Epidemiol. Infect. (2003), **131**, 1071–1076. © 2003 Cambridge University Press
DOI: 10.1017/S0950268803001304 Printed in the United Kingdom

Tuberculosis is still a major cause of cervical lymphadenopathies in adults from developing countries

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(*Accepted 7 July 2003*)

SUMMARY

To establish the frequency of infectious aetiology in Mexican adult patients with cervical lymphadenopathies (CLAs), 87 consecutive patients with enlarged cervical lymphatic nodes, HIV negative and without anti-tuberculous treatment, were selected from a tertiary-level speciality concentration hospital. Histopathological studies, investigation of acid-fast bacilli, cultures in Löwenstein–Jensen and *Mycobacterium* growth indicator tube (MGIT) media, and in-house polymerase chain reaction (PCR) with IS*6110*-based primers for *Mycobacterium tuberculosis* complex were performed in resected lymphatic nodes. Non-infectious aetiology corresponded to 45 cases (52%). Tuberculosis was suspected in 42 cases (48%) by histology and confirmed positive results were obtained by staining in 8 (19%), by culture in 23 (55%), and by PCR in 34 (81%) patients. All were confirmed after therapeutic success. In addition to the epidemiological transition process occurring in Mexico, tuberculosis remains an important cause of CLA. Histopathology with confirmatory studies including PCR can detect tuberculous aetiology.

INTRODUCTION

Cervical lymphadenopathy (CLA) can be a diagnostic challenge for the clinician because of the numerous aetiological factors that can cause this entity [1]. The corresponding histological picture in a certain number of cases is non-specific, but enlarged lymphatic nodes can be seen in metastasis of different carcinomas, in lymphoproliferative disorders, as well as in a variety of infections. In distinguishing among the causes of CLA, clinical records and epidemiological antecedents may be helpful in a number of patients, but a lack of clear features is not infrequent and special studies should be performed. When chronic infectious disease is the cause, a variety of organisms may be the aetiological agent, such as *Mycobacterium tuberculosis*, atypical mycobacteria, *Histoplasma capsulatum*, and *Coccidioides immitis*, among others [2–4]. However, tuberculosis (Tb) should be considered as most responsible in developing countries because of its high prevalence [5] and because CLAs are the most frequent extrapulmonary localization of mycobacterial infection, including in HIV-positive cases [6, 7]. In contrast, mycobacteria other than *M. tuberculosis* are now the main aetiological agents of CLA in both immunocompetent children and adults in industrialized countries [2]. Regarding diagnosis, results from Giemsa or Papanicolaou stains of smears and histopathological studies of affected lymph nodes may suggest Tb. Definitive results are usually achieved through more specific microbiological and molecular biology methods [8–11]. The aim of this study was to

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establish the frequency of infectious aetiology in adult patients with CLA living in the Mexico City metropolitan area (population approximately 20 million) and to determine the importance of mycobacteria involvement.

PATIENTS AND METHODS

Patient population

The study involved all patients with enlarged lymphatic cervical nodes who attended the Hospital General de México, a concentration tertiary-level hospital with teaching facilities from November 2000 to January 2002. A case for this study was defined as a patient $\geqslant$15 years of age with CLA, without therapy with anti-Tb drugs, and HIV seronegative. Eighty-seven cases were included in the study and all signed approved consent forms. In all cases, aetiological search of lesions with emphasis on Tb was attempted. Diagnosis of Tb was suggested by the presence of typical granuloma formation in biopsy tissue and confirmed by positive culture for *M. tuberculosis* or response to specific therapy with 1 year of follow-up.

A careful clinical record was obtained from each patient, together with routine laboratory examinations. Other studies included skin testing with purified protein derivative (PPD-RT23, Statens Seruminstitut, Copenhagen, Denmark) and chest roentgenography. A venous blood sample was obtained, serum separated, and stored until enzyme-linked immunosorbent assay (ELISA) for Tb using a crude soluble BCG extract [12] was performed. Excisional lymphatic node biopsy was performed for histopathological, microbiological, and molecular biology analyses. Presumptive non-infectious cases were studied by the same methodology to exclude any possibility of infectious aetiology. Direct observed treatment supervised (DOTS) with isoniazid, rifampin, pyrazinamid, and ethambutol was initiated in cases in which Tb was documented by any method. Each month, clinical response was evaluated for up to 12 months or until complete resolution of the process was observed.

