# The clinical manifestations, laboratory findings and causative agents of granulomatous skin reaction

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**ABSTRACT:**

**MAITRISATHIT W, SRISUTTIYAKORN C. THE CLINICAL MANIFESTATIONS, LABORATORY FINDINGS AND CAUSATIVE AGENTS OF GRANULOMATOUS SKIN REACTION. THAI J DERMATOL 2020;36:97-109.**

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**Background:** The prevalence of nontuberculous mycobacterial (NTM) infection is increasing in Thailand and cutaneous presentations of NTM infection are nonspecific and variable. The diagnosis of cutaneous NTM infection should be combined with clinical manifestations, histopathological features, tissue special stains, tissue polymerase chain reaction (PCR) and tissue culture. Thus, the diagnosis of cutaneous NTM infection is challenging; therefore, sometimes, the diagnosis and treatments can be delayed.

**Objective:** This study would like to improve the diagnosis when there was no evidence of organism from tissue biopsy, this study would like to investigate the correlations of history, clinical manifestations and histopathological features in the cases of tissue biopsy were granulomatous reaction and the final diagnosis as cutaneous mycobacterial infection. The secondary outcome is to investigate the correlation of history, clinical manifestation and histopathology between NTM granuloma and the other causes of infectious granuloma.

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Received: 2 April 2020 Revised: 08 June 2020 Accepted: 10 June 2020

**Materials and Methods:** A retrospective study of the patients with histopathological features as granulomatous reaction and suspected of cutaneous mycobacterium infection from history and clinical manifestations were obtained from the Division of Dermatology, Phramongkutklao Hospital between January 2014 and December 2016**.**

**Result:** All the 30 cases with skin biopsy compatible with granulomatous reaction were included in this study. Of the 30 cases, eight cases (26.67%) had diagnosis as NTM infection, seven cases (23.33%) had diagnosis as suspected NTM infection and 15 cases (50%) had other diagnoses. The male to female ratio was 2:3. The mean age at diagnosis was 54.4 years (range 16-94).

The history of suspected cutaneous chronic infection was significant higher in the NTM/suspected NTM infections group compared to the group with other diagnoses. The location of the lesions in NTM/suspected NTM infection was more common on extremities. All the patients presented with red-colored nodule, papule or plaque which has no statistic significant between each group.

Most of granulomatous patterns in our study are suppurative and mixed cell granuloma. There was no correlation between histopathological findings and the demonstration of organism in tissue specimens. The tissue AFB stain in NTM-infection group is positive in 37.5% (3/8 cases) which 2 of the 3 AFB-positive cases had immunocompromised status. Tissue culture was positive in 62.5% of the NTM-infection group which *M. abscessus* was the most common causative organism. The mean duration of treatments in NTM/suspected NTM infections was 6 months.

**Conclusion:** Although the tissue special stains, tissue PCR or tissue culture are the most effective diagnostic tools, the rate of positivity is low in our study. The diagnosis should be suspected in the patients with suspicious history such as history of trauma, aquatic-relate injury. The prompt treatment with broad-spectrum antibiotics should be started in suspicious patients.

**Key words:** Nontuberculous mycobacteria, granulomatous skin reactions, skin infection

## Introduction

Granulomas are discrete collections of histiocytes with or without multinucleate giant cells[1]. Granuloma has many types of histopathologic patterns which include sarcoidal, tuberculoid, palisading, suppurative and mixed cell granuloma[2].

Granulomatous inflammation is mainly divided into 2 types depended on causative factors which are infected and non-infected granulomas. Infected granuloma can be caused by various organisms such as tuberculosis, nontuberculous mycobacterium (NTM), leprosy, syphilis, deep fungal and disseminated fungal infections[3].

Nowadays, the prevalence of NTM infection is increasing in Thailand[4-5]. NTM are microorganisms that are widely distributed in the environment, including water, soil, wild animals, household, aerosol, or even in hospital environments[5-9]. Infection usually acquires from trauma, occupation and daily activities[5-7,10].

