

## Local Mast Cell Histamine and Plasma Histamine Levels in Neurofibromatosis Type 1

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Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder characterized by café-au-lait spots, neurofibromas, freckling, optic gliomas, Lisch nodules, and bone deformity (1). Neurofibromas are composed of Schwann cells, fibroblasts, perineurial cells, and mast cells. It has been reported that the interaction between NF1<sup>-/-</sup> Schwann cells and NF1<sup>+/-</sup> mast cells plays an essential role in tumour development (2). NF1<sup>-/-</sup> Schwann cells produce stem cell factor (SCF), a ligand for c-kit receptor, which can stimulate mast cell migration (3). It has already been shown that serum SCF levels are elevated in NF1 patients (4). Activated mast cells may secrete various chemical mediators, such as transforming factor- $\beta$ , histamine and heparin, in a haploinsufficient microenvironment (5). It has been suggested that mast cell stabilization could decrease neurofibroma growth (6). Tissue histamine level in neurofibroma is higher than that in normal skin (7). Nevertheless, the number of histamine-positive mast cells in neurofibromas has not yet been determined. In addition, the significance of plasma histamine in NF1 has not been elucidated. Approximately 20% of patients with NF1 have neurofibroma-associated pruritus (8), which might be associated with plasma histamine levels. Therefore, we studied histamine-positive cells and plasma histamine levels in NF1 patients with different types of neurofibromas.

### PATIENTS AND METHODS

Plasma and tissue samples from patients with NF1 were collected at the Department of Dermatology of Tottori University Hospital, Tottori, Japan between 2008 and 2009, after obtaining the informed consent of the patients. This study protocol was approved by the ethics committee of Tottori University. The diagnosis of NF1 was based on National Institutes of Health criteria (1).

The ages of the patients with NF1 (14 males and 15 females) ranged from 4 to 73 years (mean age 38.1 years) (Table I). Four patients had nodular plexiform neurofibromas (NPNs) and 5 had diffuse plexiform neurofibromas (DPNs). Seven patients had itch and 4 of these patients had atopic dermatitis (AD). Plasma samples from 14 healthy donors (7 males and 7 females; mean age 27.0 years) and 11 patients with psoriasis (10 males and 1 female; mean age 58.5 years) served as controls. We used plasma samples that were stored at 20°C until use. Plasma histamine levels were assayed by using a Histamine ELISA kit (Immuno-tech, MBL, Nagoya, Japan).

We analysed 14 neurofibromas (8 cases of cutaneous neurofibromas (CNs), 4 cases of NPN, and 2 cases of DPN). Immunohistochemistry of formalin-fixed and paraffin-embedded sections was performed using the automated slide preparation system NexES IHC staining system employing RED Map kit (Ventana Medical Systems, Inc., Tucson, AZ, USA). The sections were incubated with anti-histamine antibody (1/50

dilution, Progen, Heidelberg, Germany) for 30 min at room temperature. They were then incubated for 30 min with a universal secondary antibody (Ventana Medical Systems). Histamine-positive cells were counted at a magnification of  $\times 200$  in 10 fields. The results were expressed as positive cells/mm<sup>2</sup>.

Statistical significance of differences was determined by Mann-Whitney's *U* tests. *p*-values <0.05 were considered statistically significant.

### RESULTS

Plasma histamine levels and histamine-positive cells in neurofibromas in patients with NF1 are shown in Table I. There were many histamine-positive mast cells in CNs ( $n = 8$ ,  $32.5 \pm 27.5$  cells/mm<sup>2</sup>) and DPNs ( $n = 2$ ,

Table I. Characteristics of patients with neurofibromatosis type 1 (NF1) in this study

Case/age (years)/sex	No. of CNs	Plasma histamine (ng/ml)	Histamine positive cells/mm <sup>2</sup>	Atopic dermatitis	Itch
<i>Cutaneous neurofibroma</i>					
1/9/M	0	1.15	ND	+	+
2/10/M	0	0.62	ND		
3/10/F	0	0.99	ND		
4/14/M	1–10	1.32	ND		
5/24/F	1–10	1.31	ND	+	+
6/27/M	11–100	0.65	15.6 (T)	+	+
7/32/M	11–100	0.52	ND		
8/35/M	11–100	0.55	ND		
9/36/M	101–1000	0.17	85.2 (T)		
10/37/F	11–100	0.51	20.4 (H)		
11/38/F	101–1000	0.52	ND		
12/51/F	101–1000	0.29	10 (E)		
13/59/F	11–100	0.41	ND		
14/62/F	11–100	0.56	ND		
15/63/F	11–100	0.38	ND		+
16/66/F	101–1000	0.34	47.2 (T)		
17/67/F	101–1000	0.81	52 (T)		
18/67/M	101–1000	0.37	28 (T)		
19/68/F	11–100	0.82	ND		+
20/73/F	101–1000	0.7	1.2 (E)		
<i>Nodular plexiform neurofibroma</i>					
21/4/F	0	0.56	0 (H)		
22/15/F	0	0.61	1.2 (E)		
23/21/M	0	0.75	0 (E)		
24/35/M	1–10	0.7	0.8 (E)		
<i>Diffuse plexiform neurofibroma</i>					
25/15/M	0	1.34	ND	+	+
26/20/M	1–10	0.99	ND		
27/41/M	11–100	0.55	ND		
28/47/F	11–100	0.4	65.2 (E)		
29/58/F	101–1000	0.34	4 (T)		+

ND: not done; T: trunk; H: head; E: extremities; CNs: cutaneous neurofibromas.

