Ann Dermatol Vol. 33, No. 4, 2021
pISSN 1013-9087 · eISSN 2005-3894
https://doi.org/10.5021/ad.2021.33.4.333

ORIGINAL ARTICLE

Immunohistochemical Analysis of Prurigo Nodularis in 209 Patients: Clinicopathological Analysis between Atopic and Non-Atopic Patients and between Treatment Response Groups

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Background: Prurigo nodularis (PN) is a highly pruritic disease that significantly impairs patient quality of life. Although the mechanism that causes pruritus is not clear, one hypothesis argues that neural hyperplasia, mast cell, and Merkel cell neurite complexes may be associated with PN pathogenesis. **Objective:** The objective of this study was to analyze whether special staining outcomes differed depending on the presence of atopic dermatitis (AD) and treatment response. **Methods:** A total of 209 patients diagnosed with PN was analyzed retrospectively. Patients were divided into two groups according to presence or past history of AD and by treatment response. Histopathologic features were obtained using the following stains: Giemsa, S-100, neuron-specific enolase, cytokeratin (CK)-20, CAM5.2, and CK8/CK18. **Results:** A total of 126 patients (60.29%) had AD, and 68 (32.54%) showed clinical improvement. There were no statistically significant differences in the staining results between the PN groups with AD (PN $\bar{c}$ AD) and without AD (PN $\bar{s}$ AD). Additionally, there were no statistically significant differences in staining results between the improved and non-improved groups. **Conclusion**: Implementing the special stains helped to identify PN pathogenesis. Because there were no statistically significant differences in the special stain results between the improved and non-improved groups, we conclude that mast cell proliferation, neural hyperplasia, and Merkel cell hyperplasia may not have a significant effect on treatment response. **(Ann Dermatol 33(4) 333～338, 2021)**

-Keywords-
Atopic dermatitis, Mast cells, Merkel cells, Nerve fibers, Prurigo

INTRODUCTION

Prurigo nodularis (PN) is a highly pruritic, hyperkeratotic papule and nodular disease. PN is common in the 50 to 60 years age group; however, it can also develop in younger people in association with atopic dermatitis (AD)[1,2]. PN significantly impairs quality of life, which can cause psychological distress and sleep disturbance[3,4]. Repeated scratching of the lesions leads to excoriation and lichenification, mainly on the extensor surfaces, trunk, or lower extremities[1,5]. PN is associated with a wide range of diseases including AD, various infectious diseases, chronic kidney disease, malignancies, and neurological diseases[6-11]. Thus, PN is sometimes considered to be a clinical pattern induced by chronic pruritus and repeated scratching[5] and common histopathologic presentations include orthohyperkeratosis, acanthosis, parakeratosis, papillary dermal fibrosis with perivascular inflammatory infiltrations, and characteristic neural hypertrophy[12,13]. Several previous studies have reported changes in nerve, Merkel,

Received August 20, 2020, Revised November 25, 2020, Accepted for publication November 26, 2020

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and mast cells[12,14,15]. Although the mechanism of pruritus is not clear, one hypothesis suggests that neural hyperplasia, mast cell, and Merkel cell neurite complexes may be associated with PN pathogenesis[1,15-20]. The objective of this study was to analyze whether special staining outcomes differed according to clinical features such as presence of AD and treatment response.

MATERIALS AND METHODS

This was a retrospective study using electronic medical records. The ethical committees of Samsung Medical Center approved this study (approval number: 2002-10-009, 2009-12-053). Subjects included were patients who visited the Department of Dermatology at Samsung Medical Center from May 2012 to May 2016 and underwent skin punch biopsy with special staining under clinical diagnosis of PN. A total of 243 patients were clinically suspected of PN, and underwent skin punch biopsy and assessment through several special stains: Giemsa, S-100, neuron-specific enolase (NSE), cytokeratin (CK)-20, CAM5.2, and CK8/CK18. We excluded 34 patients who were diagnosed with diseases other than PN such as capillary hemangioma, bullous pemphigoid, or lichen simplex chronicus; thus, 209 patients were analyzed (Fig. 1).