There is wide clinical spectrum of NTM infections which including pulmonary disease, lymphadenitis, disseminated disease and skin and soft tissue infections[7].

The diagnosis of cutaneous NTM infection should be combined both clinical, histopathological features, tissue special stains, tissue polymerase chain reaction (PCR) and tissue culture[11]. However, the exploring of causative organism is difficult in some cases. Tissue culture is more specific but requires weeks or months for final report[12]. Tissue PCR is a fast and convenient method. However, the laboratory process needs careful control to prevent contamination and false-positive result[12-13].

Thus, the diagnosis of cutaneous NTM infection is challenging. Sometime, the positive results of tissue culture, tissue special stains or tissue PCR are not obtained. Around 1% of skin biopsy from Division of Dermatology, Phramongkutklao Hospital had histopathology as granulomatous features which more than half of tissue biopsies had the final diagnosis as cutaneous mycobacterial infection, but yielded negative results from special stains for acid-fast bacilli, tissue culture or tissue PCR for mycobacteria.

To improve the diagnosis when there was no evidence of organism from tissue biopsy, this study would like to investigate the correlations of history, clinical manifestations and histopathological features in the cases of tissue biopsy were granulomatous reaction and the final diagnosis as cutaneous mycobacterial infection. The secondary outcome is to investigate the correlation of history, clinical manifestation and histopathology between NTM granuloma and the other causes of infectious granuloma

## Materials and Methods

### Case selection

A retrospective study of the patients with histopathological features as granulomatous reaction and suspected of cutaneous mycobacterium infection from history and clinical manifestations were obtained from the Division of Dermatology, Phramongkutklao Hospital between January 2014 to December 2016**.** This study was approved by the institutional review boards of the Faculty of Medicine Phramongkutklao Hospital.

All the medical data and demographic data including gender, age, symptoms, duration, underlying diseases, current medical intake, clinical appearance, history of previous trauma, cosmetic procedure, or surgery, treatment, outcome of treatment, result of pathological

features together with the result of tissue special stains, tissue PCR and tissue culture were recorded.

Diagnosis of cutaneous mycobacterial infection in this study was defined as identified organism from culture or PCR or special stains from tissue biopsy or discharge material. The diagnosis of suspected cutaneous mycobacterial infection was defined as history and clinical manifestations was compatible with chronic infection such as history of trauma or chronic wound that demonstrated clinical improvement after treatment as NTM infection.

The status was defined as a complete healing by clinical manifestations, with or without a negative result of repeated tissue culture and the patient can discontinue antibiotic. Improved status was defined as the clinical improvement, i.e., the smaller skin lesions without any new lesions, and the antibiotics, however, were still continued for another period of time[14-15].

Exclusion criteria was all patients with non-infected granuloma or patients with incomplete medical records.

**Histopathological classification**

The histopathological characteristics of the skin samples were categorized as epidermal changes (acanthosis, pseudoepitheliomatous hyperplasia, exocytosis, intraepidermal abscesses and ulceration), depth of infiltrate (papillary dermis, superficial reticular dermis, deep reticular dermis and subcutaneous tissue), infiltrate distribution (superficial perivascular, superficial and deep perivascular, deep perivascular, diffuse superficial dermal infiltration, diffuse superficial and deep dermal, diffuse deep dermal infiltration, nodular superficial dermal, nodular superficial and deep dermal, nodular at deep dermal, subcutaneous infiltrate), inflammatory cells infiltrations (giants cell, foamy histiocytes, neutrophils, lymphocytes, plasma cells and eosinophils, keratin flakes), granuloma patterns (tuberculoid, sarcoid-like, palisading, suppurative, mix cell), dermal necrosis and abscesses[12,14].

Tuberculoid granuloma comprised of epithelioid histiocytes, including multinucleate forms, surrounded by a dense infiltrate of lymphocytes and plasma cells. Central caseation may be present[2]. The Langhans type of multinucleated giant cell, with a horseshoe-like arrangement of nuclei, may be observed[2].

