Erythema Induratum: A Clinicopathologic and Polymerase Chain Reaction Study

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**Background:** In Taiwan, cutaneous lesions with granulomatous lobular panniculitis, with or without vasculitis, are usually diagnosed as erythema induratum (EI), a common form of tuberculid associated with tuberculosis. However, there has been no study to elucidate the extent of this association in Taiwan. The aim of this study was to document the spectrum of the pathologic findings in EI and its association with *Mycobacterium tuberculosis*.

**Methods:** The diagnostic/inclusion criteria for EI were recurrent tender subcutaneous nodules on the legs, histopathologic findings of granulomatous lobular or septolobular panniculitis plus necrosis or vasculitis, and positive response to antituberculosis therapy. The clinicopathologic findings in the 12 cases that fulfilled these criteria were analyzed, and nested polymerase chain reaction (PCR) was used to identify *M. tuberculosis* complex DNA from formalin-fixed, paraffin-embedded sections.

**Results:** Eleven women and one man were included in the study, ranging from 18 to 70 years old (mean, 40.6 yr). The duration of the disease ranged from 10 days to 10 years (mean, 2.1 yr). Histopathology revealed granulomatous panniculitis; a diffuse lobular pattern was observed in nine cases and a focal lobular/septolobular pattern in three. Vasculitis was found in nine cases, four affecting an artery or vein, with three occurring in the patients with focal panniculitis. Ten cases showed various degrees of caseous necrosis. Eosinophils or focal eosinophilia were fairly common (10 patients). From PCR, nine patients were positive for *M. tuberculosis* complex DNA.

**Conclusions:** Type III and IV hypersensitivity reactions to *M. tuberculosis* complex were involved in the pathogenesis of EI. Our results suggest that approximately half of the cases with pathologic findings consistent with EI or nodular vasculitis in Taiwan are associated with *M. tuberculosis*.

Erythema induratum (EI), first described by Bazin [1], is characterized by chronic, recurrent, tender, indurated, and sometimes ulcerated subcutaneous nodules occurring mainly on the lower legs of women with moderate or high hypersensitivity to *Mycobacterium tuberculosis*. Histologically, EI is characterized by a lobular granulomatous panniculitis with various degrees of necrosis, vasculitis, and tuberculoid granulomas. In 1945, Montgomery et al proposed the term nodular vasculitis (NV) to designate cases with similar clinicopathologic features but not of tuberculous origin [2]. There has been great controversy regarding the etiopathogenetic role of *M. tuberculosis* in EI [3, 4], but its recent detection has provided new evidence for the tuberculous origin of EI [4–8]. It is now generally agreed that the term EI should be reserved for cases with tuberculous origin [3]. This, however, requires confirmation by positive therapeutic response to anti-tuberculosis (anti-TB) therapy or positive detection of *M. tuberculosis* DNA in individual cases. Because this information is not usually available at the time of diagnosis in clinical practice, cases are either labeled as EI, NV, or EI/NV, depending on the prevalence of TB in the region. In Taiwan, dermatologists in general regard most EI-like lesions as tuberculous in origin, based on belief and clinical experience.
However, there have been no studies undertaken to provide a basis for this belief and practice. In this study, we analyzed the spectrum of pathologic findings in cases of EI, and the association of EI with M. tuberculosis using the polymerase chain reaction (PCR).

Patients and Methods

The records for 24 cases with pathologic findings consistent with EI or NV seen between 1989 and 1998 were retrieved from the archives of the Department of Dermatology, National Cheng-Kung University Hospital. Since EI and NV show granulomatous lobular panniculitis with or without vasculitis or necrosis, and are essentially indistinguishable histopathologically, it had been our practice to label these cases pathologically as EI. Biopsy specimens were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin. Periodic acid-Schiff and acid-fast stains and polarization microscopy were performed on selected cases. The clinical and pathologic findings of these cases were reviewed. Two-, three- or four-drug anti-TB therapy (isoniazid, I; ethambutol, E; rifampin, R; pyrazinamide, P) was given to patients with a pathologic diagnosis of EI. For the purpose of this study, we focused our analysis on cases that satisfied the clinico-pathologic and therapeutic response criteria for EI according to Schneider et al [6], as follows: recurrent tender subcutaneous nodules mainly on the legs, characteristic histopathologic features with granulomatous lobular or septolobular panniculitis plus necrosis or vasculitis, and positive response to anti-TB therapy.

