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Short communication

MOLECULAR ANALYSIS OF MYCOBACTERIAL DNA IN SOME CASES OF SARCOIDOSIS: CORRELATION WITH CLINICAL COURSE AND 10 YEARS' OBSERVATION

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Abstract

We present six patients with sarcoidosis whose transbronchial biopsy specimens had been investigated, the gene of immunogenic protein MPB64 for Mycobacterium tuberculosis by polymerase chain reaction analysis. M. tuberculosis DNA was detected in three of them by PCR. We followed patients with sarcoidosis for ten years. We discussed tuberculin test results and clinical course of M. tuberculosis positive and negative patients with polymerase chain reaction. we cannot make a conclusion about a relationship between clinical course and mycobacterial DNA positivity.

Key words: *Sarcoidosis, M. tuberculosis, Polymerase chain reaction, Clinical course*

Bazı Sarkoidoz Vakalarında Mikobakteriyel DNA'nın Moleküler Analizi: Klinik Yönü ile İlişkisi ve 10 Yıllık Gözlem

Transbronşial biopsi örneklerinde M. tuberculosis, immunojenik protein MPB64 geni polimeraz zincir reaksiyonuyla araştırılan altı hasta sunuldu. PCR'la altı örneğin üçünde M. tuberculosis DNA'sı belirlendi. Sarkoidozlu hastalar on yıl izlendi. Polimeraz zincir reaksiyonuyla M. tuberculosis pozitif ve negatif hastaların tuberkülin test sonuçları ve klinik yönü tartışıldı. Klinik yönü ve mikobakteriyel DNA pozitifliği arasında ilişki olmadığına karar verildi.

Anahtar kelimeler: *Sarkoidoz, M. tuberculosis, Polimeraz zincir reaksiyonu, Klinik yönü*

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INTRODUCTION

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology. Because of pathological similarities with pulmonary tuberculosis, the role of mycobacteria in the pathogenesis of sarcoidosis has often be hypothesized (1, 2, 3). Recent studies on molecular evidence have provided a strong link between infectious agents and sarcoidosis (4, 5)

Isolation of mycobacteria in sarcoid tissues using standard microbiological techniques is generally difficult, especially in areas where tuberculosis has a high prevalance, although acid fast organisms have been identified. Discussions about the potential pathogenetic role of the cell wall deficient mycobacteria in sarcoidosis have heightened interest in recent decades (6). Almenoff et al (7) have shown acid fast cell wall deficient forms (CWDF) in the patients with active sarcoidosis. Over the last ten years molecular techniques have been used to identify mycobacterial nucleic acid in samples from patients with sarcoidosis (8, 9). Some investigators detected mycobacterial DNA in sarcoid tissues by polymerase chain reaction (PCR), but some others did not (1, 2, 10, 11).

The aim of this study is to investigate whether *M. tuberculosis* DNA could be detected in samples of sarcoidosis patients by PCR and analyze the relationship between *M. tuberculosis* DNA positivity and clinical course.

EXPERIMENTAL

Patients and Samples

Transbronchial biopsy specimens from six patients with sarcoidosis were collected from Ankara University Hospital. Each biopsy was from a different patient. Specimens from each patients were examined by routine microbiologic, histopathologic methods and PCR. For this study, we followed patients who had had mediastinal or cervical lymph node resection over a 10 year period.

DNA Extraction

DNA was extracted from transbronchial biopsy specimens as described previously (12, 13). Biopsy samples were minced and extracted DNA using proteinase-K over-night digestion, phenol/chloroform extraction and ethanol precipitation.

Amplification

Amplification was carried out using a primer set spesific for *M. tuberculosis/bovis* BCG (14, 15, 16). We used a specific 240 bp primer set of primers, a fragment called immunogenic protein MPB64: sense 5' TCC GCT GCC AGT CGT CTT CC 3' (MT1) and antisense 5'GTC CTC GCG AGT CTA GGC CA 3'(MT2).

PCR was performed with 5 µl of DNA product, 100 ng of each primer, 200 µM dNTPs, 10mM Tris HCl (pH = 8.3), 50 mM KCl, 1,5 mM $MgCl_2$, 0.01% gelatin (w/v), 0.1% Triton X-100 and 2.5 U Taq polymerase (Promega Co, Madison, WI) in a final volume of 100 µl. Amplification was carried out for 30 cycles with 2 minute denaturation at 94°C, 60°C annealing for 2 minutes and primer extension at 72°C for 2 minutes.

We used a sample of distilled water as a negative control, DNA from different bacterial specimen for specificity of the primers (*E. coli*) and a sample of *M. tuberculosis* DNA extracted from a known culture as positive control (strain H37 Rv from Institute of Hıfzıssıhha, Turkey)

in each set of specimens tested. All of the positive control group had evidence of *M. tuberculosis* and no mycobacterial DNA was detected in the negative control and *E. coli.*

Amplified PCR products were analyzed by electrophoresis in 2% agarose gels and visualized by ethidium bromide staining. Representative patterns of PCR application are shown (Figure 1).