Resected lymphatic nodes were kept at 4 °C prior to processing. Each node was divided into three portions under aseptic conditions: one was employed for histopathological studies, the second for bacteriological diagnosis, and the third for molecular biology analysis with an in-house polymerase chain reaction (PCR). For histopathology, fragments were fixed in 10% formalin, embedded in paraffin, sectioned, stained with haematoxylin–eosin, and microscopically examined to determine characteristics. Tb was suggested when typical granuloma formation with caseation, liquefaction, hyaline capsule around necrotic foci, fibrosis and epithelioid cell reaction were present [13]. Other non-Tb pathological changes were also described. Bacteriological studies were performed as recommended [14] in specimens homogenized in phosphate-buffered saline isotonic solution and decontaminated with 4% sodium hydroxide solution. After neutralization and centrifugation, each homogenate was resuspended in distilled water. Acid-fast bacilli (AFB) were identified by Ziehl–Neelsen stain. For culture, 0·2 ml aliquots of the biopsy homogenates were inoculated into bottles with MGIT fluorescent liquid medium (*Mycobacterium* growth indicator tube, Becton Dickinson, San José, CA, USA) and in Löwenstein–Jensen (LJ) solid medium slants. Vials and slants were incubated at 37 °C for up to 8 weeks and inspected weekly for growth. Acid fastness of bacterial growth in both media was assessed by Ziehl–Neelsen staining. Isolates were identified by standard biochemical tests. An in-house PCR amplification of a fragment of IS*6110* (98% sensitivity and 97% specificity), using primers IS11 (5′-CAC GCT AAT TAC CCG GTT CAT CG-3′) and IS12 (5′-ATC GCG CAG CTC GCG GCG G-3′) was performed as described elsewhere [15]. Briefly, 50 μl of reaction mixture containing 0·67 M Tris–HCl (pH 8·8), 0·016 M ammonium sulphate, 0·01 M 2-mercaptoethanol, 3 mM $MgCl_2$, 2 U of *Taq* polymerase, 200 μM each of dATP, dCTP, dGTP and TTP, and 50 pmol of each primer (final concentrations) was subjected to 40 amplification cycles of 94 °C for 30 s and 67 °C for 2 min. A 10-μl aliquot of amplified DNA was analysed by electrophoresis in 2% agarose gels. The 175-bp product DNA was stained with Gel-star (Bio-Whittaker, Walkersville, MD, USA). *Mycobacterium tuberculosis* H37Rv DNA was employed as positive control.

A 2×2 contingency table was constructed to obtain sensitivity, specificity and positive and negative predictive values. Statistical significance was determined with the χ^2 test. Correlation coefficients were calculated with 95% confidence limits (95% CI) by Spearman rank test.

RESULTS

Of 87 CLA cases studied, 42 were males and 45 females with mean age of 45·6 years (range 15–75

https://doi.org/10.1017/S0950268803001304 Published online by Cambridge University Press

Table 1. *Diagnostic methods for CLAs* (*overall results: n=87*)

Diagnostic method	Tb +	Tb −	Non-Tb +	Non-Tb −	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Concordance (%)
Lymphatic node									
Histopathology	42	0	0	45	100	100			
AFB by ZN	8	34	0	45	19	100	100	47	53
LJ culture	23	19	0	45	55	100	100	61	74
MGIT culture	23	19	0	45	55	100	100	61	74
In-house PCR	34	8	4*	41	83	90	90	80	84
PPD reactivity	21	21	6	39	50	82	80	54	65
Serum antibodies by ELISA	21	21	3	42	50	90	88	56	67

