Clinicopathological Significance among Patients with In vivo Epidermal Nuclear Staining by Direct Immunofluorescence Study

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Abstract

Context: In vivo epidermal nuclear staining (ENS) can be found in patients with autoimmune connective tissue diseases (CNTDs) and other diseases. Aims: The aim of this study was to reveal the underlying diseases and direct immunofluorescence (DIF) characters of patients with in vivo ENS and association of in vivo ENS with circulating autoantibodies. Settings and Design: A retrospective analysis was conducted involving skin biopsy specimens submitted for DIF study at the Dermatoimmunology Laboratory at Siriraj Hospital between 2002 and 2012. Subjects and Methods: The findings of DIF study, clinical manifestations, and diagnosis of patients who had positive ENS were investigated. Statistical Analysis Used: The SPSS software version 18.0. Descriptive statistics were used to report demographic data, clinical characteristics, and laboratory investigation results. Moreover, Chi-squared test or Fisher’s exact test were used to compare the categorical variables. Results: One hundred and thirty-eight out of 3735 submitted specimens (3.7%) showed positive ENS. The most common diagnosis was CNTD (79%) followed by vasculitis (10.1%). Lupus erythematosus was the most common diagnosis among CNTD (78%). No association between levels of serum antinuclear antibodies (ANA) titer and intensity of ENS (P = 0.660). However, we found that patients with positive in vivo ANA had lower prevalence of systemic involvement. Conclusions: Although lupus erythematosus was the most common diagnosis among patients with in vivo ENS, the presence of ENS does not indicate any specific diagnosis. However, patients with ENS tend to have less systemic involvement.

Key Words: Autoantibodies, epidermal nuclear staining, in vivo antinuclear antibody, in vivo epidermal nuclear staining autoantibodies

Introduction

Direct immunofluorescence (DIF) study of the skin biopsy specimen is the useful method to diagnose autoimmune vesiculobullous diseases, connective tissue diseases (CNTDs), and vasculitis. The presence of in vivo epidermal nuclear staining (ENS) represents the antinuclear antibody (ANA) deposition in the nucleus of keratinocytes which is usually found in 55% of patients with CNTD.[1,2] Most of the previous publications about in vivo ENS were conducted among Caucasians and the number of patients included in studies is limited.[3-7]

Thus, this study aimed to reveal the characters of in vivo ENS among Thai patients and to find the association of in vivo ENS with circulating autoantibodies and correlated clinical manifestations.

Subjects and Methods

Ethical approval was granted by Siriraj Institute Review Board, Siriraj Hospital, Mahidol University, Bangkok, Thailand. We retrospectively reviewed the data records of skin biopsy specimens submitted for DIF study at Dermatoimmunology Laboratory at Siriraj Hospital between January 2002 and December 2012. Those with immunoglobulin (Ig) and/or complement deposition at epidermal nucleus were included in this study.

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Clinical and immunological profiles of patients were reviewed. Definite diagnosis was based on clinical, histopathological, and immunofluorescent findings. If CNTD was suspected, the work up of such patients was done such as anti-Smith antibody, anti-ribonucleoprotein antibody, anti-double stranded DNA antibody, anticardiolipin antibody, antinuclear cytoplasmic antibody, anti-Ro antibody, anti-La antibody, lupus anticoagulant, and anti-Scl70 antibody. Patients without final definite diagnosis were excluded from the study.

DIF was done according to the standard method described previously. Skin biopsy specimens were fixed with acetone at 2°C–8°C for 10 min and air-dried. These specimens were then washed twice with phosphate buffer saline pH 7.2 for 10 min. Fluorescein isothiocyanate-conjugated rabbit antihuman IgG, IgA, IgM, C3, and fibrinogen (Dako Patt, Copenhagen, Denmark) (catalog number: F 0202, F 0204, F 0203, F 0201, and F 0111, respectively) were added. Dilution factor for IgG was 1:80. The dilution factor for other Ig, complement and fibrinogen was 1:40. Then, specimens were incubated in a moist chamber at room temperature for 30 min, and excess antibodies were washed off with phosphate buffer saline for 10 min for two times and mounted by mounting medium. The specimens were analyzed under an immunofluorescence microscopy. Interpretation included the presence and intensity of staining at structure of skin (intercellular space, basement membrane zone (BMZ), superficial and deep blood vessels, appendages, colloid bodies, and epidermal nucleus). The intensity was graded in three levels (1+, 2+ and 3+). The presence of any Ig and/or complement at epidermal nucleus meant positive in vivo ENS.

Statistical analysis was done using the SPSS software version 18.0. Descriptive statistics were used to report demographic data, clinical characteristics, and laboratory investigation results. Moreover, Chi-squared test or Fisher’s exact test were used to compare the categorical variables.

