Searching for Mycobacterium Tuberculosis the Cases with Granuloma Annulare by Amplified Mycobacterium Tuberculosis Direct Test

GRANULOMA ANNULARELI OLGULARDA AMPLİFİYE MYCOBACTERIUM TUBERCULOSIS DİREKT TEST İLE MYCOBACTERIUM TUBERCULOSİS’İN ARANMASI

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Summary

Background and objective: Existence of some clinical and histopathologic similarities between granuloma annulare (GA) and some forms of cutaneous tuberculosis motivated us to determine whether Mycobacterium tuberculosis complex was present in the skin biopsy specimens of patients with GA. We used AMTDT in order to show M.tuberculosis in GA lesions because the diagnostic identification of M. tuberculosis in skin diseases has remained difficult using conventional laboratory methods.

Place of the study: Dermatology and Pathology Departments of Kocaeli University and Karadeniz Technical University (January 1994-November 1998).

Materials and Methods: The pathology archives at Kocaeli University and Karadeniz Technical University were reviewed and 12 patients with histologically confirmed as GA were identified. Paraffin embedded skin biopsy specimens of these patients were tested for the presence of M. tuberculosis complex using Amplified Mycobacterium tuberculosis direct test. Ziehl-Neelsen and Periodic acid-Schiff staining were performed in a total of 12 specimens. At the same time patients were called and the following informations were recorded; clinical characteristics of the lesions, possible precipitating factors, medical and family history of tuberculosis; chest x-ray examination results, and hypersensitivity to intradermal injection of M. tuberculosis purified protein derivative (PPD). Routine laboratory tests including complete blood count, biochemical analysis, urine analysis, serological tests for syphilis and HIV infection were also performed.

Results: M. tuberculosis complex was detected only in one biopsy specimen of the patient with GA whose lesion resolved spontaneously without scarring. M.tuberculosis specific polymerase chain reaction and Ziehl-Neelsen staining for acid-fast bacilli were also positive in this biopsy specimen.

Conclusion: This finding could either be an incidental or M. tuberculosis is one of the factors that cause the cell mediated immune response in granuloma annulare.

Key Words: Granuloma annulare, Mycobacterium tuberculosis, Amplified Mycobacterium tuberculosis direct test

Granuloma annulare (GA) is a benign inflammatory and usually self limited dermatosis with an unknown etiology (1). But the recent data suggest that delayed type hypersensitivity may be important in the pathogenesis of GA (2). The lesions of GA consist of small, firm, asymptomatic nodules that are often grouped in a ring-like or circinate fashion (3). GA is described histologically as focal degeneration of collagen surrounded by an infiltrate composed of histiocytes and lymphocytes (4). Existence of some clinical and histopathologic similarities between GA and forms of skin tuberculosis and sarcoidosis directed us to determine whether Mycobacterium tuberculosis complex was present in the skin biopsy specimens of patients with GA.

Amplified Mycobacterium tuberculosis direct test (AMTDT) (Gen-Probe Inc., San Diego, CA/USA) is a target-amplified nucleic acid probe test for the in vitro diagnostic detection of Mycobacterium tuberculosis complex ribosomal ribonucleic acid (rRNA). This test utilises transcription mediated amplification and the hybridisation protection assay to qualitatively detect M. tuberculosis complex rRNA (5). In a study, the specificity of this test for detection of M. tuberculosis in historical paraffin-embedded pleural biopsy specimens were found 100 %, and the sensitivity were determined 52.6 % (6).

**Patients and methods**

The pathology files at Kocaeli University and Karadeniz Technical University from January 1994 to November 1998 were reviewed and 12 biopsy specimens histologically confirmed as GA were identified. Of the 12 biopsy specimens, 7 were from the archives of the Department of Pathology of Kocaeli University and 5 were from the archives of the Department of Pathology of Karadeniz Technical University. Patients were also clinically examined. The following informations were recorded; clinical characteristics of the lesions; possible precipitating factors (insect bite, trauma, tuberculin test, sun exposure, drug and infections) personal and family history of tuberculosis; chest x-ray examination result, and hypersensitivity to intradermal injection of M. tuberculosis purified protein derivative (PPD). Routine laboratory tests including complete blood count, biochemical analysis, urine analysis and serological tests for syphilis and human immune deficiency virus (HIV) infection were also performed, but the culture of M. tuberculosis was unavailable.

**Histopathologic examination**

We examined 12 paraffin-embedded skin biopsy specimens with a histologic diagnosis of GA. All biopsy specimens had been embedded in paraffin after fixation in “Holland solution” or “10 % formaldehyde” and processed by standart method. Ziehl-Neelsen and Periodic Acid Schiff staining were also performed in a total of 12 biopsy specimens.

