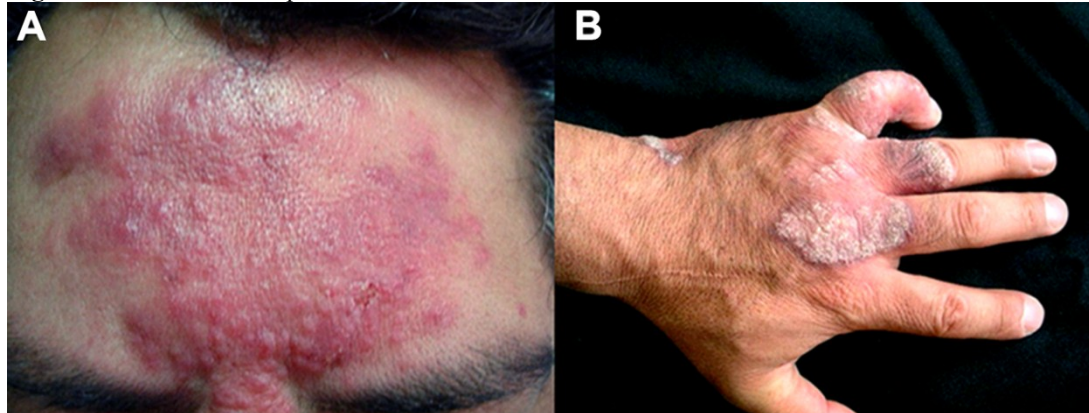
indicated and strictly supervised with Isoniazid, Rifampin, Pyrazinamide and Ethambutol in every diagnostic case of tuberculosis by any method. Clinical response was evaluated during the first year or until the resolution of the process.

The biopsy specimens were preserved at 4°C divided in three portions under aseptic conditions for histopathologic examinations, bacteriological diagnosis, and molecular biology analysis with Polymerase Chain Reaction (PCR). For the histopathologic examination, fragments were fixed in 10% formaldehyde extended to paraffin, cut and dyed via H&E stain, (HE stain / hematoxylin and eosin stain) and observed by microscope to determine their characteristics. The specimens were considered tubercular when typical granuloma formations with caseous necrosis forms, hyaline capsule, fibrosis and epithelioid cells were present.

Bacteriological studies were performed on the homogenized and concentrated specimens (Standard Petroff digestion-decontamination procedure). For the cultures, 0.1 mL of the homogenized specimens were inoculated in bottles via fluorescent liquid (BACTEC MGIT960 / Mycobacteria Growth Indicator Tube, San Jose, CA, USA) and preserved at 37°C. In the Löwenstein-Jensen solid media, specimens were incubated at 37°C for eight weeks and supervised weekly to make a growth evaluation. The presence of acid-fast bacilli was determined by Ziehl-Neelsen staining.

A PCR amplification was performed on the IS6110 fragment (97% sensitivity and 97% specificity) with IS11 (5'-CACGCTAATTACCCGCTTCATCG-3') and IS12 (5'-ATCGCGCAGCTCGCGGCGG-3'); 50 µl of reaction mixture containing 0.67 M Tris-HCL (pH 8.8), 0.016 M Ammonium Sulfate, 0.01 M 2-mercaptoethanol, MgCl₂, 2 U Taq Polymerase, 200 µM of each dATP, dCTP, dGTP y TTP; and 50 pmol from each prime (final concentrations) then subjected to 40 amplification cycles at 94°C for 30 seconds and 67°C during 2 minutes. A 10-µl DNA amplified aliquot part was made by Gel agarose electrophoresis to 2%. The DNA of the product 175-bp was dyed with GelStar (Bio Whittaker, Walkersville, MD, USA). The *Mycobacterium tuberculosis* HR37Ry DNA was used as the positive control.

A 2 x 2 contingency table was made to obtain sensitivity, specificity and positive and negative predictive values. The statistical significance was determined by the χ^2 test. The correlation

Figure 1. Cases from Hospital General de Mexico

A) Warty tuberculosis B) Lupus vulgaris

coefficients were calculated using Spearman's rank. Additionally, CI ties of 95% were estimated.

Results

Among the 95 cases, 65 patients aged between 15 and 70 years old were diagnosed with cutaneous tuberculosis, of whom 51 were female and 14 male. The predominant histopathologic lesions were scrofuloderma (42 cases, 64.6%), lupus vulgaris (12 cases, 18.5%), warty tuberculosis (six cases, 9.2%), and papulonecrotic tuberculid (5 cases, 7.6%) (Figure 1).