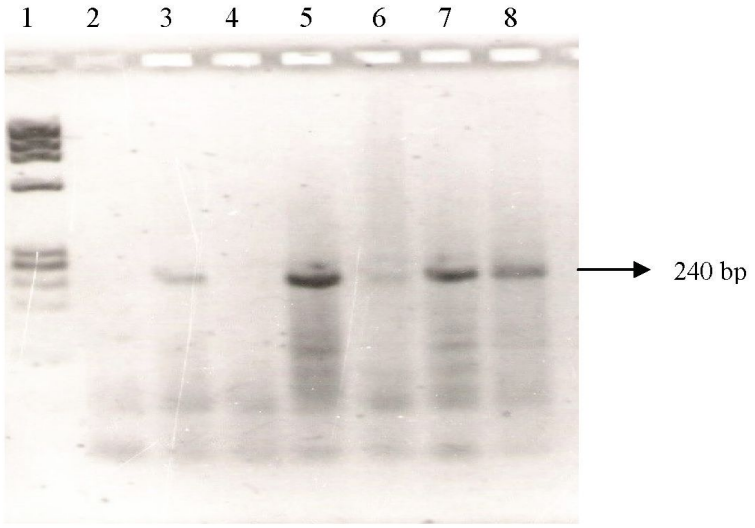

Figure 1. Representative patterns of PCR detection of an *M. tuberculosis*
1 molecular marker, 2 negative control, 3 positive control,
4 *E. coli* DNA, 5, 6, 7, 8 PCR positive (+) patients with sarcoidosis

RESULTS

We present here six patients with sarcoidosis whose samples had been investigated for *M. tuberculosis* by PCR.

Case 1: A 40 years old, female patient. In another hospital, during cholecystectomy, suspicious lesions on the liver had been seen, and several biopsies from these lesions had been taken. Histopathological examination of the biopsy specimens had revealed non-caseating granulomas. The patient was referred to Ankara University Hospital. Chest x-ray and computed tomography of the thorax of the patient showed bilateral hilar lymphadenopathy and diffuse interstitial pattern. Bronchoscopy was performed, bronchoalveolar lavage (BAL) and transbronchial biopsy (TBLB) specimens were taken. BAL CD4(+)/CD8(+) T lymphocytes ratio was 6.1. TBLB was compatible with sarcoidosis. Tuberculin skin test was negative. PCR assay for *M. tuberculosis* was negative in TBLB specimen. She had been treated with corticosteroids for a long time; because her pulmonary function tests impaired when dose of steroid had been decreased. She has been following without treatment for five years, her pulmonary function test measurements are stable.

Case 2- A 23 years old, male patient. He was admitted to our hospital because of dry cough and bilateral hilar lymphadenopathy on chest x-ray. Physical examination was normal except macolupapular lesion on the forehead. Skin biopsy from this lesion and TBLB were compatible with sarcoidosis. Tuberculin skin test was 9 mm. PCR assay for *M. tuberculosis* was positive in TBLB specimen. His skin lesion was treated with local steroids. His cough and chest x-ray resolved spontaneously.

Case 3- A 27 years old, male patient. He was admitted to our hospital because of exertional dyspnea. His physical examination was normal. Chest x-ray showed bilateral hilar lymphadenopathy and diffuse reticulonodular infiltrates. Histopathological examination of TBLB specimens revealed non-caseating granulomas compatible with sarcoidosis. Tuberculin skin test was 10 mm. PCR assay for *M. tuberculosis* was positive in TBLB specimen. He was given corticosteroid treatment for a year. He responded treatment very well. After cessation of treatment sarcoidosis did not recured.

Case 4- A 21 years old, female patient. She was referred to our hospital to investigate the etiology of erythema nodosum. Physical examination was normal except for lesions compatible with erythema nodosum on her legs. Chest x-ray revealed bilateral hilar lymphadenopaty. Histopathological diagnosis of the TBLB was sarcoidosis. Tuberculin skin test was negative. PCR assay for *M. tuberculosis* was positive in TBLB specimen. Her illnes resolved spontaneously.

Case 5- A 35 years old, female patient. She was consulted from Eye Department. She was getting treatment for uveitis. Physical examination was normal. Chest x-ray showed bilateral hilar lymphadenopathy. Tuberculin skin test was negative. TBLB was compatible with sarcoidosis. PCR was negative for *M. tuberculosis*. She was not treated with systemic steroids. Her chest x-ray improved spontaneously.

Case 6- A 38 years old, female patient. She was admitted to hospital because of dry cough and generalized arthralgia. Physical examination was normal. Chest x-ray showed bilateral hilar lymphadenopathy. Tuberculin skin test was 10 mm. BAL CD4(+)/CD8(+) T lymhocytes ratio was 5.8. Histopathological diagnosis of the biopsy specimens was sarcoidosis. PCR was negative for *M. tuberculosis*. She was given nonsteroid antiinflammatory drugs. She improved without corticosteroids.

