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Olfactory mucosal findings and clinical course in patients with olfactory disorders following upper respiratory viral infection*†

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SUMMARY

Seventy patients with olfactory disorders following upper respiratory viral infection (URVI) were studied clinically, and the olfactory mucosa of 13 patients was biopsied using special biopsy forceps. The specimens were examined using immunohistochemical staining for neuron-specific enolase (NSE), S-100 protein (S-100), cytokeratin (CK), and proliferating cell nuclear antigen (PCNA). Although the clinical course of URVI-olfactory disorders was not very good, overall a high proportion of Alinamin® intravenous injection test-positive patient's recovered their sense of smell. Immunohistochemical study of the biopsy specimens revealed a decrease in the number of olfactory receptor cells and nerve bundles. In a few cases the olfactory neuroepithelium was replaced by metaplastic squamous epithelium. Sometimes different types of degeneration were found in the same specimen. No PCNA-immunoreactivity was detected in the olfactory epithelium. The result generally correlated with the degree of degeneration of the olfactory mucosa, because regeneration of the olfactory receptor cells is suspected to be extremely slow or rare in humans. Alinamin® test-positive patients had many olfactory receptor cells. These findings suggest that olfactory mucosal biopsy and the Alinamin® intravenous injection test are useful methods of determining the prognosis in post-URVI olfactory disorders.

Key words: olfactory mucosa, viral airway infection, olfactory neuroepithelium

INTRODUCTION

During upper respiratory viral infection (URVI) many people complain of some degree of olfactory impairment. After the infection, most people recover normal olfactory function. This type of olfactory loss is suspected to be caused by nasal mucosal swelling which prevents odorant molecules from reaching the olfactory receptor cells. In a few patients, however, olfactory loss remains after infection and continues for a long period, or sometimes even permanently, despite treatment. This type of olfactory disorder has been noted clinically by many authors (Schaupp, 1971; Henkin et al., 1975; Doty, 1979) and is said to have a neural basis. The cause is suspected to be damage to olfactory receptor cells and/or olfactory filae. Furthermore, it has been confirmed experimentally that some viruses causing upper respiratory infection have an affinity for olfactory receptor cells, and that olfactory epithelium can be destroyed by intranasal virus infection (Kanoh, 1986). We have been performing olfactory mucosal biopsies since 1983 and have already reported the olfactory mucosal findings in a few cases of olfactory disorder following URVI (Yamagishi et al., 1988, 1990, 1992). It was apparent from these studies that the number of olfactory receptor cells and nerve bundles was decreased in post-URVI olfactory disorders. Furthermore, it was assumed that the prognosis might be affected roughly by the degree of degeneration. In this paper we present clinical data on 70 patients who had post-URVI olfactory disorders from 1984 to 1991, and we describe the results of more detailed morphological examinations of the olfactory mucosa of several patients using immunohistochemical techniques, and analyse the relationship between the degree of degeneration and clinical outcome. Moreover, the goal of this study is to evaluate whether it is possible to determine the prognosis of patients at the first visit or not.

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PATIENTS AND METHODS

Patients

Seventy patients with post-URVI olfactory disorders were evaluated clinically at the Smell Clinic of the University of Niigata School of Medicine, between 1984 and 1991.

Samples were obtained from 13 of these patients who had visited the clinic between 1987 and 1991. All patients were examined by endoscopy, polytomography, and routine olfactory testing. The patients consisted of 18 males and 52 females, aged 23 to 72 years (mean: 50 years). Most patients were in their forties and fifties (Figure 1). The severity of the olfactory disorders is shown in Table 1. Thirty-seven patients (52.9%) complained of anosmia, 19 (41.4%) of hyposmia, and four (5.7%) were normal. Nine (12.9%) were found to have dysosmia, six (8.6%) reported parosmia, and three (4.3%) had cacosmia.

