**Interleukin 6 in Hyposmia**

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Loss of smell (hyposmia) is a symptom reflective of multiple chronic disease processes involving multiple organ systems and somatic processes including endocrine, vitamin, trace metal, metabolic, neurological, neurodegenerative, hematological, immunological, and other organ systems and processes.  Hyposmia can reflect local changes in the oral or nasal cavities affecting olfactory receptors, in the nerves connecting receptors to the brain, or in the brain itself. The systemic changes associated with these major pathological processes include hyposmia as a major symptom. Descriptions of hyposmia associated with these pathological processes have been made by many prior investigators.

We have attempted to understand the changes in secretions of the multiple organ systems in the multiple pathological processes responsible for initiation and perpetuation of hyposmia. Thus, we have reported that decreases in specific biochemical moieties are associated with hyposmia onset and that their replacement has corrected this symptom. For example, lack of thyroid hormone induces hypothyroidism with its associated systemic symptoms, one of which is hyposmia; administration of thyroid hormone corrects the systemic symptoms of hypothyroidism and the associated hyposmia. Zinc deficiency induces multiple systemic symptoms and hyposmia, which is manifested by decreased gustin (carbonic anhydrase VI) secretion; administration of zinc ion supplementation to these patients corrects both these systemic symptoms and the associated hyposmia, this latter effect being associated with the zinc-induced in-

**IMPORTANCE** Olfaction is a complex sensory process that has not been fully studied. Elevated plasma levels of interleukin 6 (IL-6) have been found in patients with several acute and chronic diseases but have not been reported in patients with smell loss (hyposmia).

**OBJECTIVE** To determine IL-6 levels in patients with hyposmia.

**DESIGN** Retrospective study. All measurements were made without reference to the origin of any collected sample.

**SETTING** An ambulatory private practice at The Taste and Smell Clinic in Washington, DC.

**PARTICIPANTS** Fifty-nine consecutive patients who presented to the clinic between 2005 and 2008 for evaluation and treatment of various degrees of hyposmia were studied. Nine volunteers with normal sensory function served as controls.

**MAIN OUTCOMES AND MEASURES** Levels of IL-6 were measured in samples of plasma, urine, saliva, and nasal mucus.

**RESULTS** All biological fluid samples studied contained IL-6. Mean (SEM) levels in plasma, saliva, and nasal mucus in patients were significantly higher than in controls (0.95 [0.10] vs 0.12 [0.03] pg/mL, 0.57 [0.05] vs 0.30 [0.01] pg/mL, and 29.7 [3.8] vs 11.6 [0.5] pg/mL, respectively; all \( P < .001 \)). The concentration of IL-6 in nasal mucus in patients was significantly higher than in controls and was more than 30 times higher than in any other biological fluid. Mean (SEM) levels in urine were not significantly different: 0.92 (0.41) pg/mL for patients and 1.26 (0.41) pg/mL for controls (\( P > .50 \)).

**CONCLUSIONS AND RELEVANCE** Compared with controls, IL-6 in patients was significantly elevated in plasma, saliva, and nasal mucus. Because IL-6 is a proinflammatory cytokine, these changes can relate to local or systemic inflammatory processes, which can be a cause or a result of pathological processes associated with hyposmia. These results support the concept that hyposmia has a biochemical basis and IL-6 may play a role in biochemical pathological processes underlying hyposmia and its treatment.
crease in carbonic anhydrase VI secretion. The multiple biochemical moieties of these diverse organ systems that correct hyposmia in these various pathological processes have been determined to be growth factors that stimulate olfactory epithelial stem cells to initiate maturation and renewal of the sensory cells responsible for normal olfaction.

Olfaction is a complex process comprising multiple components including receptors, nerves, and brain. The local and systemic components of this complex process have not been fully explored. Cell-signaling processes are critical in any complex sensory system such as olfaction, and we and others have investigated several aspects of these signaling processes, some involving adenyl cyclases, sonic hedgehog, and some cytokines. Among these cytokines, we were interested in determining levels of interleukin 6 (IL-6) in patients with hyposmia because no prior studies of this type among these patients have been reported.