Sarcoidal granuloma comprised of aggregates of epithelioid histiocytes, with sparse peripheral lymphocytes or plasma cells. Palisaded (necrobiotic) granulomas comprised of epithelioid histiocytes aligned as a rim around a central area of degenerated collagen[2]. Suppurative granulomas comprised of neutrophils within, and sometimes among or surrounding, aggregates of epithelioid histiocytes[2]. Mix cell granuloma comprised of neutrophils and lymphocytes

and/or plasma cell and/or eosinophils among or surrounding, aggregates of histiocytes.

The appearance of bacilli was classified on a semiquantitative scale: grade 0 (negative); grade I (sparse) and grade II (clumps)[12]. All cases were reviewed by dermatopathologists.

**Statistical analysis**

This is the retrospective study. The subjects were divided into three groups; cases with NTM infection, suspected NTM infection and cases with other diagnosis and their data were assembled separately. A Fisher's exact test was used to compare differences in categorical data while continuous variables were compared by a Mann-Whitney U test. A p-value of $< 0.05$ was considered statistically significant. All analyses were performed using Statistics for Windows, version 23 (IBM SPSS Inc., Chicago, IL, USA). Suspected sample sized in this study were 40 cases base on previous study in Taiwan[16].

**Results**

**Patient demographic and clinical characteristics**

All the 30 cases with skin biopsy compatible with granulomatous reaction were included in this study. All clinical manifestations and courses of the diseases were demonstrated in Table 1. Of the 30 cases, eight cases (26.67%) had diagnosis as NTM infections and seven cases (23.33%) had diagnosis as suspected NTM infection and 15 cases (50%) had other diagnoses. The male to female ratio was 2:3. The mean age at diagnosis was 54.4 years (range 16-94).

Of the 8 cases with NTM infection, 1/8 cases (12.50 %) had both positive tissue AFB and PCR for NTM, 2/8 cases (25%) had positive tissue AFB stain solely and 5/8 cases (62.50%) had only positive tissue culture for NTM (Table 2).

Of the 7 cases with suspected NTM infection, they had history and clinical manifestations compatible with chronic infection, and the clinical symptoms improved after received antibiotic treatments for NTM infection.

Of the 15 cases which had other diagnoses, the diagnoses were acute bacterial infection, folliculitis, Majocchi granuloma, tuberculosis verrucose cutis and secondary syphilis.

The general demographic data was not statistically significant between patient with NTM/suspected NTM infection and patient with other diagnoses as shown in table 1.

There were more cases with immunocompromised status in NTM/suspected NTM infection groups but it showed no statistic significant (Table 1). All of 4 immunocompromised hosts in NTM/suspected NTM infection, 2 cases were received immunosuppressive agents (cyclosporin, methotrexate), 1 case were post chemotherapy and 1 case had HIV infection (CD4+ < 250 cells/mm3).