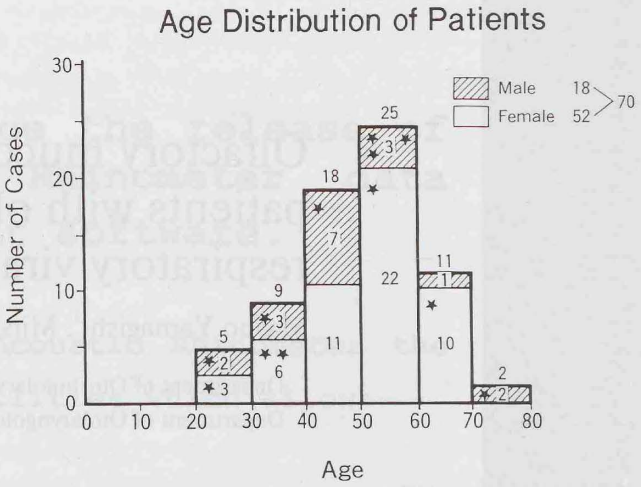


Figure 1. Age distribution of patients with post-URVI olfactory disorders. Most patients are in their forties and fifties. Asterisks show the patients from whom the olfactory mucosa were biopsied.

Table 1. Degree of post-URVI olfactory disorder.

degree	No.	%
normal	4	5.7
moderately hyposmic	15	21.4
severely hyposmic	14	20.0
anosmic	37	52.9
total	70	100

normal: -1.0–2.5; moderately hyposmic: 2.6–4.0; severely hyposmic: 4.1–5.5; anosmic: >5.6 (as measured by T&T olfactometry)

Twenty-four patients (34.3%) complained of taste disturbance, but all were flavour disorders.

Detailed data on the olfactory testing of the biopsied patients are shown in Table 2. Ten patients complained of anosmia or hyposmia alone, and two patients had accompanying dysosmia (cacosmia or parosmia), and one patient complained of dysosmia only. Patients were treated with oral vitamin B_{12}, adenosine triphospate and betamethasone nasal drip.

Olfactory testing

Olfactory testing was performed using an odour-threshold and identification test (T&T olfactometry) and the intravenous thiamine propyl disulfate (Alinamin®) injection test. These are widely used in Japan (Takagi, 1987). T&T olfactometry uses five odorants, i.e., ß-phenylethylalcohol, methylcyclopentenolone, isovaleric acid, γ-undecalactone and skatole. Each odorant is prepared in eight concentrations except for methylcyclopentenolone, which is prepared in seven concentrations through 1:10 serial dilutions. Each bottle of odorant is given a numerical value from -2 to 5 according to its concentration. The average odour threshold level and the identification level are calculated, and the degree of olfactory disorder is classified. If a patient can not identify the highest concentration, it is counted as 6. As mentioned, the Alinamin® test was also used. This involves intravenous injection of 5 mg/ml thiamine propyl disulfate (Alinamin®) and tests for the presence of the sense of garlic odour, which reaches the pulmonary alveoli after intravenous

Table 2. Characteristics of patients enrolled in the T&T olfactometry and intravenous tests.

patients	age	sex	complaint	DET/RET	response	interval after onset	duration of therapy	therapy
1	54	M	anosmia	5.8/5.8	-	16m	3m	B_{12}, BET
2	55	F	anosmia	5.8/5.8	-	20d	3m	B_{12}, ATP, BET
3	38	F	hyposmia	5.8/5.8	-	3m	3m	B_{12}, BET
4	58	M	hyposmia	4.0/4.6	+	11m	3m	B_{12}, BET
5	72	M	hyposmia	5.6/5.8	+	5m	2m	B_{12}, ATP, BET
6	62	F	anosmia	5.0/5.8	-	1m	3m	B_{12}, ATP, BET
7	32	F	anosmia	5.8/5.8	+	2m	3m	B_{12}, BET
8	26	F	anosmia	5.8/5.8	-	2m	3m	B_{12}, ATP, BET
9	59	M	anosmia	5.8/5.8	-	2.5m	5m	B_{12}, ATP, BET
10	47	M	anosmia	5.8/5.8	-	3m	1m	B_{12}, ATP, BET
11	59	F	dysosmia	0.6/1.0	+	5m	7m	B_{12}, ATP, BET
12	28	M	anosmia + dysosmia	5.8/5.8	-	8m	11m	B_{12}, ATP, BET
13	38	M	anosmia + dysosmia	3.4/3.8	+	5m	2m	B_{12}, ATP, NCA, BET