Tb, Tuberculosis; PPV, positive predictive value; NPV, negative predictive value; AFB, acid-fast bacilli; ZN, Ziehl-Neelsen stain; LJ, Löwenstein-Jensen; MGIT, *Mycobacterium* growth indicator tube; ELISA, enzyme-linked immunosorbent assay.
* PCR was positive in two cases of Hodgkin's lymphoma, one breast adenocarcinoma and one lung adenocarcinoma.

years). Seventy-seven (88·5%) patients presented unilateral lymphadenopathy, 51 of right cervical lymphatic chain and 26 left, and 10 had bilateral affection. Predilection for right side ($P=0{\cdot}01$) was especially noticeable in cases in which Tb was diagnosed [30/42 (71%)] in relation to patients with other pathological disorders [19/45 (46%)].

Diagnosis was done in excised lymph node biopsies beginning with histopathological studies. According to findings, cases were grossly classified as non-infectious (lymphoproliferative) and chronic infectious (caseating granulomata). In the former group, 45 cases (52%) were included as follows: 20 with lymphatic metastasis of carcinoma (13 from lung, all with intra-thoracic abnormalities, 5 from breast, 1 from stomach, and 1 colonic), 19 with non-specific lymphoreticular hyperplasia, and 6 with lymphoma (5 with Hodgkin's and one non-Hodgkin's). In 42 cases (48%), lesions showed caseating granulomata compatible with tuberculous infection. From a clinical standpoint, it was considered that all histopathological positives were true Tb cases until proved otherwise; therefore, standard chemotherapy was initiated in all cases. In this group, up to 70% of patients had a history of non-specific symptoms such as fever, asthenia, weight loss, cough, and dyspnoea, 24 (57%) were males and 18 (43%) were females, and 7 (17%) had typical lesions in chest radiograph. Microbiological studies were positive only in lymph node biopsies from this Tb group: AFB were demonstrated in 8 samples (19%) and positive growth in MGIT and LJ media was observed in 23 samples (55%), all corresponding to *M. tuberculosis*. Except for a reduction in the time required to detect mycobacterial growth, no differences in efficiency of liquid or solid culture media were found. In-house PCR had the highest diagnostic yield; the test was positive in 34/42 cases (81%) with Tb, and also in 4/45 (8%) in the non-Tb group.

Concerning other tests, PPD skin reactivity (cut-off $\leqslant$10 mm induration) was positive in 27 (31%) of 87 cases, 21 in the Tb group (50%), and in 6 of the non-Tb group (13%). Antimycobacterial antibodies by ELISA were positive for 24 of 87 serum samples (28%), 21 in Tb patients (50%), and in 3 in the non-Tb group (7%).

Considering overall results, with Tb plus non-Tb cases, concordance for PCR was 84%, the highest in comparison with LJ and MIGT 73·6%, ELISA 66·7%, PPD 65%, and AFB 52·8% (Table 1).

DISCUSSION

Rapid recognition and accurate characterization of aetiology of CLAs are obligatory for the institution of appropriate treatment regimens [13]. The findings of this study revealed that in a cohort of Mexican patients with CLA 48% corresponded to infectious aetiology with histopathological characteristics compatible with Tb. In Mexico, nearly 18 900 new cases of Tb were confirmed in 2001 (18·7 per 100 000) [16]; although the actual number of extrapulmonary forms is unknown in the country, within the setting that this investigation was carried out approximately 30% of Tb cases are extrapulmonary. CLA being the most frequent with >40% of these cases. Confirmation of

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Tb diagnosis was obtained in all 42 suspected cases, in 17 by positive results from bacteriological tests and PCR with a fragment specific for the *M. tuberculosis* complex, in another 17 cases by PCR only, in 6 other cases only by culture, and in the remaining 2 cases after good therapeutic results with specific drug treatment. The figure of 48% for Tb aetiology is higher than the 13–41% reported in developed countries [3, 17] but is the same or very close to the figures found for India [18, 19], a developing country similar to Mexico. In our series, mycobacteria other than Tb were not identified as a cause of CLA; further, such a finding could be conceivable in the context of the process of epidemiological transition currently occurring in Mexico [20].