**Table 1** Clinical characteristics and courses of cases with NTM infection and cases with other diagnoses

| Clinical characteristics | Other diagnoses (n=15) | NTM (n=8) | Suspect NTM (n=7) | P-value |
|---|---|---|---|---|
| **Sex** | | | | |
| Male | 7 (46.7%) | 2 (25%) | 3 (42.9%) | 0.629 |
| Female | 8 (53.3%) | 6 (75%) | 4 (57.1%) | 0.629 |
| **Age at diagnosis (years (max, min))** | 56 (27,62) | 61.5 (53, 70) | 60 (56, 64) | 0.437 |
| **Immunocompromised hosts** | 1 (6.7%) | 3 (37.5%) | 1 (14.3%) | 0.191 |
| **Occupation** | | | | |
| Merchant | 1 (6.7%) | 0 (0%) | 0 (0%) | 1 |
| Soldier | 2 (13.3%) | 0 (0%) | 2 (28.6%) | 0.23 |
| Private business | 1 (6.7%) | 0 (0%) | 0 (0%) | 1 |
| Student | 2 (13.3%) | 0 (0%) | 0 (0%) | 0.724 |
| Housekeeper | 3 (20%) | 0 (0%) | 4 (57.1%) | 0.030 |
| Employed | 4 (26.7%) | 3 (37.5%) | 1 (14.3%) | 0.762 |
| Government service | 2 (13.3%) | 5 (62.5%) | 0 (0%) | 0.012 |
| **History suspected cutaneous infection** | 8 (53.3%) | 7 (87.5%) | 7 (100%) | 0.060 |
| - Aquatic-relate injury | 0 (0%) | 1 (12.5%) | 2 (28.6%) | 0.064 |
| - Not improved after treatment with ATB in 1-4 wk | 6 (40%) | 0 (0%) | 3 (42.9%) | 0.072 |
| - Insect bite | 2 (13.3%) | 5 (62.5%) | 2 (28.6%) | 0.050 |
| - Tattoo | 0 (0%) | 1 (12.5%) | 0 (0%) | 0.500 |
| **Location** | | | | |
| Upper extremities | 5 (33.3%) | 4 (50%) | 3 (42.9%) | 0.799 |
| Lower extremities | 4 (26.7%) | 3 (37.5%) | 3 (42.9%) | 0.687 |
| Central part of body (face, neck, scalp, trunk, groin) | 6 (40%) | 1 (12.5%) | 1 (14.3%) | 0.352 |
| **Side** | | | | |
| Left | 3 (20%) | 3 (37.5%) | 3 (42.9%) | 0.520 |
| Right | 5 (33.3%) | 4 (50%) | 4 (57.1%) | 0.568 |
| Both | 6 (40%) | 1 (12.5%) | 0 (0%) | 0.122 |
| Not evaluated | 1 (6.7%) | 0 (0%) | 0 (0%) | 1 |
| **Morphology** | | | | |

| Clinical characteristics | Other diagnoses (n=15) | NTM (n=8) | Suspect NTM (n=7) | P-value |
|---|---|---|---|---|
| Nodule | 5 (33.3%) | 4 (50%) | 1 (14.3%) | 0.465 |
| Papule | 5 (33.3%) | 2 (25%) | 2 (28.6%) | 1 |
| Plaque | 4 (26.7%) | 1 (12.5%) | 4 (57.1%) | 0.216 |
| Verrucous papule and plaque | 0 (0%) | 1 (12.5%) | 0 (0%) | 0.500 |
| Verrucous plaque | 1 (6.7%) | 0 (0%) | 0 (0%) | 1 |
| Color: erythematous | 15 (100%) | 8 (100%) | 7 (100%) | N/A |
| **Duration of symptom (weeks (max, min))** | 4(4,8) | 4 (2.52, 12) | 8(8, 12) | 0.058 |
| **Number of lesions** | | | | |
| 1 | 5 (33.3%) | 3 (37.5%) | 3 (42.9%) | 1 |
| 2 | 0 (0%) | 1 (12.5%) | 2 (28.6%) | 0.064 |
| 3 | 0 (0%) | 1 (12.5%) | 0 (0%) | 0.500 |
| Multiple | 8 (53.3%) | 3 (37.5%) | 2 (28.6%) | 0.631 |
| Not evaluated | 2 (13.3%) | 0 (0%) | 0 (0%) | 0.724 |
| **Systemic involvement** | | | | |
| Arthritis | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| Lung | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| Others | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| **Duration of treatment (weeks) Median (IQR) (max, min)** | 3 (2,4) | 24(12,28) | 24 (12, 24) | 0.005 |
| **Cure** | 10 (66.7%) | 5 (62.5%) | 4 (57.1%) | 1 |
| **Recurrent** | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| **Not improve** | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| **Loss to follow-up / Refer** | 3 (20%) | 3 (37.5%) | 3 (42.9%) | 0.520 |

Kruskal-Wallis Test and Fisher's exact test.

NTM; nontuberculous mycobacterial

The NTM /suspected NTM infection groups had significant higher number of cases with history of suspected cutaneous infection compare to patient with other diagnoses (Table 1). The most common history was insect bite.