Results

The total number of 3735 specimens were submitted for DIF at Dermatoimmunology Laboratory at Siriraj Hospital during January 2002 and December 2012. One hundred and thirty-eight specimens which showed positive ENS were included in this study [Figure 1]. The majority of patients with in vivo ENS were women (90.6%) and age of onset of disease ranged from 12 to 82 years. Mean age of onset were 42.5 years. CNTD was the most prevalent diagnosis (79.0%) followed by vasculitis (10.1%). Among patients with CNTD, systemic lupus erythematosus (SLE) was the most common diagnosis followed by cutaneous lupus erythematosus (CLE) [Table 1]. Other definite diagnoses included eczema, lichen planus, exfoliative dermatitis, psoriasis, vitiligo, erythema nodosum, sweet’s syndrome, subcutaneous panniculitis-like T-cell lymphoma, sarcoidosis, granuloma annulare, erythema multiforme, linear morphea, follicular mucinosis, and alopecia areata.

Of 126 patients, serum ANA was detected in almost all patients (124 patients, 98.4%) with titers varying from 1:40 to 1:2,560. Most patients had high serum ANA titer (1:640 or higher). Speckled pattern was the most frequent ANA pattern in serum (82.8%). Homogenous, rim, and nucleolar pattern were detected in 13.1%, 12.3% and 12.3% of tested patients, respectively. Intensity of ENS among patients with high serum ANA titer was 1+ in 91.7%, 2+ in 7.3%, and 3+ in 1%, respectively. All patients with serum ANA titer

![Figure 1: Immunoglobulin G deposited in the nucleus of keratinocytes (epidermal nuclear staining) (DIF, ×40)](image)

Table 1: Demographic data of patients with in vivo epidermal nuclear staining (n=138)

| Demographic data                                      | n (%)     |
|-------------------------------------------------------|-----------|
| Gender                                                |           |
| Female                                                | 125 (90.6)|
| Male                                                  | 13 (9.4)  |
| Age of onset (mean±SD, year)                          | 42.5±16   |
| Diagnosis                                             |           |
| Connective tissue disease                              | 109 (79.0)|
| Systemic lupus erythematosus                          | 47 (43.1) |
| Cutaneous lupus erythematosus                         | 38 (34.9) |
| Overlapping syndrome                                  | 10 (9.1)  |
| Systemic sclerosis                                    | 4 (3.7)   |
| Dermatomyositis                                       | 4 (3.7)   |
| Mixed connective tissue disease                       | 4 (3.7)   |
| Antiphospholipid syndrome                             | 1 (0.9)   |
| Undifferentiated connective tissue disease            | 1 (0.9)   |
| Vasculitis                                            | 14 (10.1) |
| Others                                                | 15 (10.9) |

SD: Standard deviation
lower than 1:640 had 1+ intensity ENS. There was no statistically significant association between serum ANA titer and intensity of ENS \( (P = 0.660) \) [Table 2]. Among 124 patients with positive serum ANA, 63.7% had either SLE or CLE, 16.1% were diagnosed with other CNTD than SLE/CLE, and 10.5% had vasculitis. Two patients who had negative serum ANA were female aged 27 and 38 years old, respectively. They were diagnosed with localized CLE without any systemic involvement. Other serum autoantibodies found in patients with \( \text{in vivo} \) ENS are shown in Table 3.

The most common immunoreactant deposit at ENS was IgG (135/138 patients, 97.8%). Considering the location of immune deposition from DIF, half of patients who showed only ENS had SLE or CLE. If deposit also presented at both ENS and BMZ, the diagnosis of SLE or CLE was more likely (74.0%). The presence of ENS and superficial blood vessel staining was more prevalent in vasculitis and other CNTD than SLE or CLE \( (P = 0.002) \) [Table 4]. Deposition at DEJ or superficial blood vessel were not different in patients with SLE and CLE \( (P = 0.471 \text{ and } 0.774, \text{ respectively}) \).

Among patients with SLE, cutaneous symptoms were the most common presentation (87.2%). Cutaneous presentations were discoid rash (54.8%), photosensitivity (45.2%), oral ulcer (42.9%), and malar rash (38.1%). One-third of patients had arthritis (33.3%). Hematologic and renal abnormalities were presented in 22.0% and 19.0%, respectively. Serositis and neurological involvement were infrequent (2.5% and 2.4%, respectively).