DET: odour detection threshold; RET: odour recognition threshold; B_{12}: vitamin B_{12} per os; NCA: nicotinic acid per os; BET: betamethasone nasal drip; ATP: adenosine triphosphate

injection and is exhaled through the choanae, thereby reaching the olfactory mucosa.

Biopsy technique and tissue preparation for immunohistochemical staining

The olfactory cleft was inspected with an micro-endoscope, and Takahara's forceps for ear microsurgery and its modified types (Figure 2) were inserted carefully without anaesthesia. When the tip reached the tegmen, it was pressed softly and the forceps closed.

Small specimens obtained from the patients were immediately immersed in Bouin's fluid for 2 h, dehydrated in a graded ethanol series, and embedded in paraffin wax. Dewaxed sections were treated with methanol containing 0.3% hydrogen peroxide for 15 min to block intracellular peroxidase. Next, sections were incubated overnight with the primary antibodies at room temperature. The sera to rabbit anti-neuron-specific enolase (NSE), S-100 protein (S-100), and mouse anti-proliferating cell nuclear antigen (PCNA) (DAKO, Glostrup, Denmark) were used at a dilution of 1:900 (NSE and S-100) and 1:50 (PCNA). The specimen were further incubated with secondary immunoglobin antibody and rabbit- or mouse-peroxidase-anti-peroxidase complex (PAP) or streptavidin-biotin complex (SABC) for 1 h. Reacted specimens were exposed to diaminobenzidine or aminoethylcarbazole.

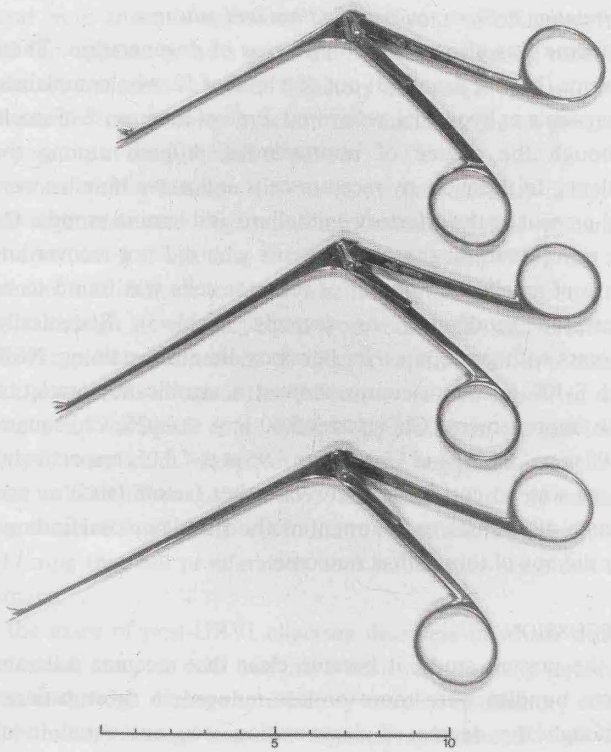


Figure 2. Takahara's forceps for ear microsurgery and its modified types: Grasping-type forceps in three different sizes are used for olfactory mucosal biopsy.

Classification of degenerated olfactory mucosa

The residual olfactory receptor cells and olfactory nerve bundles were counted using hematoxylin-eosin staining and immunostaining for NSE and S-100 protein at high magnification. If different types of area were present in the same specimen, each area was evaluated separately.

RESULTS

Clinical outcome

Fifty-five out of 70 patients were treated over three months. Twelve (21.8%) of the treated patients recovered their sense of smell to almost normal and 12 (21.8%) recovered slightly (Table 3). The recovery rate of Alinamin® test-positive patients was high while negative patients had an extremely poor recovery rate (Chi-square: 12.81, $p < 0.001$; Table 4).