In this data series analysis, tuberculosis was present on the face in 7 cases (10.7%), on the neck in 27 cases (41.5%), on the torso in 20 cases (30.7%), and in more than one anatomical region in 11 cases (16.9%).

Bacteriologic studies

ZN staining revealed positive results in 9 skin biopsy specimens (13.8%) with sensitivity of 14%. Using BACTEC MGIT 960, nine cases (13.8%) were positive with sensitivity of 14%, while L-J examination identified four positive (6.15%) with sensitivity of 6%. Strains corresponded to *Mycobacterium tuberculosis* complex (Table 1).

PCR amplifications

In this study, PCR obtained better results for diagnosis of cutaneous tuberculosis, identifying 48 positive samples (73.8%) with sensitivity of 74%, specificity of 91%, PPV 94%, and NPV 64% (Table 2).

PPD test

The cutaneous reactivity to PPD (10 millimeters or above induration) was detected in 17 patients (26%) with cutaneous tuberculosis, and only three patients presented non-TB cutaneous lesions (10%).

Compatible with other types of tuberculosis than cutaneous

In addition to the cutaneous location, different types of tuberculosis were found: six cases with pulmonary tuberculosis, four cases with lymph node tuberculosis and one patient with miliary tuberculosis. The presence of cutaneous tuberculids in lower limbs was especially noticeable in 100% of the above-mentioned cases. All the cases with histopathological anomalies with positive characteristics were clinically considered as cutaneous tuberculosis. Chemotherapy with anti-TB drugs was necessary in 65 cases (Table 3).

Not compatible with tuberculosis

In this study we found 30 patients (17 female and 13 male, between 20 and 70 years old) with lesions that did not correspond to cutaneous tuberculosis. Of these, 11 cases proved compatible with melanoma, seven with lymphoma, six with mycosis, and two with lupus erythematosus; four cases reacted to foreign substances.

Discussion

After some decades in which a decreased incidence of tuberculosis was noticeable in some countries, re-emergence of the disease has been observed over the last years, particularly in people with low economic resources, those infected with

Table 1. Cutaneous tuberculosis (65 cases)

Clinical form	Z-N	L-J medium	MGTI	PPD	PCR
Scrofuloderma	7	3	5	11	35
Lupus vulgaris	1	0	4	3	8
Warty tuberculosis (TBCV)	1	1	0	2	3
Papulonecrotic tuberculid	0	0	0	1	2
Total	9	4	9	17	48

Abbreviations: Z-N: Ziehl-Neelsen stain (acid-fast stain), L-J: Lowenstein-Jensen growth medium, MGTI: Mycobacteria Growth Indicator Tube, PPD: test for Purified Protein Derivative, PCR: polymerase chain reaction

Table 2. Diagnostic methods for cutaneous tuberculosis patients

Diagnostic method	Sens	95% CI for Sens	Spec	95% CI for Spec	PPV	95% CI for PPV	NVP	95% CI for NVP	Conc
AFB with ZN	0.14	(0.05,0.22)	1	1	1	1	0.35	(0.25,0.45)	0.09
MGIT culture	0.14	(0.05,0.22)	1	1	1	1	0.35	(0.25,0.45)	0.09
L-J culture	0.06	(0,0.12)	1	1	1	1	0.42	(0.25,0.45)	0.09
PCR	0.74	(0.63,0.85)	0.91	(0.81,1.01)	0.94	(0.88,1.01)	0.64	(0.5, 0.78)	0.05
Reactivity to PPD	0.26	(0.15,0.37)	0.9	(0.79,1.01)	0.85	(0.69,1.01)	0.36	(0.25,0.47)	0.11

(total results, $n = 95$) Abbreviations: Sens = sensitivity, Spec = specificity, Conc = concordance, TB = tuberculosis, PPV = positive predictive value, NPV = negative predictive value, AFB = acid-fast bacillus, ZN = Ziehl-Neelsen stain, LJ = Löwenstein-Jensen growth medium, MGTI = Mycobacteria Growth Indicator Tube, PCR = polymerase chain reaction