When patients visited the clinic, the physician first took their medical history including AD history. Next, skin biopsy was performed with special stains. On the same day, oral antihistamines with topical 0.1% methylprednisolone and topical 0.1% tacrolimus were prescribed. Two weeks later, the physician checked the skin biopsy results and adjusted the antihistamines as needed. Patients were followed-up every two to four weeks for six months, and antihistamine prescriptions were adjusted as needed. Signs of improvement included diminished itching or relieved cutaneous lesions such that the antihistamine treatment frequency could be reduced or stopped. Patients with minimal clinical symptom change in their skin lesions or who required additional antihistamines during a follow-up appointment were considered to have no improvement in their condition. Treatment with topical 0.1% methylprednisolone and topical 0.1% tacrolimus ointment was maintained for all patients for six months. Patients were divided into two groups according to presence or past history of AD and according to treatment response. Histopathologic features were obtained from hematoxylin and eosin (H&E) stained slides and from the other six special stains. The chi-square test, Fisher's exact test, and logistic regressions were used, and all statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA).

RESULTS

A total of 209 patients were included in the analyses. Males accounted for 52.63% of the study sample. The average age of onset for all patients was 52.96 years. The average age of onset was 50.19 years for female patients and 55.45 years for male patients, which is significantly different ($p=0.017$). The majority of patients (126/209; 60.29%) had AD or a history of AD. The average AD patient age was 52.78 years, and the average age of patients without AD was 53.24 years; this difference was not statistically significant ($p=0.838$). Among the 209 patients diagnosed with PN after skin biopsy, 75 were lost to follow-up within 3 months. While 68 patients experienced

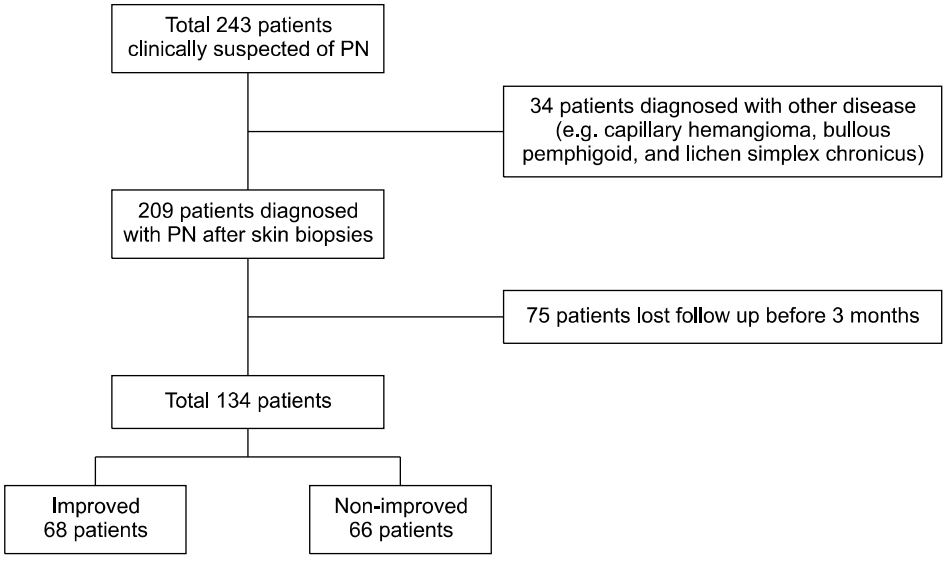

Fig. 1. Patient flowchart. PN: prurigo nodularis.

clinical improvement, 66 patients showed no improvement within 6 months (Fig. 1). Clinical improvement was observed in 68 patients (32.54%) (Table 1), but the improvement status of the 75 patients who were lost to follow-up is unknown. Thus, when analyzing the 134 patients with more than 3 months of follow-up data, 50.75% showed clinical improvement.

Skin punch biopsy with six special stains was conducted. Giemsa staining was performed to identify mast cell proliferation, and S-100 and NSE were used to identify neural hyperplasia. CK-20, CAM5.2, CK8/CK18 stains were used to observe Merkel cell hyperplasia. With Giemsa staining, mast cell proliferation was seen in 8.1% of patients (Table 2). S-100 stain was positive for nerve proliferation in 15.3% of patients and NSE was positive in 27.3% of patients, with neural hyperplasia being observed in 32.1% (n=67; Table 2). CK-20, CAM5.2, and CK8/CK18 were positive in 0.96%, 17.7%, and 43.1% of patients, respectively, and Merkel cell hyperplasia was observed in 61.2% (n=128; Table 2).

