The Effect of Human Olfactory Biopsy on Olfaction: A Preliminary Report

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Normal human olfactory function is subject to a wide variety of factors. Although biopsy of human olfactory neuroepithelium has been reported by several researchers, there are no studies which have evaluated the effect of this procedure on olfactory function. In this retrospective study, we sought to determine if tissue removal from the olfactory cleft has an adverse influence on the sense of smell. Nineteen subjects underwent bilateral olfactory testing and subsequent endoscopic olfactory mucosal biopsy. All subjects were retested 6 weeks to 1 year after olfactory neuroepithelial biopsy. No statistical difference was found between olfactory tests performed before or after biopsy. These data suggest that biopsy of human olfactory neuroepithelium has no discernible adverse effect on the ability to smell.

INTRODUCTION

Normal human olfactory function is subject to a variety of factors that are not yet well understood. In an effort to further understand the human sense of smell, several investigators have sampled tissue from the olfactory cleft in living subjects. From these tissue studies, researchers have proposed the existence of a fourth cellular type, described mechanisms to explain persistent anosmia after head injury and observed the histopathologic changes present in certain disease states. In addition, in vivo olfactory tissue sampling eliminates postmortem artifact.

There are, however, a number of variables which make reliable olfactory tissue sampling from living subjects challenging. The olfactory epithelium is believed to be a dynamic tissue and is known to have an uneven distribution of receptor cells admixed with patches of respiratory epithelium. The degree of variability in neuroepithelial distribution may be related to many factors, such as age, gender, smoking habits, and exposure to viruses. Therefore, successful retrieval of human olfactory neuroepithelium often requires multiple biopsies from a single subject. It is, in part, dependent on its relative inaccessibility and the experience of the surgeon.

In vivo tissue sampling from the olfactory cleft carries inherent, albeit small, risks when performed by an experienced surgeon. The risks include bleeding, potential for cerebrospinal fluid leakage with secondary intracranial infection, and intracranial injury. Another potential adverse outcome is reduced olfactory function.

Although olfactory tissue sampling is performed using an array of techniques and instrumentation, its potential detrimental effect on olfactory function has not been evaluated. The purpose of this retrospective study is to determine if olfactory tissue sampling has an adverse influence on olfactory function.

MATERIALS AND METHODS

Study subjects for this retrospective project were identified from an ongoing controlled prospective investigation of human olfactory epithelium from patients with early Parkinson's and Alzheimer's disease. All but two of these study subjects have been included in a prior report.

Informed consent was obtained from all study subjects. Nineteen subjects were available for endoscopically guided tissue sampling from the olfactory cleft. The study groups consisted of 7 control subjects (CON), 8 subjects with Parkinson's disease (PD), 3 subjects with Alzheimer's disease (AD), and 1 with Pick's disease (included in the AD group).

All but two of the control subjects received cognitive screening tests which included the mini-mental status examination (MMSE) and Picture Identification Test (PIT). Each subject's sense of smell was evaluated with the standardized University of Pennsylvania Smell Identification Test (UPSIT) and with the phenethyl alcohol (PEA) olfactory threshold test. Administration of these tests preceded the biopsy procedure by no more than 2 months, and was most frequently performed on the day of tissue
sampling prior to biopsy. Some subjects also had olfactory test data available which antecedent the aforementioned prospective investigation of human olfactory epithelium.

Endoscopically guided olfactory cleft tissue sampling was undertaken. Repeated unilateral tissue sampling was performed in all but one subject who had bilateral sampling. Tissues were analyzed for the presence of olfactory neuroepithelium by histological or immunohistochemical techniques and were investigated by electron microscopy.

Repeat psychophysical evaluations were performed 6 or more weeks after the biopsies were taken. Most patients were followed with serial olfactory testing. Unilateral UPSIT scores were obtained by occluding one naris with impermeable tape and administering one half of the 40 test items to the opposite naris. Since these data are not part of the routine analysis of all study subjects examined at the University of Pennsylvania Smell & Taste Center, they are available only on a subset of subjects prior to their olfactory biopsy. Either bilateral or unilateral PEA threshold testing was performed in all subjects after tissue sampling. Olfactory threshold testing is measured on a logarithmic scale using negative log values. Therefore, the lower the negative integer, the more sensitive the subject is to the odor.