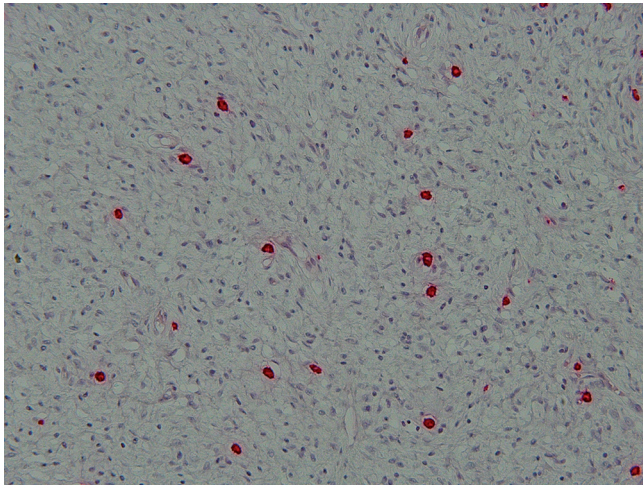


Fig. 1. Histamine-positive cells in cutaneous neurofibroma ( $\times 200$ ) corresponding to  $0.5 \text{ mm}^2$  (case 16).

$34.6 \pm 43.3 \text{ cells/mm}^2$ ) (Fig. 1). Weber et al. (9) reported that mast cell populations were high at peripheral skin sites or superficial skin layers in healthy human skin. We compared the number of positive cells in CNs at different body sites and found low numbers in the hand and foot (peripheral skin sites) in our study of NF1 patients. The sizes of CNs (cases 6, 9, 10, 16, 17, 18) were approximately 1 cm and CNs were located in superficial areas. In contrast, CNs in cases 12 and 20 were larger ( $>2\text{--}3 \text{ cm}$ ) and were located in deeper dermis. On the other hand, the number of mast cells in DPN located in a superficial area (case 28) was large, whereas that located in a deep area (case 29) was small. Few histamine-positive mast cells were seen in NPNs ( $n=4$ ,  $0.5 \pm 0.6 \text{ cells/mm}^2$ ), and the cell density was low compared with that in CNs or DPNs (not shown).

Plasma histamine levels in patients with NF1 were not different from those in healthy donors. NF1 patients were divided into 3 groups (CN,  $n=20$ ; NPN,  $n=4$ ; DPN,  $n=5$ ) with mean  $\pm$ SD histamine levels of  $0.65 \pm 0.33$ ,  $0.66 \pm 0.1$  and  $0.72 \pm 0.43 \text{ ng/ml}$ , respectively. Plasma histamine levels were not correlated to the number of CNs.

Although 7 (24.1%) of the 29 patients had itch, 4 of them had AD. Plasma histamine level in 7 patients with neurofibroma-associated pruritus was  $0.86 \pm 0.42 \text{ ng/ml}$ , which was not different from that in healthy donors. Plasma histamine level in 4 patients with AD was slightly elevated ( $1.11 \pm 0.33 \text{ ng/ml}$ ) compared with that without AD, but was not different from that in healthy donors.

## DISCUSSION

Large numbers of degranulated mast cells bearing IgE surface membrane receptors have been detected in neurofibroma (10). Interestingly, elevated serum IgE levels (29.3%; 22/75) were observed in patients with

NF1 (11). Moreover, Fabricant & Todaro (12) reported increased serum levels of nerve growth factor, which induces mast cell degranulation and mediator release. In fact, Riccardi (6) demonstrated that blockade of mast cell secretion by the use of ketotifen, an antihistamine drug, resulted in a decrease of pruritus with NF1.

We first performed immunohistochemical study of histamine in different types of neurofibroma. There were many histamine-positive cells in CNs and DPNs. In contrast, few positive cells were detected in NPNs. We speculate that the number of histamine-positive cells is related to size of the tumour. If the tumour is small and is located in a superficial area, the number of histamine-positive cells might be large. With regard to NPNs, the stroma of the tumour was mucinous and cell density was extremely low in all cases compared with that in CNs or DPNs. Therefore, the density of cells is also related to the variance of histamine-positive cells. In addition, histamine staining might be influenced by the activation/degranulation status of cells in neurofibromas.

We hypothesized that plasma histamine levels might be elevated in patients with NF1. However, plasma histamine levels in patients with NF1 were not different from those in healthy donors. In addition, plasma histamine levels were not associated with the number of CNs or type of neurofibroma.

Although our results are compatible with previously reported results (8), plasma histamine levels in 7 patients with neurofibroma-associated pruritus were not different from those in healthy donors. Even in patients with AD, the level was not elevated. Elevated plasma histamine levels were found in patients with psoriasis (13), which was confirmed by us ( $1.9 \pm 2.0 \text{ ng/ml}$ ;  $p < 0.01$ ). In contrast, elevated plasma histamine level have been found only in patients with severe AD (14, 15). The results of our study indicate that local histamine is only active on cutaneous levels in NF1.

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*The authors declare no conflicts of interests.*

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