Table 3. Outcome of patients.

outcome	No.	%
normally recovered	12	21.8
slightly recovered	12	21.8
stationary	31	56.4
total	55	100

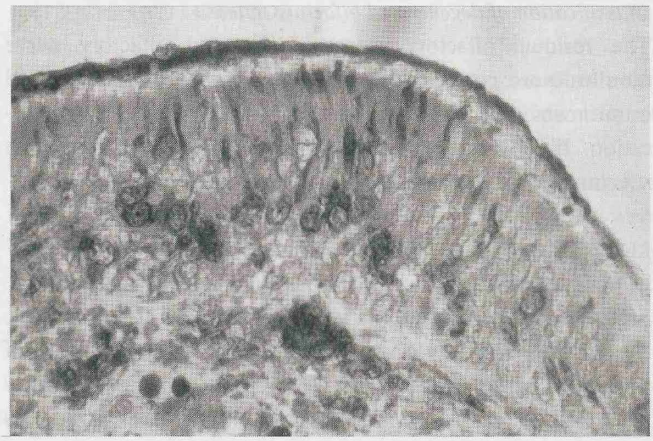
Table 4. Recovery rate according to Alinamin® test.

	positive		negative	
outcome	No.	%	No.	%
normally recovered	11	45.8	1	3.2
slightly recovered	6	25.0	6	19.4
stationary	7	29.2	24	77.4
total	24	100	31	100

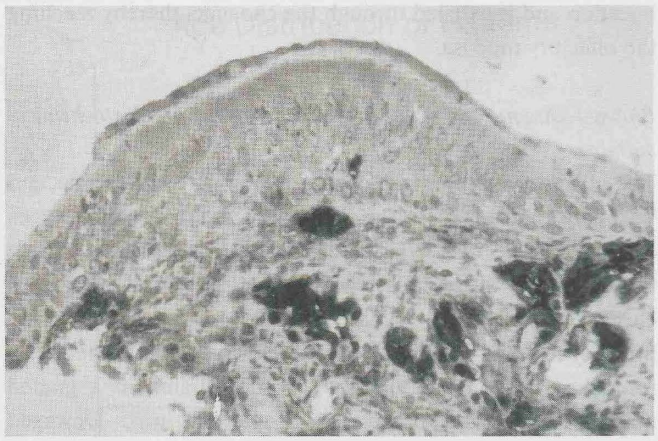
Olfactory mucosal findings

Hematoxylin-eosin staining of samples from patients with post-URVI olfactory disorders revealed three patterns in the olfactory epithelium. In the first pattern, the basic three-layered arrangement of supporting cells, olfactory receptor cells and basal cells was retained, and the large round nuclei of olfactory receptor cells were present in the middle portion of the olfactory epithelium, although the number of receptor cells differed from sample to sample. NSE- and S-100-immunoreactivity was found in the residual receptor cells in the epithelium and nerve bundles in the lamina propria (Figures 3a, b). In the second pattern, the olfactory epithelium was thinner and consisted only of supporting cells and basal cells (Figure 4).

NSE-immunoreactivity could not be found in the epithelium, and a small number of S-100-immunoreactive nerve bundles were seen in the lamina propria. In the third pattern, the olfactory neuroepithelium was replaced by metaplastic squamous epithelium (Figure 5). In some cases, a single specimen contained different patterns of degeneration in different areas. In nine patients, the receptor cells and nerve bundles



A



B

Figure 3. Olfactory mucosa of patients. A: fundamental structure is preserved and NSE-immunoreactive receptor cells are relatively preserved (×400); B: A number of S-100-immunoreactive olfactory nerve bundles are present in lamina propria and Bowman's glands are intermingled (×200).