The relationship between special staining outcomes and AD history was analyzed. Giemsa stain yielded a statistically significant difference between the PN groups with AD (PN $\bar{c}$ AD) and PN groups without AD (PN $\bar{s}$ AD) (p=0.0280; Table 3). In logistic regression models, Giemsa stain was expressed significantly less in PN $\bar{c}$ AD patients than PN $\bar{s}$ AD patients (odds ratio=0.0347). The relationship between special staining outcomes and treatment response were also analyzed. When analyzing both 209 patients including patients who lost follow up, and 134 patients without them, we found no statistically significant differences between the improved and non-improved groups (Table 3).

DISCUSSION

PN is known to be induced by pricking and scratching, secondary to itching[13]. A number of pathways have been suggested to explain heightened perception to itch and touch in PN patients[13]. These include neurochemical changes in cutaneous nerves in the lesions, the spinal cord, and the central nervous system[13]. PN lesions show nerve hyperplasia and elevated numbers of cutaneous nerve fibers, as indicated by positive staining for neuropeptides such as substance P and calcitonin gene-related peptide (CGRP)[1]. A previous report indicated that increased CGRP and substance P-immunoreactive nerve fibers is a characteristic feature of PN[1]. Nerve growth factor, which is released from mast cells[21], is overexpressed in PN lesions and may induce hypersecretion of neuropeptides, such as substance P and calcitonin CGRP, from sensory fibers, resulting in neuropeptide deposition in pruritic nodules[15,17,22]. Neuropeptides promote mast cell degranulation and release of mediators like histamines, which not only induces itching in the skin, but also increases fibroblast proliferation and collagen synthesis that causes collagen remodeling[20,23]. Increased Merkel cell density has also been reported to be associated with neuronal proliferation in PN lesions because they are compo-

Table 1. Clinical characteristics of patients with prurigo nodularis (total=209)

Clinical feature	Value
Mean age of onset (yr)	
Total	52.96 (15.93)
Male	55.45 (14.60)
Female	50.19 (17.00)
Sex	
Male	110 (52.63)
Female	99 (47.37)
Male:female	1.1:1
Atopic dermatitis	
Atopic	126 (60.29)
Non-atopic	83 (39.71)
Treatment response	
Improvement	68 (32.54)*
No improvement	66 (31.58)
Follow-up loss	75 (35.89)

Values are presented as mean (standard deviation) or number (%). *When 75 patients who were lost to follow-up were excluded, 50.75% clinically improved.

Table 2. Special staining results for patients with prurigo nodularis

Special stain	No. of specimens with positive stains (n=209)	No. of specimens with positive stains (n=134)
Mast cell proliferation		
Giemsa	17 (8.1)	13 (9.7)
Total	17 (8.1)	13 (9.7)
Neural hyperplasia		
S-100	32 (15.3)	19 (14.2)
NSE	57 (27.3)	35 (26.1)
Total	67 (32.1)	41 (30.6)
Merkel cell hyperplasia		
CK-20	2 (1.0)	2 (1.5)
CAM5.2	37 (17.7)	24 (17.9)
CK8 and CK18	90 (43.1)	58 (43.3)
Total	128 (61.2)	83 (61.9)

Values are presented as number (%). NSE: neuron-specific enolase, CK: cytokeratin.