Of the original 24 EI/NV cases, 12 were excluded because they did not receive or respond to adequate anti-TB therapy. Of these cases, two failed complete courses of three- or four-drug combined anti-TB therapy; five cases showed spontaneous resolution; and the remaining five patients had persistent lesions either without treatment (2 patients) or after treatment with prednisolone (2 patients) or potassium iodide (1 patient). PCR results in these 12 cases were negative, except for one in the spontaneously healing subgroup.

The pathologic variables in the 12 patients who satisfied the strict diagnostic criteria of EI were analyzed and correlated with the PCR results. The histologic variables, including panniculitis, vasculitis and size of affected vessels, the presence of well-organized granulomas and the degree of the granulomatous infiltration, giant cells, neutrophils, plasma cells, or eosinophilic infiltration, necrosis, and fibrosis were scored semi-quantitatively from 0 to ++ (0 = absent, + = limited, ++ = marked).

Results

The clinical data are summarized in Table 1. The patients consisted of 11 women and one man, with a mean age of 40.6 years (range, 18–70 yr). Tender, erythematous or violaceous, and occasionally ulcerated subcutaneous nodules or indurations were noted on the shins in six patients, calves in three, shin and calf in one, thighs in two, and back in one. Patients had had the lesions for 10 days to 10 years (mean, 2.1 years) before diagnosis. None had any personal or family history of active TB infection. One patient had an abnormal chest roentgenogram. Tuberculin test with purified protein derivative (PPD: 1 TU) was strongly positive in all 11 patients who were tested. Mycobacterial culture was negative in three cases studied.

To detect M. tuberculosis complex DNA, we used a nested PCR assay with IS6110 as the target for amplification [9–11]. Five 10-mm-thick sections were cut from each paraffin-embedded tissue block. The sections were deparaffinized and digested with proteinase K-containing buffer as described by Thierry et al [10]. DNA extraction was performed using standard phenol/chloroform techniques. Multiple precautions were observed to avoid contamination. The primers used for PCR amplification of IS6110 were: pt8 5'-GTGGCGGATG- GTCGCAAGAGAT-3' and pt 9 5'-CTCGATGCCCTC- ACGTTCA-3' (amplicon 541 base pairs, bp); and TB2A 5'-GACCAGACCGAAAGATCCCG-3' and TB2B 5'- GGTCGTAGTGGCGATGGG-3' (amplicon 259 bp). As an internal control for adequacy and integrity of template DNA, β-actin was amplified in each case according to the protocol of Fuqua et al [11]. The primers for β-actin were: #1 5'-ATCATGTTTGGAC- CCTTCAA-3' and #2 5'-CATCTCTTGGTCGAA- CTCGA-3' (amplicon 317 bp). Positive controls included DNA extracted from cultured M. tuberculosis ATCC 25177 (H37Rv) and from M. bovis BCG (from the Tokyo 172 strain), respectively. In addition, 19 specimens of non-TB lesions, including seborrheic keratosis, squamous cell carcinoma, lymph node, intradermal nevus, dermatofibroma, sarcoidal granulomatous dermatitis, and 13 specimens of polyarteritis nodosum, were used as negative controls. To detect inhibiting substances in DNA samples, we added approximately 10 copies of purified M. tuberculosis DNA to the DNA samples of the cases in which IS6110 was not detected, prior to PCR. To determine the sensitivity of the assay, serial 10-fold dilutions of purified M. tuberculosis DNA were amplified. The detection limit was 10 copies of the IS6110 sequence per reaction.
Table 1. Clinicopathologic data in 12 erythema induratum patients with complete response to anti-tuberculosis therapy