Table 3. Histopathological features of cutaneous tuberculosis

Histopathological features of cutaneous tuberculosis (n = 65)							
Lesion	Histology	SCF	LV	TBVC	TCP	Total	%
Granuloma		32	6	3	2	43	66
Cellular content	Epitheloid cells	16	5	4	3	28	43
	Giant cells	25	9	5	4	43	66
	Plasma cells	14	8	5	2	29	44
	Lymphocytes	12	4	3	2	21	32
Caseous necrosis		38	2	2	2	44	67
Inflammatory cell infiltration	Nonspecific	28	8	6	4	46	70
	PMN cells	10	6	2	4	22	33
	Abscess	7	4	3	2	16	24
	Perivascular	3	7	1	2	13	20
	Perinadnexal	1	3	0	0	4	6

Abbreviations: SCF = scrofuloderma, LV = lupus vulgaris, TBVC = warty tuberculosis (tuberculosis verrucosa cutis), TCP = papulonecrotictuberculid (tuberculosis cutis papulonecrotica), PMN = Polymorphonuclear cells

HIV, and subjects with chronic degenerative diseases. This infectious and contagious disease has a predominant presence in underdeveloped countries. Each year approximately three million people die because of tuberculosis. However, the incidence of this disease has increased also in developed countries, as observed in Western Europe and the United States where it was believed that it had been controlled.

Cutaneous tuberculosis has spread worldwide, though in Europe it represents less than 1% of cutaneous pathologies. In some countries such as India, Pakistan and others in Asia and Africa, the incidence of cutaneous tuberculosis is increasing [8,9]. Early diagnosis and the exact identification of *Mycobacterium tuberculosis* in skin lesions are necessary for an appropriate treatment. The results of this study demonstrate that among a cohort of adult Mexican patients with cutaneous lesions, 68.4% had histopathological characteristics compatible with tuberculosis.

In this cohort, it was demonstrated that the majority of the patients with confirmed cutaneous tuberculosis were young adults, with a major prevalence among women whose predominant histopathologic lesions had been scrofuloderma, a finding shared with other published studies [10].

This study also shows that the most common location of cutaneous tuberculosis was the face, followed by the neck and torso. A history of pulmonary tuberculosis or presence of other lesions was detected in only 6% of the examined patients. In addition to pulmonary dissemination, ganglion chains were also found, and only one patient was diagnosed with miliary tuberculosis. In fact, the majority of cutaneous tuberculosis lesions were of a local nature [11].

The bacteriological tests had scanty results. Z-N stains were positive in just 9 cases, and growth was obtained in 13 cultured specimens. The identification of *Mycobacterium* is necessary for a definitive diagnosis of tuberculosis. However, skin tissue is slightly favorable to the reproduction of the bacilli (unlike pulmonary tissue), and the tuberculous bacillus shows inefficient growth when conventional culturing techniques are used. Better and faster results were obtained with the radiometric BACTEC 460TB method, though in the case of cutaneous tuberculosis the results were scant.

During the assay, the polymerase chain reaction had the highest positive results (48 of 65), proving once more to be the best diagnostic method with

relative high sensitivity (71%). This method offers an earlier diagnosis than the others [12,13,14].

False negative PCR results were found in 17 cases, which could be due to the non-uniform distribution of *Mycobacterium*, or to the presence of inhibitory substances in the tissue specimens. Among the 30 biopsies negative for tuberculosis, three had false positive PCR results, but their histopathological and bacteriological examinations were negative, and this could be explained by DNA contamination [15].

The PPD test was positive only in 26% of the tuberculosis patients, while 10% were reactors of the non-tuberculosis group. The PPD reactors among the tuberculosis group were lower than those in the general Mexican population (41%), probably caused by malnutrition and/or an immunologic anergy status.

The results of this study suggest that histopathology is still the best option for an initial diagnosis of cutaneous tuberculosis. In cutaneous tuberculosis, as in other extrapulmonary manifestations, the application of new cultivation methods is important to identify *Mycobacterium* to exclude non-tuberculosis etiology that may be related to the pathogenesis of the disease and the failure of anti-TB drugs [16].

Conclusions

The information revealed in this study suggests that, in some communities with high prevalence and incidence of tuberculosis, there are enough causes to suspect the illness among patients. Clinical examinations involving both histopathologic and microbiologic studies in conjunction are necessary for the definitive diagnosis of cutaneous tuberculosis. Though isolation of *Mycobacterium* from biopsy is low, this must be performed for classification studies and drug-sensitivity examinations in cases that test positive.

In this series of cases, histological compatibility with tuberculosis was the major diagnostic method. The DNA amplification with PCR offers an adequate way to support the medical and histopathologic diagnosis of cutaneous tuberculosis.

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