It is well known that IL-6, a proinflammatory cytokine, is overproduced in a spectrum of clinical illnesses and conditions including cardiovascular disease, osteoporosis, arthritis, type 2 diabetes mellitus, renal disease, hepatitis, schizophrenia, preeclampsia, various neoplasms, periodontal disease, frailty, stress, and functional decline. Increased IL-6 level has been reported in these conditions, mainly in blood plasma. Increased IL-6 level has been reported in both plasma and ventricular fluid following acute but not chronic head injury. Increased IL-6 level has been reported in cerebrospinal fluid following traumatic brain injury and to trigger nerve growth factor secretion in astrocytes. Increased IL-6 level has been reported in blood plasma of patients with persistent sciatric pain, and IL-6 mRNA level has been reported increased in rat spinal cord following peripheral nerve injury. Increased IL-6 level has been reported in plasma and in saliva of some patients with burning mouth syndrome (BMS) but not in others; no IL-6 differences were reported in these patients with and without associated depression and perceived pain. However, stress hormones have been reported to regulate IL-6 expression in various ovarian carcinoma cells through a Src-dependent mechanism. These results suggest that both specific and nonspecific factors elicit changes in IL-6 in several biological fluids in several disease processes including neurological, inflammatory, and psychological stress.

To evaluate IL-6 in patients with hyposmia, we undertook an investigation of IL-6 levels in samples of plasma, urine, parotid saliva, and nasal mucus obtained from patients with hyposmia and compared these levels with similar measurements obtained in a group of controls. We previously published a preliminary study of some of these results, and we now present a comprehensive study.

Methods

Subjects
Participants in the study were 59 patients, 26 men and 33 women, aged 10 to 86 years, with a mean (SEM) age of 54 (2) years, who presented to the Taste and Smell Clinic in Washington, DC, between 2005 and 2008 with various degrees of smell loss. Patients were recruited from referrals from otolaryngologists and other physicians throughout the entire United States, with many from the Washington, DC, region. All studies were performed in a retrospective manner. Diagnoses of these patients included 24 with postinfluenza-like hyposmia and hypogeusia (PIHH), with or without rhinitis; 39 with allergic rhinitis, with or without congenital smell loss; 6 with hyposmia related to idiopathic causes; 5 with head injury; 4 with drug-induced hyposmia; 3 with phantogeusia and hypogeusia, and 3 with hypogeusia and BMS. All patients had loss of smell as manifested by subjective statements and by olfactometry measurements in which impaired smell function was determined in each patient. Olfactometry was performed using standard psychophysical techniques with 4 odors (pyridine, nitrobenzene, thiophene, and amyl acetate). These techniques were validated by performance in a double-blind clinical trial. Olfactory impairment was determined when a patient had impaired detection thresholds (DTs) and/or recognition thresholds (RTs) (RT elevated above normal) and/or decreased magnitude estimation (ME) levels (below normal levels) for 1 or more of the 4 odors.

By use of these techniques, smell loss was confirmed in each patient, with 12 exhibiting type I hyposmia (the most severe form of hyposmia, with DT, 0 and ME, 0 for all patients for all odors), 44 with type II hyposmia (the next most severe form of hyposmia, with DT, RT, and ME below normal for all patients), and 3 exhibiting type III hyposmia (the least severe form of hyposmia, with DT and RT normal but ME below normal) (see Henkin and Henkin et al for details).

Study participants also included 9 controls, 5 men and 4 women, aged 39 to 76 years with a mean (SD) age of 60 (8) years. All controls were healthy and not taking any prescribed medications. All volunteers were determined to have normal smell function by subjective statements and by normal results on olfactometry testing.

The study was conducted under a protocol approved by the institutional review board of the Georgetown University Medical Center; informed consent was obtained from each study participant.