**Discussion**

DIF in patients with CNTD, either from lesions or normal skins, usually reveals epidermal nuclear staining.\(^2\)\(^4\) This phenomenon was previously thought

| Table 2: Serum antinuclear antibodies titer in patients with \( \text{in vivo} \) epidermal nuclear staining \((n=126)\) |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Serum ANA titer | n (%): 1+ | 2+ | 3+ | \( P \) |
| 1:40            | 4 (3.2) | 4 (3.2) | 0 | 0 | 0.660 |
| 1:64            | 1 (0.8) | 1 (0.8) | 0 | 0 | 0 |
| 1:100           | 2 (1.6) | 2 (1.6) | 0 | 0 | 0 |
| 1:160           | 9 (7.1) | 9 (7.1) | 0 | 0 | 0 |
| 1:250           | 1 (0.8) | 1 (0.8) | 0 | 0 | 0 |
| 1:640           | 15 (11.9) | 15 (11.9) | 0 | 0 | 0 |
| 1:1280          | 2 (1.6) | 1 (0.8) | 0 | 1 (0.8) | 0 |
| 1:2560          | 83 (65.9) | 75 (59.5) | 8 (6.3) | 0 | 0 |
| Negative        | 2 (1.6) | 2 (1.6) | 0 | 0 | 0 |
| Unknown         | 7 (5.5) | 7 (5.6) | 0 | 0 | 0 |

ANA: Antinuclear antibodies, IgG: Immunoglobulin G, ENS: Epidermal nuclear staining
to be in vitro phenomenon resulted from high serum anti-ribonucleoprotein antibody titer. However, later publication demonstrated that ENS was in vivo phenomenon and caused by penetration of antibodies through live mononuclear cells by Fc-receptor-mediated endocytosis. Furthermore, binding of autoantibodies to antigen on epidermal cells leads to posterior internalization and antibodies can diffuse into living cells.

The prevalence of in vivo ENS in CNTD varies among studies. ENS was found in 32%–66% of patients with SLE, 10.7%–100% of patients with mixed connective tissue diseases (MCTD), 33.3%–56% of patients with systemic sclerosis. Wells et al. studied 33 patients with positive in vivo ENS and reported that SLE was the most prevalent diagnosis (69.7%), followed by MCTD (9.1%), Sjogren’s syndrome (6.1%), dermatomyositis (6.1%), progressive systemic sclerosis (6.1%), and rheumatoid arthritis (3.0%). In our study, SLE (43.1%) or CLE (34.9%) was also the most prevalent diagnosis. Taken together the results, it may be suggest that other evidence of autoimmune diseases should be sought in patients with positive ENS. Similar to previous studies, we also found that 98.4% of patients with ENS showed positive serum ANA. Thus, further serum ANA testing should be done in these ENS positive patients.

Focusing on intensity of ENS and level of serum ANA titer, Wells et al. found that intensity of ENS could not predict the serum ANA titer. Similarly, almost all patients in our study had ENS with 1+ intensity regardless of serum ANA titer and we did not find the correlation between intensity of ENS and level of serum ANA.

All patients with SLE had positive serum ANA. However, high serum ANA titer did not correlate with any cutaneous manifestation such as malar rash, discoid rash, and photosensitivity. In addition, there were no relationships between high titer of serum ANA and hematologic, renal, neurological involvement, or serositis. Previous report among Thai patients with SLE showed that hematologic abnormalities are the most frequent systemic involvement (76.2%) followed by nephropathy (66.2%), arthritis (53.9%), neuropathy (19.2%), and serositis (19.2%). Compare to our study, all patients with positive in vivo ANA had lower prevalence of systemic involvement, and arthritis is the most common (33.3%). Further studies are needed to clarify whether positive in vivo ENS could predict any systemic involvement in SLE patients.

To our knowledge, this study was conducted in the largest number of patients with in vivo ENS. However, the limitations are that patients with negative in vivo ENS were not included and full panel autoantibodies were not tested in all patients.

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Conflicts of interest
There are no conflicts of interest.

What is new?
Serum antinuclear antibody titer was not correlated with disease severity, but patients with epidermal nuclear staining tend to have less systemic involvement.

### Table 4: Diagnosis in patients with in vivo epidermal nuclear staining and staining at basement membrane zone and/or superficial blood vessels

| Diagnosis                              | Location of positive staining (n=138), n (%) |
|----------------------------------------|------------------------------------------|
|                                        | ENS only | ENS and BMZ | ENS and SBV | ENS, BMZ and SBV |
| Total                                  | 22 (15.9) | 50 (36.2) | 5 (3.6) | 61 (44.2) |
| Systemic/cutaneous lupus erythematosus | 12 (54.5) | 37 (74.0) | 1 (20.0) | 35 (57.4) |
| Other connective tissue diseases       | 5 (22.7)  | 6 (12.0)  | 2 (40.0) | 11 (18.0) |
| Vasculitis                             | 1 (4.5)   | 0          | 2 (40.0) | 11 (18.0) |
| Others                                 | 4 (18.2)  | 7 (14.0)  | 0          | 4 (6.6)   |

P=0.002. ENS: Epidermal nuclear staining, BMZ: Basement membrane zone, SBV: Superficial and/or deep blood vessels

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