<table>
<thead>
<tr>
<th>Case No</th>
<th>Age/Sex</th>
<th>Location</th>
<th>Pathology</th>
<th>Duration</th>
<th>PPD (cm)</th>
<th>PCR</th>
<th>Treatment</th>
<th>Follow-up (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45/F</td>
<td>Thigh, shin</td>
<td>LP/V/N</td>
<td>6 mo</td>
<td>1.7</td>
<td>+</td>
<td>IE (8 wk)</td>
<td>9, NR</td>
</tr>
<tr>
<td>2</td>
<td>46/F</td>
<td>Shin</td>
<td>LP/V/N</td>
<td>10 yr</td>
<td>3x4</td>
<td>-</td>
<td>IERP (6 wk)</td>
<td>9, NR</td>
</tr>
<tr>
<td>3</td>
<td>30/F</td>
<td>Calf</td>
<td>LP/V/N</td>
<td>3 yr</td>
<td>2.2</td>
<td>-</td>
<td>IER (22 wk)</td>
<td>6, NR</td>
</tr>
<tr>
<td>4</td>
<td>56/F</td>
<td>Calf</td>
<td>LP/V/N</td>
<td>10 d</td>
<td>1x1</td>
<td>+</td>
<td>IER (15 wk)</td>
<td>5, NR</td>
</tr>
<tr>
<td>5</td>
<td>34/F</td>
<td>Calf, shin</td>
<td>LP/V</td>
<td>2 yr</td>
<td>2x2</td>
<td>+</td>
<td>IER (21 wk)</td>
<td>7, NR</td>
</tr>
<tr>
<td>6</td>
<td>25/F</td>
<td>Calf</td>
<td>LP/N/V</td>
<td>7 yr</td>
<td>ND</td>
<td>+</td>
<td>IER, IERP* (26 wk)</td>
<td>3, NR</td>
</tr>
<tr>
<td>7</td>
<td>29/F</td>
<td>Calf</td>
<td>LP/N/V</td>
<td>2 mo</td>
<td>4.5</td>
<td>+</td>
<td>IER, IE† (29 wk)</td>
<td>3, NR</td>
</tr>
<tr>
<td>8</td>
<td>43/F</td>
<td>Back</td>
<td>LP/N</td>
<td>2 mo</td>
<td>2.8</td>
<td>-</td>
<td>IER (19 wk)</td>
<td>3, NR</td>
</tr>
<tr>
<td>9</td>
<td>51/F</td>
<td>Shin, ankle</td>
<td>LP/N</td>
<td>2 mo</td>
<td>2</td>
<td>+</td>
<td>IER (31 wk)</td>
<td>2, NR</td>
</tr>
<tr>
<td>10</td>
<td>70/F</td>
<td>Thigh, shin</td>
<td>LP/N/V</td>
<td>10 d</td>
<td>1.4</td>
<td>+</td>
<td>IER (22 wk)</td>
<td>2, NR</td>
</tr>
<tr>
<td>11</td>
<td>18/M</td>
<td>Shin</td>
<td>LP/N/V</td>
<td>2 yr</td>
<td>3x2</td>
<td>+</td>
<td>IER (17 wk)</td>
<td>1, NR</td>
</tr>
<tr>
<td>12</td>
<td>40/F</td>
<td>Shin</td>
<td>LP/N</td>
<td>2 mo</td>
<td>1.5</td>
<td>+</td>
<td>IERP (26 wk)</td>
<td>3, NR</td>
</tr>
</tbody>
</table>

*Relapse after IER therapy for 12 weeks; continued with IERP therapy for 16 weeks. †Therapy for 15 weeks with IER then IE for 14 weeks due to rifampin-induced morbilliform eruption. PPD = tuberculin test; PCR = detection of Mycobacterium tuberculosis complex DNA by polymerase chain reaction; LP = lobular panniculitis; V = vasculitis; N = necrosis; I = isoniazid; E = ethambutol; NR = no recurrence; ND = not determined; R = rifampin; P = pyrazinamide.

All skin lesions cleared completely after 6 weeks to 31 weeks (mean, 20.2 weeks) of anti-TB therapy. Seven patients were treated with standard three-drug therapy (1300 mg/day, E 15 mg·kg⁻¹·d⁻¹, R 600 mg/day) for 15 to 31 weeks (mean, 21 weeks). One patient was treated with two drugs (I, E) for 8 weeks. One was treated with three-drug therapy for 15 weeks and then two drugs for 14 weeks due to a rifampin-induced morbilliform eruption. Two patients received four-drug therapy for 6 weeks and 26 weeks. One patients had a relapse after 12 weeks of three-drug therapy and was then treated with four drugs for 16 weeks.