The location of lesions in NTM /suspected NTM infections was more common on extremities and less likely on central part of body which had no statistical significance compare to cases with other diagnoses. Numbers of the lesion had no

statistical significance between NTM /suspected NTM infections and other diagnosis. Interestingly, the bilateral distribution of the lesions was more common in cases with other diagnoses but there was no statistical significance compare to NTM /suspected NTM infections (Table 1).

All the lesions in all groups had red in color and commonly presented with nodule, papule or plaque. The mean duration of symptom was 8 weeks in suspected NTM infections, 4 weeks in NTM infections and 4 weeks in cases with other diagnoses, respectively. None of the cases were reported to have systemic involvement (Table 1).

Twelve cases in NTM /suspected NTM infections were treated with combined antibiotic therapy (clarithromycin and ciprofloxacin, clarithromycin and doxycycline, clarithromycin and ethambutol and clindamycin, clarithromycin and ciprofloxacin and ethambutol, clarithromycin and ciprofloxacin and rifampicin). Only one case received single therapy with doxycycline. Nine cases (9/15, 60%) were cured while 6 cases (6/15, 40%) had loss of follow up (Table 1).

Thirteen cases with other diagnoses (13/15, 86.66%) received treatment with single antibiotic agents (augmentin, dicloxacillin, levofloxacin, benzathine penicillin G), antifungal agents (itraconazole) or anti-tuberculosis agents according to the causes of the diseases. Two cases with other diagnoses (2/15, 13.33%) were loss follow up after skin biopsy and did not receive any treatment. Thirteen cases which received treatment, ten cases (10/15, 66.7%) were cured and 1 case (1/15,6.67%) was lost of follow up and 2 cases (2/15,13.33%) were died from his/her underlying cardiac disease.

The mean duration of treatment in both NTM and suspected NTM infections was 24 weeks (range from 4-28 weeks) which was significant longer compare to cases with other diagnoses which had mean duration of treatment about 3 weeks (range 1-24 weeks) as shown in table 1.

**Histopathological findings**

Epidermal acanthosis is the most common histologic finding (Table 2). Other histopathological features showed no statistical significance between the 2 groups as shown in table 2. More number of abscess formation was found in cases with other diagnoses but no statistical significance was identified (Table 2).

Positive tissue culture for mycobacterium was found in 5 cases which identified as *Mycobacterium abscessus* in 4 cases and *Mycobacterium haemophilum* in 1 case. Positive tissue PCR for mycobacterium was found in 1 case.