Table 3. Relationship between special staining results and clinical factors

Variable	Mast cell proliferation			Neural hyperplasia			Merkel cell hyperplasia					
	Giemsa positive	*p*-value	Odds ratio	S-100 positive	*p*-value	NSE positive	*p*-value	CK-20 positive	*p*-value	CAM 5.2 positive	*p*-value	CK8 and CK18 positive	*p*-value
Atopic dermatitis (n=209)	17	0.0280*	0.0347*	32	0.9088	57	0.6651	2	0.6377	37	0.9097	90	0.8323
Present (n=126)	6 (35.3)			19 (59.4)		33 (57.9)		1 (50.0)		22 (59.5)		55 (61.1)	
Not present (n=83)	11 (64.7)			13 (40.6)		24 (42.1)		1 (50.0)		15 (40.5)		35 (38.9)	
Treatment response (n=209)	17	0.7742		32	0.1619	57	0.8565	2	0.1048	37	0.7099	90	0.4962
Improved (n=68)	5 (29.4)			7 (21.9)		18 (31.6)		2 (100)		13 (35.1)		27 (30.0)	
Non improved (n=141)	12 (70.6)			25 (78.1)		39 (68.4)		0 (0)		24 (64.9)		63 (70.0)	
Treatment response (n=134)	13	0.3512		19	0.1907	35	0.9252	2	0.4964	24	0.7114	58	0.3962
Improved (n=68)	5 (38.5)			7 (36.8)		18 (51.4)		2 (100)		13 (54.2)		27 (46.6)	
Non improved (n=66)	8 (61.5)			12 (63.2)		17 (48.6)		0 (0)		11 (45.8)		31 (53.4)	

Values are presented as number only or number (%). NSE: neuron-Specific Enolase, CK: cytokeratin. *Statistically significant ($p<0.05$).

nents of the neurocutaneous system response to light touch[18]. The authors recognized that PN pathophysiology is closely related to nerve, Merkel, and mast cells. Skin biopsies were performed from patients with clinically suspected PN, including special stains for the three cell types above, to determine if they were actually associated with PN.

Our study results were reviewed in context with the previous literature. CK-20, CAM5.2, and CK8/CK18 staining were performed to evaluate Merkel cell hyperplasia, which is a characteristic feature of PN. In a previous study, 75% of tissues tested positive in CK8 staining, indicating elevated Merkel cell counts in these tissues[18]. In our study, 61.2% of patients showed Merkel cell hyperplasia via CK-20, CAM5.2, CK8, and CK18 staining. Another report showed dermal nerve hyperplasia in 96% of patients using S-100 staining[19]. In this study, S-100 and NSE stains were used to verify nerve hyperplasia: 37 patients were S-100 positive, 57 patients were NSE positive, and 23 patients were positive for both stains. Therefore, 34.0% of patients (n=71) were found to have nerve hyperplasia. With Giemsa staining, 8.1% of patients showed mast cell proliferation. In contrast to a previous study that identified no mast cell proliferation with H&E staining[12], we observed far more mast cell proliferation with Giemsa staining. When analyzing the relationship with AD, only Giemsa stain showed statistically significant difference between PN $\bar{c}$ AD and PN $\bar{s}$ AD. Giemsa stain was expressed less in the PN $\bar{c}$ AD than the PN $\bar{s}$ AD group, which is inconsistent with previous literature that reported mast cells were elevated in PN $\bar{c}$ AD patients[24,25]. However, since mast cells are also known to be elevated in PN patients independent of AD, the result could be different from the change in the mast cell only by AD[20].

Among the 209 patients analyzed, 32.54% showed improvement with treatment using antihistamines, topical methylprednisolone, and topical tacrolimus. After excluding the 75 patients who were lost to follow-up within three months, 50.75% showed clinical improvement. Also, there were no statistically significant differences in special staining results between the improved and non-improved groups (Table 3), which indicates that mast cell proliferation, neural hyperplasia, and Merkel cell hyperplasia might not be critical factors for predicting treatment response.

This study may have a selection bias, because it consists of patients at a single tertiary hospital. Also, since this is a retrospective study, the degree of improvement was not evaluated with numerical scales or patient surveys, but according to medication change.

This is the first study to examine the relationship between

special staining outcomes and clinical features of PN. Through special staining analyses, neural hyperplasia, Merkel cell hyperplasia, and mast cell proliferation were observed, and our results are consistent with the previous literature. Lastly, we did not find any statistically significant differences in special staining results between the PN $\bar{c}$ AD and PN $\bar{s}$ AD groups nor between the improved and non-improved groups. Therefore, we concluded that mast cell proliferation, neural hyperplasia, and Merkel cell hyperplasia may not have a significant effect on treatment response or presence of AD.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

FUNDING SOURCE

None.

DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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