Histopathologically, all biopsy specimens showed a primarily lobular granulomatous panniculitis with various degrees of necrosis and vascular damage. In nine cases, there was diffuse lobular panniculitis involving all lobules (Fig. 1) or widespread patchy panniculitis. In the remaining three cases, there was focal lobular or septolobular panniculitis. Vasculitis was found in nine cases, and arteries or veins were affected in four of these. The affected blood vessels showed inflammatory infiltrates in the vessel wall, occlusion by fibrin platelet thrombi, or extensive necrosis of the vessel walls (Fig. 2), resulting in palisaded granuloma in some foci. The granulomas were well organized in nine cases and three were palisaded with central necrosis. Tissue necrosis was noted within the granulomas or subcutaneous lobules in 11 cases, 10 with various degrees of caseous necrosis, three with coagulative fat necrosis, and one with lipomembranous necrosis. The inflammatory infiltrates were mixed, consisting of lymphocytes, histiocytes, neutrophils, and eosinophils. Langhans’ or foreign body giant cells were seen in eight of the 12 cases. Neutrophils were present in nine cases, and were more numerous in areas of vasculitis or acute or severe caseous necrosis. Abscess formation was noted in one lesion. Plasma cells were noted in two cases. Eosinophils or focal eosinophilia was present in 10 cases. The epidermis appeared normal.

![Fig. 1](https://example.com/fig1.png)

*Fig. 1. The lesion in Case 7 shows diffuse lobular granulomatous panniculitis involving all lobules. (H & E, x 40)*
Erythema Induratum in most cases. However, direct extension of panniculitis with involvement of the deep dermis by granulomatous inflammation was noted in seven cases. Acid-fast stain was negative in five cases examined.

Polymerase chain reaction
PCR showed positive results for M. tuberculosis complex DNA in nine of the 12 cases (Fig. 3). All negative controls, including polyarteritis nodosa (13 specimens), gave negative results (data not shown). There was no sample inhibition.

Discussion
EI is not rare in some populations and accounts for 22% to 80% of cutaneous tuberculosis tuberculids, depending on the population [12, 13]. It represented 93.3% of all tuberculids in a recent Hong Kong study [14]. TB is still an important public health issue in Taiwan, with a 0.44% annual risk of infection by M. tuberculosis from 1996 to 1998. This is higher than the 0.1% rate reported in other industrialized countries [14]. Cutaneous TB and tuberculids are not uncommonly seen in our hospital in southern Taiwan. During the study period (1989–1998), 13 cases of cutaneous TB, seven cases of papulonecrotic tuberculid, and 24 cases of EI were diagnosed. The clinical features in the present series of EI were similar to those in other reports [6, 15, 16].

Rademaker et al reported 26 cases of EI that showed varying histologic features but predominantly those of vasculitis with paraseptal panniculitis [15]. Recently, Schneider and Jordaan provided a more detailed account of the histologic findings in a series of 20 EI lesions [17]. Primary vasculitis was present in 85% of the lesions, with large vessels affected in 45% and smaller vessels in 40%. Two morphologic patterns of panniculitis are recognized, focal septolobular (25%) with vasculitis of septal arteries or veins, and diffuse septolobular (75%) with vasculitis of smaller vessels, focal to extensive necrosis and prominent granulomatous inflammation. The spectrum of histologic findings in our series was very similar to that reported by Schneider and Jordaan. Most previous reports on EI do not mention eosinophils in the infiltrate. We found eosinophils or focal eosinophilia in most EI lesions in our series, a finding also noted by Schneider and Jordaan [17].

It has been speculated that EI represents a hypersensitivity reaction to mycobacterial antigens [14, 17]. The finding of necrotizing neutrophilic vasculitis in the present study further supports an initial type III hypersensitivity reaction in EI [16, 17]. The subsequent perivascular infiltration of T-lymphocytes and macrophages and the occasional occurrence of granulomatous vasculitis indicate that type IV hypersensitivity is also involved in the pathogenesis of the vascular changes in EI [17, 18].

The positive detection rates of M. tuberculosis complex DNA in EI/NV skin lesions range from 25% to 77% in various studies [4–6, 14, 19]. Four of these studies reported on EI patients [5, 6, 14, 19], but Baselga et al included cases of EI and NV [4]. The higher detection rates have been attributed to the higher sensitivity of PCR and/or Southern blot assays [4, 14]. M. tuberculosis complex DNA was detected in 75% of our cases. We likewise suspect that this relatively high rate is attributable, in part, to the high sensitivity of the nested PCR assay used in our study. The detection limit was 10 copies of the IS6110 sequence in our study, which is nearly as sensitive as the eight copies in the study by Baselga et al [4]. The likelihood of false positivity in our series was low because all negative