**Table 2** Histopathological Parameters in cases with NTM infection and cases with other diagnoses

| Histopathological Parameters | Other diagnoses (n=15) | NTM (n=8) | Suspect NTM (n=7) | P-value |
|---|---|---|---|---|
| **Epidermal change** | | | | |
| 1. Acanthosis | 3 (20%) | 3 (37.5%) | 4 (57.1%) | 0.21 |
| 2. Pseudoepitheliomatous hyperplasia | 3 (20%) | 0 (0%) | 1 (14.3%) | 0.653 |
| 3. Exocytosis | 1 (6.7%) | 0 (0%) | 1 (14.3%) | 0.724 |
| 4. Intraepidermal abscess | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| 5. Ulceration | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| 6. No epidermal change | 9 (60%) | 5 (62.5%) | 2 (28.6%) | 0.406 |
| **Depth of infiltrate** | | | | |
| 1. Papillary dermis | 7 (46.7%) | 1 (12.5%) | 1 (14.3%) | 0.189 |
| 2. Superficial reticular dermis | 10 (66.7%) | 5 (62.5%) | 6 (85.7%) | 0.671 |
| 3. Deep reticular dermis | 13 (86.7%) | 7 (87.5%) | 5 (71.4%) | 0.677 |
| 4. Subcutaneous tissue | 5 (33.3%) | 3 (37.5%) | 1 (14.3%) | 0.671 |
| **Infiltrate distribution** | | | | |
| 1. Superficial perivascular | 0 (0%) | 1 (12.5%) | 0 (0%) | 0.500 |
| 2. Superficial and deep perivascular | 1 (6.7%) | 0 (0%) | 1 (14.3%) | 0.724 |
| 3. Deep perivascular | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| 4. Diffuse superficial dermal infiltration | 1 (6.7%) | 0 (0%) | 1 (14.3%) | 0.724 |
| 5. Diffuse superficial and deep dermal | 2 (13.3%) | 2 (25%) | 1 (14.3%) | 0.821 |
| 6. Diffuse > deep dermal infiltration | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| 7. Nodular superficial dermal | 1 (6.7%) | 1 (12.5%) | 1 (14.3%) | 1 |
| 8. Nodular superficial and deep dermal | 4 (26.7%) | 2 (25%) | 1 (14.3%) | 1 |
| 9. Nodular at deep dermal | 5 (33.3%) | 3 (37.5%) | 3 (42.9%) | 1 |
| 10. Subcutaneous infiltrate | 6 (40%) | 2 (25%) | 1 (14.3%) | 0.589 |
| **Inflammatory cells** | | | | |
| 1. Lymphocytes | 15 (100%) | 8 (100%) | 7 (100%) | N/A |
| 2. Histiocytes | 15 (100%) | 8 (100%) | 7 (100%) | N/A |
| 3. Giant cells | 9 (60%) | 4 (50%) | 3 (42.9%) | 0.718 |
| 4. Foamy histiocytes | 3 (20%) | 1 (12.5%) | 0 (0%) | 0.785 |

| Histopathological Parameters | Other diagnoses (n=15) | NTM (n=8) | Suspect NTM (n=7) | P-value |
|---|---|---|---|---|
| 5. Neutrophils | 14 (93.3%) | 8 (100%) | 7 (100%) | 1 |
| 6. Plasma cells | 3 (20%) | 0 (0%) | 1 (14.3%) | 0.653 |
| 7. Eosinophils | 2 (13.3%) | 2 (25%) | 2 (28.6%) | 0.592 |
| 8. Keratin flakes | 1 (6.7%) | 0 (0%) | 0 (0%) | 1 |
| **Granuloma** | | | | |
| 1. Tuberculoid | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| 2. Sarcoid-like | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| 3. Palisading | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| 4. Suppurative | 5 (33.3%) | 2 (25%) | 0 (0%) | 0.281 |
| 5. Mix cell | 10 (66.7%) | 6 (75%) | 7 (100%) | 0.281 |
| **Dermal necrosis** | 3 (20%) | 2 (25%) | 3 (42.9%) | 0.493 |
| **Abscess** | 5 (33.3%) | 1 (12.5%) | 0 (0%) | 0.214 |
| **Positive stain** | 0 (0%) | 3 (37.5%) | 0 (0%) | 0.022 |
| **AFB stain** | | | | |
| Grade 0 | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| Grade 1 | 0 (0%) | 3 (37.5%) | 0 (0%) | 0.022 |
| Grade 2 | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| **Culture** | | | | |
| Bacterial | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| TB | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| NTM | 0 (0%) | 5 (62.5%) | 0 (0%) | 0.001 |
| Fungus | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| **PCR** | | | | |
| Bacterial | 0 (0%) | 0 (0%) | 0 (0%) | N/A |
| TB | 1 (6.7%) | 0 (0%) | 0 (0%) | 1 |
| NTM | 0 (0%) | 1 (12.5%) | 0 (0%) | 0.500 |
| Fungal | 0 (0%) | 0 (0%) | 0 (0%) | N/A |

Kruskal-Wallis Test and Fisher's exact test.

*(Some cases had more than one histopathological feature.)

NTM; nontuberculous mycobacterial

**Discussion**

Nowadays, the prevalence of NTM infection is increasing in Thailand[4]. NTM are microorganisms that are widely distributed in the environment, including water, soil, wild animals, the household, aerosols, or even in hospital environments[5-9]. Infection is usually acquired from trauma, occupation and daily activities[5-7,10]. The cutaneous presentations of NTM infection are nonspecific and variable.