Procedures
At initial clinical evaluation, samples of blood plasma were collected from each patient by means of venipuncture, placed into tubes containing 100 μL of zinc-free heparin and chilled in ice, and centrifuged at 3000 rpm for 10 minutes. The plasma was then removed and stored at −20°C until assayed. The 24-hour output of urine from each patient was collected in timed relationship to collection of blood plasma. Urine volume was measured and an aliquot stored at −20°C until assayed. A sample of parotid saliva was collected from each patient immediately after blood collection by means of placement of a Lashley cup over Stensen’s duct with lingual stimulation with reconstituted lemon juice (Real Lemon; Borden) as previously described. Saliva was collected in plastic tubes during an 8-minute to 12-minute period and chilled in ice. Samples were stored at −20°C until assayed. Samples of nasal mucus were collected from each patient directly from the nasal cavity in 50-mL wide-mouthed plastic tubes as previously...
Results

Levels of IL-6 were measured in all biological fluids studied (Table 1). Comparison of IL-6 levels in each biological fluid in controls, despite their small number, with those in patients demonstrated large, consistent, and significant differences (Table 1). Levels of IL-6 in plasma, saliva, and nasal mucus in patients were significantly higher than in controls, but levels in urine were not significantly different. Mean levels of IL-6 in nasal mucus in patients were 2.6 times those in controls, mean levels in saliva were 1.9 times those in controls, and mean plasma levels were 7.9 times those in controls.

Comparison of IL-6 levels among patients categorized by etiology of smell loss with controls demonstrated that mean plasma IL-6 level was significantly higher in all categories of patients compared with controls; the highest levels were found in patients with BMS, the next in those with head injury, the third highest in patients with PIHH, and the lowest in patients with allergic rhinitis (Table 2). Mean urine IL-6 levels in patients were similar to that in controls in all patient categories except in patients with congenital hyposmia, in whom levels were significantly lower than in controls. Mean saliva IL-6 level was significantly above that in controls in patients with BMS, head injury, and PIHH, with the highest level in patients with BMS. Mean nasal mucus IL-6 level was elevated in patients with head injury, BMS, allergic rhinitis, phantogeusia, and PIHH but was significantly elevated only in patients with BMS and PIHH. Levels of IL-6 in nasal mucus were highest in patients with head injury and BMS.

Comparison of IL-6 levels in controls in plasma, urine, saliva, and nasal mucus revealed a specific hierarchy (Table 1) different from that found in patients. Levels of IL-6 in nasal mucus were higher than in any other biological fluid, being more than 10 times the concentrations found in urine, saliva, or in blood plasma. Levels were next highest in urine, then saliva, and lowest in plasma. The concentration ratio of nasal mucus to plasma was 97:1, of nasal mucus to saliva, 34:1, and nasal mucus to urine, 9:1.

Comparison of IL-6 levels in patients in plasma, urine, saliva, and nasal mucus also yielded a hierarchy of levels but with a somewhat different set of ratios than that found in controls. The highest level of IL-6 was also found in nasal mucus, which had more than 30 times the levels found in saliva, urine, or plasma. The next highest levels were found in plasma and urine (levels were similar) and the lowest level in saliva. The concentration ratio of nasal mucus to plasma was 31:1, approximately one-third of that found in controls; nasal mucus to saliva was 52:1, approximately 1.5 times the ratio in controls; and nasal mucus to urine was 32:1, approximately 3.5 times the ratio found in controls.

There were no significant differences among IL-6 levels in plasma, saliva, or nasal mucus with smell loss type and patient diagnosis (determined by means of ANOVA; results not shown). These results differ from those of previous studies, which demonstrated a significant inverse relationship between levels in saliva and nasal mucus of cyclic nucleotides (cyclic adenosine monophosphate and cyclic guanosine monophosphate) with severity of smell loss. In those studies, the more severe the smell loss, the lower the level of

Table 1. Interleukin 6 Levels in Plasma, Urine, Parotid Saliva, and Nasal Mucus in Patients With Hyposmia and in Controls With Normal Sensory Function