In our cohort, the majority of Tb patients were young adults but there did not appear to be a preference for females, as reported in other studies [8, 18, 21]. The most common presentation of CLA was unilateral with consistent predilection for right side, 71% for Tb, while in non-Tb only 46% presented at that location. History of pulmonary Tb or presence of other foci were detected in only 19% of Tb cases, supporting a local nature of the disease [8, 22–24].

Other diagnostic methods for CLA such as fine-needle aspiration (FNA) yielded good results [7, 9] and this has been proposed to replace excisional biopsy. However, FNA has demonstrated some diagnostic limitations [25], while study of biopsies from lymphatic nodes gives comprehensive information concerning the pathogenic process and the aetiology involved [8, 24]. This is an important issue because differential diagnosis of CLA is broad, Tb is often not suspected, and confident preliminary results can only be obtained through histopathological studies of biopsies.

Although identification of mycobacteria is mandatory for a definitive diagnosis of Tb, conventional techniques such as AFB microscopic observation and culturing employed in this study were disappointing due to the relative low sensitivity, i.e. 19 and 55%, respectively. Bacteriologically negative cases may be explained because of the low number of bacilli in samples. In this trial, the PCR-based amplification test demonstrated a relatively low sensitivity of 83%, but its feasibility and simplicity to perform in an ordinary laboratory signified a major improvement over standard techniques. False-negative persistent results of PCR were found in eight of our cases; in the six with positive culture for *M. tuberculosis* this may be due to non-uniform distribution of mycobacteria or the presence of inhibitory substances in the samples [26], and in the other two cases by infections with non-tuberculous mycobacteria. Of 45 biopsies from non-Tb patients, four had positive PCR results but negative bacteriological studies. Mycobacterial DNA contamination was not a likely cause for this result because different personnel and laboratories were routinely used during the process, leading to a very low probability of false-positive results. Since *M. tuberculosis* can be present in individuals without Tb symptoms in endemic areas [27], these four cases could be true positives, considering that PCR positivity is not always indicative of active infection [28].

In this study, immunological tests as diagnostic tools for Tb were not reliable, and were without any definitive correlation with results in other tests for Tb. PPD skin reactivity was positive in only 50% of Tb cases, while 13% were reactors in the non-Tb group. The percentage of PPD reactors in the Tb group was surprisingly low, slightly higher than the 41% reported in the Mexican general population [29, 30], resulting probably from their low nutritional condition together with the anergy associated with the infection [31]. Only 6 non-Tb cases were PPD reactors, all non-malignant. In the search for anti-mycobacterial antibodies, 50% of Tb patients were negative, including 12 cases in which cultures were positive. In addition, a high cut-off value was used (optical density, three times background) to eliminate an overall false-positive rate; 7% in the non-Tb group were ELISA-positive and also PPD-positive, probably due to an immune response elicited from dormant bacteria of a previous primary infection.

Results of this trial currently suggest that histopathology is a good option as an initial diagnostic procedure for CLA. When possible, excisional biopsy should be preferred over FNA because it gives comprehensive information. In patients from developing countries with an important prevalence of *M. tuberculosis* infection, this agent must come under immediate suspicion when infectious aetiology is present. It is recommended that when infectious CLA is strongly suspected, the patient should be notified and treated for Tb until histopathological, bacteriological and molecular biology studies confirm another aetiology. Microbiological evaluation and clinical correlation should always be carried out for definitive diagnosis. Although isolation of *M. tuberculosis* from biopsies is infrequent, culture of the causative agent must be intended for further drug susceptibility testing. Amplification assay has disadvantages regarding

sensitivity; however, its low cost, the short time to achieve results, and the easy performance of the test [32], render PCR an adequate tool to support clinical and histopathological diagnosis of Tb CLA. In conclusion, as we found 49% of all CLA cases studied to be tuberculous, it is important in an endemic Tb area that Tb is strongly suspected when infectious aetiology has been detected. PCR must be used as a complementary and confirmatory test in doubtful cases.

ACKNOWLEDGEMENTS

The authors are indebted to Guadalupe Granados, Raquel Escobar and Guadalupe Torres for their excellent laboratory assistance.

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