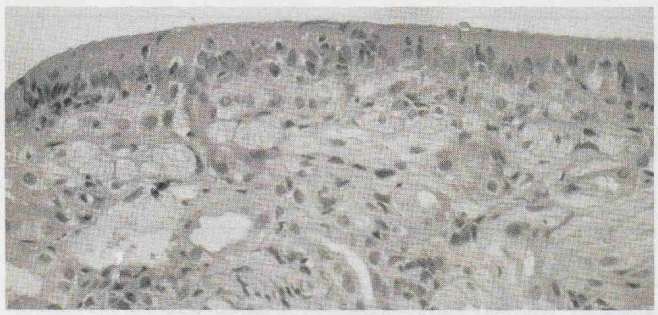


Figure 4. Olfactory receptor cells have disappeared, and the epithelium consists of supporting and basal cells only (×400).

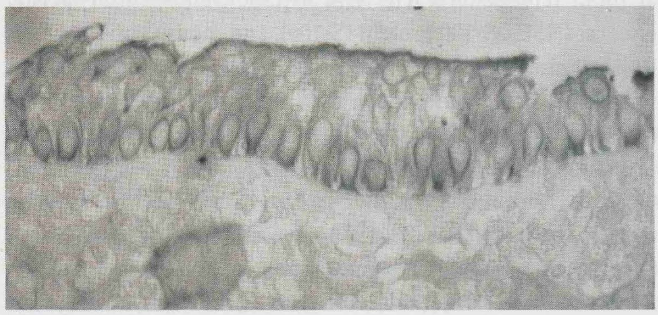


Figure 5. Olfactory neuroepithelium is replaced by squamous metaplasia (×400).

were preserved more or less on hematoxylin-eosin and immunohistochemical staining, in two patients they were found only on hematoxylin-eosin or immunostainlng, and in one case, they were not found anywhere. In most cases, the immunohistochemical findings corresponded to the hematoxylin-eosin findings, but in a few cases they differed.

Relationship between olfactory testing and mucosal findings

The number of olfactory receptor cells in hematoxylin-eosin and NSE-immunostained specimens, as well as the number of nerve bundles immunostained for S-100 were counted and the results were evaluated in relation to olfactory testing. Analysis was performed by the method. A p value of 0.1 was viewed as significant. According to Tables 2 and 5, pairwise comparisons were made between two patients groups of >++ and <++. If different grades were seen in the same specimen, then the mean values were calculated. There was no correlation between the number of residual receptor cells or nerve bundles and results of T&T olfactometry. On the other hand, in patients who had a positive response to the Alinamin® test many receptor cells remained. In comparisons of the residual receptor cells in hematoxylin-eosin staining and NSE-immunostaining patients with grade >++ showed a significant correlation with Alinamin® test positive response (Chi-square: 3.25 at $p < 0.1$; and Chi-square: 1.24 at $p < 0.025$, respectively). There was no correlation between the residual nerve bundles in S-100 immunostaining and Alinamin® test response (Chi-square: 2.18 at $p > 0.1$).

Correlation between mucosal findings and outcome

Outcome was also affected by degree of degeneration. Three patients (Nos. 5, 7, and 13) out of a total of 12, who complained of anosmia or hyposmia, recovered some of their sense of smell, although the degree of improvement differed among the patients. In these, many receptor cells and nerve bundles were still present in the olfactory epithelium and lamina propria. On the other hand, in the nine patients who did not recover any sense of smell, the number of receptor cells was found to be decreased moderately or severely (Table 5). Statistically, patients with grade > ++ in hematoxylin-eosin staining, NSE- and S-100 immunostaining showed a significant correlation with improvement (Chi-square: 5.60 at $p < 0.025$; Chi-square: 12.00 at $p < 0.001$; and Chi-square 4.95 at $p < 0.05$, respectively). There was no correlation between other factors (such as age, interval after onset, or treatment method) and mucosal findings, nor did any of these affect outcome.