**Amplified Mycobacterium Tuberculosis Direct Test (AMTDT)**

The samples were tested for the presence of M. tuberculosis complex using the Mycobacterium Tuberculosis Direct (MTD) test (Gen-Probe Inc., San Diego, CA/USA) in the Microbiology Department of Karadeniz Technical University.

Specimens in paraffin blocks were sliced and deparaffinized in 3 ml of xylene for 10 min at room temperature. The xylene was removed by centrifugation of the sample at 5000 rpm for 10 min. The pellet was washed by 95 % ethanol and three times with sterile distilled water and dissolved in 1 ml TE buffer (10 mM Tris-HCl, PH 8.0; mM EDTA, PH 8.0) (7).

The samples were decontaminated following the N-acetyl-L-cysteine NaOH procedures (8). The AMTDT was performed in three steps according to the instructions on the package insert of the first version of the kit. Briefly, lysis was induced by adding 50µL of digested sample to 200µL of specimen dilution buffer in a lysing tube, and the mixture was sonicated in a water bath sonicator (Gen-Probe, Germany) for 15 min at room temperature. For amplification, 25µL of reconstituted amplification reagent was placed in a bottom of a reaction tube and covered with 200µL of mineral oil. Fifty microliters of lysate
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was transferred to the amplification tube, incubated at 95 °C for 15 min, and then cooled at 42 °C for 5 min. After addition of an enzyme reagent mix (25 µL), the mixture was incubated at 42 °C for 2 h. Twenty microliters of the termination reagent was added to each amplification tube, and the mixture was kept at 42 °C for additional 10 min. Detection was achieved by adding 100 µL of the reconstituted acridium-labelled probe reagent to each tube and incubating the mixture at 60 °C for 15 min. The selection reagent (300 µL) was then added, and the mixture was reincubated at 60 °C for 10 min. All temperature-controlled incubation steps were carried out in heating blocks, and all runs included AMTDT positive and negative controls. The tubes were cooled at room temperature for 5 to 10 min prior to being read in a luminometer (Leader 50; Gen- Probe, Germany) with the cut off value set at 30,000 relative light units (RLUs). Thus, samples showing > 30,000 RLUs were considered positive, and those showing < 30,000 RLUs were considered negative.

*M. tuberculosis*-specific polymerase chain reaction was done in sample which was positive in AMTDT to confirm the positivity. The primers MT1 (5' CCT GCG AGC GTA GGC GTC GG 3') and MT2 (Biotin- 5' CTC GTC CAG CGC CGC TTC GG 3') directed against IS6110 sequence of *M. tuberculosis*, *M. bovis* and *M. simiae* were used (9). One hundred twenty three base pair product was visualised by ethidium bromide staining on agarose gel electrophoresis (Figure 1).

**Results**

All of the patients had localized type of GA. None of them had a personal or family history of tuberculosis. Tuberculin tests showed an erythematous induration of larger than 1.5 X 1.5 cm in three patients. There was no evidence of pulmonary tuberculosis in 12 patients according to the chest x-ray examination results. Serological tests for syphilis and HIV infection were negative in all of the patients. Complete blood count, biochemical analysis and urine analysis were normal in all of the cases except fasting plasma glucose was high in 3 cases. Characteristic histopathologic findings such as collagen degeneration or palisading granuloma were seen in 11 biopsy specimens, in one biopsy specimen neutrophil leucocytes was detected in the centre of the granuloma. *M. tuberculosis* complex was detected only in one patient with GA whose lesion resolved spontaneously without scarring (Case No: 8). Ziehl-Neelsen staining for acid-fast bacilli was also positive in this case; but negative in the rest. *M. tuberculosis* specific polymerase chain reaction (PCR) was done in this case to confirm the positivity and the result of PCR was also positive.

The clinical features, histopathologic and laboratory findings and the results of AMTD test of the patients are outlined in Table 1.

**Discussion**

Numerous hypotheses have been proposed to explain the pathogenesis of GA, such as immune-
complex vasculitis, a disorder of fibrinolysis, a derangement of phagocyte function, a disorder of catabolic enzyme release and a cell-mediated immune reaction. Nowadays the identification of T lymphocytes in the cutaneous infiltrate supports the role of cell mediated immune reaction in the pathogenesis (10). GA has also been reported to develop at the site of resolved herpes zoster and occasionally in association with necrobiosis lipoidica (4), sarcoidosis (4,11) and AIDS (3).