<table>
<thead>
<tr>
<th>Biological Fluid</th>
<th>Interleukin 6 Level, Mean (SEM), pg/mL</th>
<th>Controls (n = 9)</th>
<th>Patients (n = 59)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>0.12 (0.03)</td>
<td>0.95 (0.10)</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>1.26 (0.41)</td>
<td>0.92 (0.17)</td>
<td>&gt; .50</td>
<td></td>
</tr>
<tr>
<td>Saliva</td>
<td>0.30 (0.01)</td>
<td>0.57 (0.05)</td>
<td>.001</td>
<td></td>
</tr>
<tr>
<td>Nasal mucus</td>
<td>11.6 (0.5)</td>
<td>29.7 (3.8)</td>
<td>.001</td>
<td></td>
</tr>
</tbody>
</table>

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cyclic nucleotides (levels were lowest in patients with anosmia, second lowest in patients with type I hyposmia, third lowest in patients with type II hyposmia, and highest in patients with type III hyposmia).

**Discussion**

To our knowledge, the present study is the first to measure the levels of IL-6 in patients with hyposmia, as well as IL-6 elevations among patients with hyposmia compared with controls. If these findings were to relate to similar results found in rheumatoid arthritis, then elevated IL-6 could be considered a possible causal factor for initiation of hyposmia reflective of local and/or systemic immunological and/or inflammatory changes in blood, saliva, or nasal mucus. This hypothesis is consistent with finding smell loss among patients with inflammatory rheumatoid arthritis. Among patients with hyposmia, chronic lymphocytic inflammation has been reported in nasal mucous membranes of patients with PIHH. Elevated IL-6 level has been previously reported in nasal laverage fluid from patients with naturally acquired viral rhinitis. Paraviruses and other viruses have been found in turbinate epithelial cells of patients with postviral olfactory dysfunction. Manifestation of herpes virus infection has been found in olfactory bulb neurons in mice as long as 200 days after they were initially experimentally infected, as well as in astrocytes in the suspected portal of entry. However, historical changes that may occur in the olfactory epithelium, transmitting nerves, or brain under these conditions have not been investigated. Treatment of rheumatoid arthritis with IL-6 inhibitors has been associated with diminution of both inflammation and IL-6 elevation. If IL-6 elevation in hyposmic patients were related to the cause of their hyposmia, then treatment with IL-6 inhibitory drugs might be associated with improvement of their smell function.

It is well known that an increase in the concentration of many substances locally or systemically can directly inhibit smell function, including zinc, cadmium, drugs of several types, and several other chemical moieties. Elevated IL-6 level could act as an endogenous substance regulating olfactory neuronal activity because it has been shown to regulate neuronal and glial cell activity. Thus, elevated IL-6 levels among patients with hyposmia and chronic head injury may relate to neurological as well as to inflammatory changes.

Finding elevated levels of IL-6 in both plasma and saliva in some patients with BMS suggests not only a response to an inflammatory process but also a possible neurological process as well. Suggestion of a neurological rather than an inflammatory mechanism responsible for the pyrosis in BMS is consistent with the lack of obvious signs of oral inflammation among these patients. Some investigators have considered BMS a trigeminal small fiber neuropathological condition, and treatment with antioxidants and y-aminobutyric acid agonist drugs, or repetitive transcranial magnetic stimulation has been reported to alleviate this condition. However, although elevated IL-6 levels have been measured previously in some patients with BMS, other investigators have found neither elevated IL-6 level nor tumor necrosis factor.

Results of the present study also show that IL-6 levels in nasal mucus are higher than those in plasma, urine, or saliva. This seems to be the first direct comparison of IL-6 levels in these biological fluids and the first demonstration that levels of IL-6 in nasal mucus in patients with hyposmia and in controls are increased relative to those in plasma, urine, or saliva. This finding is logically consistent with the abundance of microbial and antimicrobial agents normally present in nasal mucus. Active inflammatory agents in nasal mucus include bacteria, viruses, fungi, and other substances including histamine, whereas anti-inflammatory agents found include lysozyme, lactoferrin, and albumin. However, con-

### Table 2. Interleukin 6 Levels in Plasma, Urine, Parotid Saliva, and Nasal Mucus in Patients With Hyposmia and Controls With Normal Sensory Function