The diagnosis of cutaneous NTM infection should be combined with clinical and histopathological features, tissue special stains, tissue PCR and tissue culture[11]. Thus, the diagnosis of cutaneous NTM infection is challenging; therefore, sometimes, the diagnosis and treatments can be delayed.

In our study, all the demographic data and histopathologic features showed no different between the cases with NTM /suspected NTM infections and cases with other diagnoses. The findings of female gender predominantly in the cases with NTM infection and the clinical manifestations with erythematous papules, nodules and plaques were similar compare to previous studies with cutaneous NTM infection[4,12,15,16].

Only history of suspected cutaneous infection can differentiate the cases with NTM /suspected NTM infections and cases with other diagnoses. The most common history was insect bite and then followed by aquatic-relate injury as shown in table 1.

The insect bite associated cutaneous NTM infection has been reported in a single report which associated with *Mycobacterium ulcerans* infection[17]. Insect bite associated with invasive fungal infection has also been reported[18]. Local edema which can impair local lymphatic drainage and excoriation or erosion of the skin following scratching at insect bite site are the possible mechanism of superimpose infection[18].

The lesions of NTM /suspected NTM infections tend to occur on extremities more than central part of the body which was similar to the previous reports from Thailand[15,16].

The histopathological findings showed no difference between NTM infection and cases with other diagnoses. No correlation between histopathological findings and the demonstration of organism in tissue specimens was found.

No difference between demographic data and histopathologic features between NTM infection and suspected NTM infection (Table 1).

The tissue AFB stain was positive in 3/8 (37.5%) cases in our study and 2/3 (66.67%) cases which positive AFB stain were immunocompromised hosts. The rate of positive AFB stain in our study was closed to previous report which found positive AFB stain on tissue specimen between 11-27%[14,19]. The demonstration of AFB stain also

relates to immunologic status of host which smaller in normal hosts[14].

Only 5/8 (62.5%) cases in our study had positive tissue culture which is lower rate compare to previous study from Thailand which rate of tissue culture positive was 100%[15].

Most of the positive culture in our study was *M. abscessus* similar to previous report from Thailand and Taiwan[15,16]. In contrast to the reports from Europe which had different mycobacterium species[6,9,10,14]. This can be explained by different environment, climate, human cultural and habit that can impact the species of mycobacterium[5,6,7].

The lower rate of positive tissue culture in our study may be form different laboratory quality and may be from inappropriate collection and specimen handling. Another possible explanation is partial antibiotic treatment prior to the diagnosis.

According to histopathological findings, most of granulomatous patterns in our study are suppurative and mixed cell granuloma which was similar to previous study from Thailand[15]. None of our cases has sarcoidal or palisading granuloma, in contrast to the study from Bartralot R, et al and Hsiao C-H, et al which also found number of patients with sarcoidal and palisading granuloma. These may be explained from difference in mycobacterium species[14,16].

Only longer treatment duration showed statistical significance in NTM /suspected NTM infections compared to cases with other diagnoses. The mean duration of treatments in our study was 6 months. The main combination therapy with macrolide-based regimens and ciprofloxacin were also similar to previous study[15].

There were some limitations in this study due to the retrospective review. Some clinical and laboratory data were incomplete. The small number of cases of NTM /suspected NTM infections may limit the statistical power of the analysis. In our study, some data tended to have positive relationship, but they showed no statistical significance.

Although the tissue special stains, tissue PCR or tissue culture are the most effective diagnostic tools, the rate of positivity is low in our study. The diagnosis of cutaneous mycobacterial infection should be made in the patients with suspicious history such as history of trauma, insect bite or aquatic-relate injury. The prompt treatment with broad spectrum antibiotics should be started in suspicious patients. Clarithromycin is an effective empirical treatment for NTM infections, where its combination with ciprofloxacin is the most commonly used regimen[7,15,16].

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