Fig. 2. A large blood vessel in the subcutaneous septum in Case 4 shows occlusion by fibrin platelet thrombi with necrosis and inflammatory infiltrates in the vessel wall. (H & E, x 200)

Fig. 3. Detection of Mycobacterium tuberculosis complex DNA by polymerase chain reaction (PCR) amplification of IS6110. Lane 1: 100-bp marker; lane 2: negative control; lane 3: positive control with a 259 bp-amplicon of IS6110; lanes 4–11: representative cases with positive results; lanes 13, 15, 17: Cases 2, 3, 8, showing negative results; lanes 12, 14, 16: Cases 2, 3, 8, after addition of 10 copies of M. tuberculosis DNA prior to PCR to test for sample inhibition. The positive results indicate a lack of inhibiting substances in these three samples.
controls, including 13 cases of polyarteritis nodosa, gave negative results. It is worth noting that the cases of polyarteritis nodosa gave negative results, because this disease is in the differential diagnosis of EI both clinically and histologically.

*M. tuberculosis* complex DNA was not detected in three of our patients despite the fact that their lesions had been cleared by anti-TB therapy. It is possible that these might represent false negatives as the result of insufficient mycobacterial DNA in the samples, degradation of DNA by buffered formalin, presence of inhibiting substances in the extracted DNA samples, or the sensitivity of the PCR procedure. However, adequate amplification of the internal β-actin gene suggested that the extracted DNA samples were not degraded. Sample inhibition was also excluded in our study. It is possible that the lesions in these three cases might be caused by an agent other than *M. tuberculosis* and that the lesions resolved spontaneously. However, in a study of seven cases of EI, atypical mycobacterium DNA was not detected [20].

We analyzed the relationship of PCR results with the clinical data and pathologic findings and found no significant differences between PCR-positive and -negative groups with respect to the age of patients, location and duration of the lesions, PPD reactivity, and presence of necrosis and vasculitis (Table 2). Baselga et al, however, noted that the presence and degree of necrosis were significantly higher in the PCR-positive group [4]. This discrepancy might be attributable to the fact that the subjects studied by Baselga et al consisted of EI and NV patients, while the present study focused only on patients presumed to have EI with a complete response to anti-TB therapy.

Of the 12 cases that were excluded from this study because they did not receive or respond to anti-TB therapy, only one had a positive PCR result. The skin lesions resolved spontaneously. This case could be regarded as having spontaneous resolution of TB-related EI. If we add this case to the 12 cases in the study group, then about half of our original 24 EI/NV cases were associated with *M. tuberculosis* infection. These findings support our belief that most cases with pathologic findings of EI in our patient population are probably of tuberculous origin.

Although the major laboratories in Taiwan now provide clinicians with PCR assay for *M. tuberculosis*, the assay is still not routinely utilized because of technical costs and issues. Since most people in Taiwan have received BCG vaccination in childhood, a positive tuberculin test does not necessarily indicate tuberculous infection in vaccinated individuals, but a strong reaction is considered consistent with a diagnosis of EI. On the other hand, a negative tuberculin test practically rules out tuberculous infection, unless the patient is anergic. For practical purposes, we routinely perform a tuberculin test on these patients and treat patients with a positive tuberculin test with three-drug anti-TB therapy for 3 to 4 months. Since many patients show a good response to the anti-TB therapy and may discontinue the treatment prematurely on their own, it is important to stress to patients that a complete treatment course is required to achieve a cure. One patient in the exclusion group failed a course of three-drug anti-TB therapy followed by a course of four-drug therapy. She had a negative tuberculin test and negative PCR results on three separate specimens. This experience suggests that anti-TB therapy is probably not indicated in patients with negative tuberculin tests in Taiwan.

### Table 2. Histopathologic features and the results of polymerase chain reaction (PCR) study in 12 cases with erythema induratum

<table>
<thead>
<tr>
<th>Histopathologic/clinical features</th>
<th>PCR-positive (n = 9)</th>
<th>%</th>
<th>PCR-negative (n = 3)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well-organized granulomas</td>
<td>5</td>
<td>56</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Presence of necrosis</td>
<td>8</td>
<td>89</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>7</td>
<td>78</td>
<td>2</td>
<td>67</td>
</tr>
<tr>
<td>Presence of giant cells</td>
<td>5</td>
<td>56</td>
<td>3</td>
<td>100</td>
</tr>
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</table>

References