The relationship between GA and diabetes mellitus has been extensively investigated. Some reports show no evidence of any interrelationship but others indicate that the disease, especially in its atypical generalized form, should be included among those skin disorders associated with diabetes mellitus. A survey of 100 patients releaved diabetes in 21 % of generalized GA and in 10 % of localized GA (2). In our study fasting plasma glucoses were high in 3 patients of localized GA.

There is no evidence for other putative causes of GA including tuberculosis, trauma, streptococcal infection and collagen disorders (1).

Mycobacteria are acid-fast intracellular bacteria. Protective immunity against intracellular bacteria is generally a local event which results from granuloma formation (12). Mycobacteria are frequently suggested of causative agents in various granulomatous dermatoses. Some studies reported the presence of \textit{M. tuberculosis} in patients with sarcoidosis. In these studies the authors thought that sarcoidosis might be resulted from an immune response initiated by \textit{M. Tuberculosis} (13-15). Although the idea \textit{M. tuberculosis} might play a role in the etiology of GA has been discarded, there are some similarities of its clinical presentation and histopathologic characteristics with cutaneous tuberculosis and sarcoidosis. These similarities oriented us to investigate whether \textit{M. tuberculosis} could be detected in GA lesions. We used AMTDT in order to show \textit{M. tuberculosis} in GA lesions because the diagnostic identification of \textit{M. tuberculosis} in skin diseases has remained difficult using conventional laboratory tests (i.e microscopy and culture) (16). The staining of smears for acid-fast bacilli is not a sensitive method; and mycobacterial culture takes time as long as 3 to 8 weeks (17). So we think to perform the AMTD test which is very specific in order to detect \textit{M. tuberculosis} in the biopsy specimens. In our study \textit{M. tuberculosis} complex was detected only in one biopsy specimen of the patient with GA whose lesion resolved spontaneously without scar-

\begin{table}
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\caption{The clinical features, histopathologic and laboratory findings and the result of AMTD test of the patients.}
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Case & Age and Sex & Site & Histologic characteristics & fasting plasma glucose & PPD & AMTD test \\
No & & & & & & & \\
\hline
1 & 2, M & Shoulder and hand & palisading granuloma, collagen degeneration & 260 & 17 mm & negative \\
2 & 54, F & Hand & collagen degeneration, dispersed giant cells, histiocytic infiltrate & negative \\
3 & 58, M & Hand & nuclear dust, neutrophyl leucocyte in the centre of the granuloma & negative \\
4 & 60, M & Posterior of the ears & dispersed giant cells, collagen degeneration, histiocytic infiltrate & 120 & 15 mm & negative \\
5 & 61, F & Hand & collagen degeneration, dispersed giant cells, histiocytic infiltrate & 72 & 20 mm & negative \\
6 & 5, F & Hand and foot & degenerate collagen in the centre of palisading granuloma & 100 & & negative \\
7 & 34, F & Hand and foot & degenerate collagen, palisading granuloma & 86 & 10 mm & negative \\
8 & 27, F & Hand & necrobiosis in collagen fibers, groups of histiocytes & 13 mm & & positive \\
9 & 6, M & Foot & focal collagen degeneration, granulomatosus inflammation & & & negative \\
10 & 62, F & Forearm & collagen degeneration, multinuclear giant cells & & & negative \\
11 & 49, M & Hand & collagen degeneration, histiocytic infiltrate & 206 & 10 mm & negative \\
12 & 3, F & Foot & collagen degeneration, nuclear dust, perivascular chronic inflammation & 95 & 13 mm & negative \\
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ring. This finding could either be an incidental event or *M. tuberculosis* may be a causative factor for the cell-mediated immune response in GA.

The relationship between GA and *M. tuberculosis* had previously been speculated, (1) but we could not find any study regarding the presence of *M. tuberculosis* in GA lesions in the literature except the role of inciting agents such as tuberculosis skin test was reported in GA (2). Probably *M. tuberculosis* is one of the factors that cause this cell mediated immune response in GA.

Sometimes epitheloid nodules simulating sarcoidosis may be found in patients with clinical lesions of GA (4). These findings explain the difficulty to distinguish some cases of cutaneous sarcoidosis from GA. In this time the authors suggested that the clinician must follow up the patient for a long time, because sarcoidosis may occur in the skin before visceral involvement is detected (4). Some authors also propose that some GA lesions may be precursor lesions to cutaneous sarcoidal granulomas (18). We could not notice any cutaneous sarcoidal lesion in our patient with granuloma annulare in four years follow up who gives a positive reaction in AMTD test.

REFERENCES


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