DISCUSSION

In the present study, it became clear that receptor cells and nerve bundles were more or less reduced in most patients, although the degree of degeneration was not equal in all patients. Based on these findings, post-URVI olfactory disorders are the result of damage to peripheral olfactory neurons. Clinical recovery of olfaction occurred in a few patients who retained a relative large number of olfactory receptor cells and nerve bundles. Conversely, in patients with small numbers of

Table 5. Findings of the olfactory mucosa and clinical course.

	number of receptor cells		number of nerve bundles	
patients	H.E.	immunostaining of NSE	immunostaining of S-100 protein	clinical course
1	S.M.	(-)	(-)	poor
2	(++)	(+)	(+)	poor
3	(+++), (+)	(++), (-)	(+++), (-)	poor
4	(+), (+)	(+), (-)	(++), (+)	poor
5	(+++), (++)	(+++), (+++)	(++), (-)	improved
6	(-)	(-)	(+)	poor
7	(+++), (+++)	(+++), (+++)	(+++), (+++)	improved
8	(+), (-)	(-), (-)	(-), (-)	poor
9	(-), S.M.	(-), (-)	(+), (-)	poor
10	(+++), (-)	(++), (-)	(++), (-)	poor
11	(+), (-)	(++), (-)	(+), (+)	dysosmia continued
12	(+), (-)	(+), (+)	(+), (+)	poor; dysosmia continued
13	(++)	(+++)	impossible*	improved; dysosmia continued

Number of the olfactory receptor cells and nerve bundles in a specimen of 0.1 mm length: (-): no receptor cell; (+): a few (2, 3-10); (++): moderate (11-20); (+++): many (≥21); SM: squamous metaplasia
* Only epithelium could be obtained

receptor cells and nerve bundles, or squamous metaplasia, improvement tended not to occur. On the basis of these findings, it appears that only patients who have minor olfactory mucosa damage can recover their olfactory function.

The Alinamin® intravenous injection test is only used in Japan. In this study, most Alinamin®-positive patients recovered their olfaction and retained their receptor cells, although most of them were anosmic according to the results of olfactometry, and this appears to be a good method for evaluating residual olfactory neuronal function. Olfactory mucosal biopsy and the Alimamin® intravenous injection test made an overall prognosis possible at the first visit of patient.

Previous studies in animals have shown that olfactory receptor cells regenerate after degeneration following physical or chemical destruction (Nagahara, 1940; Graziadei et al., 1979). But in humans it is difficult to know whether regeneration occurs or not, although it was suspected from our light- and electron microscopical examinations of olfactory epithelia from patients with post-URVI anosmia that precursor cells do appear in the basal area (Yamagishi et al., 1989; 1992). Based on our previous study in many kinds of olfactory disorders, regeneration appears to be unlikely (Yamagishi et al., 1990). In this study, no immunoreaction to anti-PCNA was found in the olfactory epithelium, indicating that cell proliferation does not occur very much in humans.

In the cases of post-URVI olfactory disorders in which smell recovered, receptor cells may have been only partially injured, such as through damage to olfactory vesicles or olfactory cilia. Such minor damage may be repaired spontaneously or in response to treatment.

Dysosmia (parosmia and/or cacosmia) has been reported to accompany post-URVI olfactory disorders. Henkin et al. (1975) noted that these dysosmias developed in approximately half of their post-URVI patients, whereas Leopold et al. (1988) found these smell distortions in less than 10% of their study population. Among the 70 patients examined in the present study, dysosmia was found in 12.9%. Dysosmia appeared in the biopsied patients who had slightly or moderately impaired olfactory mucosa. It was not observed, however, in the patients who had completely destroyed mucosa. The results indicate that dysosmia may be attributable to the impaired residual receptor cells which still are connected to higher olfactory centre.

Although post-URVI olfactory disorders are difficult to treat and the improvement rate is not high, a few patients do recover, and it is important to make a prognosis and predict the response to the treatment. Olfactory mucosal biopsy specimens are not very large and may not display all of the changes actually present in the olfactory mucosa. Nevertheless, the results of this study indicate that olfactory mucosal biopsy combined with the intravenous Alinamin® injection test at the first visit is a useful method of estimating damage to olfactory function and is available for making an overall prognosis in post-URVI olfactory disorders.

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