<table>
<thead>
<tr>
<th>Etiology of Hyposmia</th>
<th>Plasma IL-6 Level, Mean (SEM), pg/mL</th>
<th><em>P</em> Value vs Control</th>
<th>Urine IL-6 Level, Mean (SEM), pg/mL</th>
<th><em>P</em> Value vs Control</th>
<th>Saliva IL-6 Level, Mean (SEM), pg/mL</th>
<th><em>P</em> Value vs Control</th>
<th>Nasal Mucus IL-6 Level, Mean (SEM), pg/mL</th>
<th><em>P</em> Value vs Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIHH (n = 24)</td>
<td>1.03 (0.15)</td>
<td>&lt;.001</td>
<td>1.22 (0.38)</td>
<td>&gt;.05</td>
<td>0.51 (0.05)</td>
<td>&gt;.05</td>
<td>29.7 (5.2)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Allergic rhinitis (n = 7)</td>
<td>0.62 (0.12)</td>
<td>&lt;.001*</td>
<td>0.71 (0.16)</td>
<td>&gt;.05</td>
<td>0.39 (0.06)</td>
<td>&gt;.05</td>
<td>39.5 (18.2)</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Congenital (n = 7)</td>
<td>0.72 (0.15)</td>
<td>&lt;.001*</td>
<td>0.29 (0.06)</td>
<td>&lt;.025b</td>
<td>0.59 (0.14)</td>
<td>&gt;.05</td>
<td>19.6 (8.3)</td>
<td>&gt;.05*</td>
</tr>
<tr>
<td>Idiopathic (n = 6)</td>
<td>1.03 (0.15)</td>
<td>&lt;.001*</td>
<td>1.00 (0.40)</td>
<td>&gt;.05</td>
<td>0.55 (0.07)</td>
<td>&gt;.05</td>
<td>13.6 (4.7)</td>
<td>&gt;.05*</td>
</tr>
<tr>
<td>Head injury (n = 5)</td>
<td>1.47 (0.56)</td>
<td>&lt;.001*</td>
<td>0.83 (0.26)</td>
<td>&gt;.05</td>
<td>0.68 (0.18)</td>
<td>&lt;.02</td>
<td>54.4 (23.7)</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Drug induced (n = 4)</td>
<td>0.70 (0.10)</td>
<td>&lt;.001*</td>
<td>0.78 (0.26)</td>
<td>&gt;.05</td>
<td>0.53 (0.05)</td>
<td>&gt;.05*</td>
<td>10.8 (4.0)</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Phantogeusia (n = 3)</td>
<td>0.56 (0.08)</td>
<td>&lt;.001*</td>
<td>0.44 (0.21)</td>
<td>&gt;.05</td>
<td>0.45 (0.13)</td>
<td>&gt;.05</td>
<td>31.5 (23.0)</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>BMS (n = 3)</td>
<td>2.20 (0.60)</td>
<td>&lt;.001*</td>
<td>0.90 (0.50)</td>
<td>&gt;.05</td>
<td>1.40 (0.70)</td>
<td>&lt;.001</td>
<td>50.9 (11.5)</td>
<td>&lt;.005</td>
</tr>
<tr>
<td>Control (n = 9)</td>
<td>0.12 (0.03)</td>
<td>Ref</td>
<td>1.26 (0.41)</td>
<td>Ref</td>
<td>0.30 (0.01)</td>
<td>Ref</td>
<td>11.6 (0.50)</td>
<td>Ref</td>
</tr>
</tbody>
</table>

Abbreviations: BMS, burning mouth syndrome; IL-6, interleukin 6; PIHH, postinfluenza-like hyposmia and hypogeusia; Ref, reference.

*P* < .05 vs BMS.

<.05 vs PIHH.

<.01 vs PIHH.

<.001 vs BMS.
trary to this supposition, the highest level of IL-6 that we found in nasal mucus was not in patients with allergic rhinitis—in which these agents might be expected to be most active—but in patients with head injury and BMS, in whom no active local nasal inflammatory process presumably occurs although both patient groups exhibit hyposmia. Consistent with these disparate findings, a group of investigators found higher IL-6 levels in nasal mucus in controls rather than in patients with allergic rhinitis.