DISCUSSION

Sarcoidosis is a granulomatous inflammatory disorder with unknown etiology. It is supposed that different etiological factors could be responsible for different patients with sarcoidosis (17). Parkes et al were among the first to provide evidence for an infectious cause of sarcoidosis (18). Mycobacterial infections can be seen in tissues of patients with sarcoidois and mycobacteria have been considered as a etiologic agent (4). It is speculated that *Mycobacterium tuberculosis* may play a role in the pathogenesis of sarcoidosis at least in some cases due to clinically and the histological similarity between the sarcoidosis and tuberculosis. There is an intense controversy concerning the role of mycobacteria in sarcoidosis (3).

The use of molecular methods has shown the presence of mycobacterium in recent studies. It is determined mycobacteria by PCR analysis in tissues of patients with sarcoidosis at a lot of studies (4) Popper et al (19) detected mycobacterial 65 kDa antigen in 11 from lung biopsy specimens (35 cases) with sarcoidosis, but IS6110 sequence of the *Mycobacterium tuberculosis* was not found in any of the 11 cases. Some authors used various DNA sequences for detecting mycobacteria in the tissue of sarcoidosis patients (20). A group used to 16S rRNA, rpoB, and IS6110 sequences in patients with sarcoidosis for determining mycobacterial DNA. The study reported mycobacterial DNA using rpoB in 24% of sarcoidosis specimens and 16S rRNA in 48% of the same specimens, but *M. tuberculosis* DNA were not found for all of them IS6110 (21). Our study uses MPB 64 protein coding gene for *M. tuberculosis*. It determines *M. tuberculosis* DNA in three of six transbronchial specimens in sarcoidosis patients using PCR. In contrast, several studies did not detect mycobacterial DNA from clinical samples in patients with sarcoidosis (11, 22, 23, 24). Ghossein et al also failed to detect mycobacterial DNA in formaldehyde fixed paraffin embedded tissues from 10 patients with sarcoidosis (10).

Most of studies investigating the role of mycobacteria in sarcoidosis originate from developed countries. *M. tuberculosis* DNA was detected in lung, skin or lymph node of sarcoidosis patients using nested PCR in Spain (25). In another study (26) was not found *M. tuberculosis* DNA in any of the all samples from Japanese patients with sarcoidosis, but *M. tuberculosis* were found in 8% of the samples from European patients with sarcoidosis. It is necessary studies from countries with high tuberculosis prevalance. There are a lot of case reports that shown a link between sarcoidosis and *M. tuberculosis* in Turkey (27, 28).

We evaluated our six patients with sarcoidosis whose samples investigated for *M. tuberculosis* by PCR. We can not discuss the etiological role of mycobacteria in sarcoidosis with this very limited patient number. We aimed to discuss clinical course of patients with positive or negative PCR results. PCR was positive for *M. tuberculosis* in three patients. Tuberculin skin test was negative in one of them, 9 and 10 mm in others. In case 6; tuberculin test was 10 mm, but PCR assay was negative. It seems that there is not an association between tuberculin positivity and PCR positivity. We think that studies other than case reports are necessary to discuss if there is an association between tuberculin positivity and PCR positivity. It is known that tuberculin anergy in sarcoidosis is not influenced by the rate of Mantoux positivity in the general population (29). So prevalance of tuberculin sensitivity in the population is not important to investigate this association. Clinical course of sarcoidosis is not different in PCR positive (mycobacterial DNA) and PCR negative patients. Fite et al. (25) did not find clinical differences between mycobacterial DNA-positive and mycobacterial DNA-negative for sarcoidosis patients as our results as. Two of PCR positive patients improved spontaneously, other patient was given corticosteroids. One of PCR negative patients was given corticosteroids for a long time, but illness in others was resolved spontaneously. Asymptomatic patients with sarcoidosis do not require therapy. When therapy is required for pulmonary sarcoidosis, corticosteroids have to use (30). A response to corticosteroid therapy does not establish the diagnosis of sarcoidosis.

The pathogenetic role of *M. tuberculosis* in sarcoidosis is controversial. Some studies showed that *M. tuberculosis* DNA is to be only sporadically in the granulomatous lesions of sarcoidosis and doesn't establish a correlation between *M. tuberculosis* DNA positivity and the pathogenesis of sarcoidosis (31). In contrast, several studies demonstrated that *M. tuberculosis* may play important pathogenetic role in the some sarcoidosis patients and allow in differentiating tuberculosis and sarcoidosis (4, 5, 10, 32, 33).

After following ten years for patients of sarcoidosis, we think that we cannot make a conclusion about a relationship between clinical course and mycobacterial DNA positivity with these case reports; it is necessary to design studies comparing PCR positive and negative patients with sarcoidosis.

CONCLUSION

This study determined *Mycobacterium tuberculosis* DNA by PCR in tuberculin skin test positive and negative of transbronchial biopsy samples from sarcoidosis patients. *Mycobacterium tuberculosis* DNA is therefore unlikely to be a factor in the pathogenesis of this disease.

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