Possible mechanism(s) of the relationship(s) of IL-6 signaling to loss of smell are multiple. Interleukin 6 is part of a complex and sophisticated signaling system that plays multiple roles in body metabolism. It is an inflammatory cytokine that drives acute-phase proteins including C-reactive protein and fibrinogen, both proteins that are induced by systemic inflammation. It is a major factor in differentiation of B cells into antibody-producing plasma cells. It influences nuclear factor κB and adenosine triphosphate–ubiquitin-dependent proteolytic pathways; it can activate tumor necrosis factor and thereby activate apoptotic pathways that could directly inhibit smell function. Neurepoietin, an IL-6–related cytokine that affects signaling through ciliary neutrophic factor receptor, could directly inhibit smell function because inhibition of several ciliary factors has induced smell loss in patients with Kartagener and Bardet-Biedl syndrome. Patients with Castleman disease produce IL-6, and treatment that inhibits either IL-6 or IL-6 receptor activity alleviates symptoms of the disease. Interleukin 6–deficient mice are incapable of mounting an inflammatory response. After binding to its receptor, the IL-6 receptor complex activates glycoprotein 130 signaling in cells that would not normally express IL-6 receptor, a mechanism known to play a role in pathophysiologic characteristics of chronic inflammatory disorders.

These results suggest that both specific and nonspecific factors may increase IL-6 levels in both acute and chronic disease processes. These include neurological and inflammatory processes and processes involving psychological stress.

There are limitations associated with performance of the present study. The number of controls studied was small, although there was only a small variation in measurements made in their biological fluids. The numbers of participants in the patient groups evaluated were also relatively small, which contributed to the variability in the measurements obtained. This study was performed with the participation of a consecutive group of hyposmic patients; this group may not reflect the complex entirety of hyposmic patients. Indeed, no patient in this group had chronic sinusitis, which amplifies the importance of elevated IL-6 level in nasal mucus among these patients. Whereas immunoassay measurements may vary widely, the levels of variation exemplified by the relatively small variation among all the SEMs obtained illustrate the validity and reliability of the specific immunoassay measurements used in this study. To our knowledge, this is the first study of any type in which IL-6 measurements were obtained in several biological fluids (plasma, urine, saliva, and nasal mucus) in a similar time-based study and compared in both patients with chronic disease processes and controls. These results offer insight into the signaling processes present among some patients with hyposmia that may influence some of the complex processes responsible for their sensory changes.

Future studies are required to reveal how the complex mechanisms underlying IL-6 signaling relate to the cause or the result of smell loss. Indeed, drug treatment of elevated levels of IL-6 or its receptor in several diseases has been useful in inhibiting symptoms of these disease processes. Whether this therapy would be useful in treatment of patients with hyposmia is unknown. However, our findings support the concept that hyposmia has a biochemical basis and that discovery of the role(s) that IL-6 may play in hyposmia offers an opportunity to learn more about its biochemical pathologic processes and to establish new methods to treat it.

In conclusion, increased IL-6 secretion in patients with hyposmia suggests that it may play a role in the initiation and perpetuation of this symptom. The role of IL-6 in hyposmic patients in noninflammatory states such as head injury remains unclear. However, this discovery of elevated IL-6 level in hyposmia offers an opportunity to learn more about both the biochemical pathologic characteristics of hyposmia and the possible establishment of new methods to treat the pathologic processes associated with this symptom.

ARTICLE INFORMATION
Submitted for Publication: November 29, 2012; final revision received February 27, 2013; accepted April 16, 2013.

Author Contributions: All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Henkin.

Acquisition of data: All authors.

Analysis and interpretation of data: Henkin, Schmidt.

Drafting of the manuscript: Henkin, Schmidt.

Critical revision of the manuscript for important intellectual content: Henkin, Schmidt, Velicu.

Statistical analysis: All authors.

Administrative, technical, and material support: Schmidt.

Study supervision: Henkin.

Conflict of Interest Disclosures: None reported.

REFERENCES