Multivariate analysis of variance was used for data analysis. Data analysis included a comparison between bilateral UPSIT scores obtained before and after biopsies within each group. Since multiple test intervals were available from the longitudinal parent project, postbiopsy psychophysical tests separated from the prebiopsy tests by the greatest possible interval of time were compared in order to maximize possible differences. Postbiopsy, unilateral UPSIT test scores from the biopsy side were compared to data from the contralateral side. Also, bilateral olfactory threshold scores from prebiopsy and postbiopsy test dates were compared.

RESULTS

Six subjects were considered to have poor olfactory function prior to biopsy (i.e., bilateral UPSIT score < 25). The number of tissue samples obtained from each subject averaged 2.8 with a range of 2 to 5. Tissue analysis has been completed on 36 (69%) of 52 specimens sampled from 18 of 19 subjects. The site of tissue sampling is known for 15 subjects. Olfactory mucosal specimens were documented to contain neuroepithelium by electron microscopy (EM) or immunohistochemistry in 21 (58%) of 36 specimens and in 16 (89%) of 18 subjects who have been evaluated. An intermediate voltage electron micrograph (IVEM) of a “typical” biopsy of olfactory epithelium from a patient with Parkinson’s disease is shown in Figure 1. Until recently, one of the study subjects had been improperly diagnosed with Alzheimer’s disease and is now known to have Pick’s disease. Thus, this study subject is included in the data analysis as an Alzheimer’s subject.

The postbiopsy psychophysical test results separated from the prebiopsy tests by the greatest interval of time are compared in Table I. The mean length of this interval is 11 months (range 1.5 to 18 months). Among each of the three study groups, no statistical differences were found in prebiopsy or postbiopsy bilateral UPSIT scores. Similar results were found in
TABLE I.
Analysis of Means: Psychophysical Tests Separated by the Longest Time Interval.

<table>
<thead>
<tr>
<th></th>
<th>Bilateral UPSIT (Max = 40)</th>
<th>Bilateral PEA</th>
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<tbody>
<tr>
<td></td>
<td>Pre Bx</td>
<td>Post Bx</td>
</tr>
<tr>
<td>CON (N = 5)</td>
<td>37.6 ± 2.3</td>
<td>37.6 ± 2.2</td>
</tr>
<tr>
<td>PD (N = 7)</td>
<td>27.7 ± 5.5</td>
<td>25.4 ± 3.7</td>
</tr>
<tr>
<td>AD (N = 4)</td>
<td>20.0 ± 5.6</td>
<td>20.0 ± 5.9</td>
</tr>
<tr>
<td>Dementia (N = 11)</td>
<td>24.9 ± 6.5</td>
<td>23.5 ± 5.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Unilateral Post Bx UPSIT (Max = 20)</th>
<th>Unilateral Post Bx PEA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bx Side</td>
<td>Non-Bx Side</td>
</tr>
<tr>
<td>CON (N = 7)</td>
<td>17.7 ± 1.1</td>
<td>17.6 ± 1.40</td>
</tr>
<tr>
<td>PD (N = 4)</td>
<td>14.5 ± 2.4</td>
<td>12.8 ± 0.96</td>
</tr>
<tr>
<td>AD</td>
<td>N = 2</td>
<td>N = 2</td>
</tr>
<tr>
<td>Dementia (N = 6)</td>
<td>12.8 ± 3.5</td>
<td>10.0 ± 4.3</td>
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the prebiopsy and postbiopsy bilateral PEA scores. Comparisons between the postbiopsy unilateral UPSIT scores from the biopsy side and the nonbiopsy side showed no significant difference. Comparisons between the postbiopsy PEA scores from the biopsy side and the control nonbiopsy side also showed no meaningful difference.

The best olfactory performance ratings after tissue sampling are compared to the best test scores which are available from before biopsy (Table II). This analysis was performed because, on a given day, an individual's olfactory ability might be somewhat diminished by a large number of variables. Thus, a subject's best performance could be viewed as most reflective of their truest olfactory abilities. The mean time interval separating the best prebiopsy and postbiopsy bilateral UPSIT scores is 8.7 months (range 3 to 26 months). The same mean interval separating the best prebiopsy and postbiopsy bilateral PEA values is 10.6 months (range 2 to 26 months). The best paired unilateral UPSIT and PEA scores was defined as the pair of right and left unilateral scores obtained on the same day with their highest combined score (e.g., r = 14 1 = 20, where combined score is 34). Although no statistical differences are found within groups, some variation between individual test scores in a given subject is seen (Tables I and II).

DISCUSSION

This retrospective analysis sought to determine if discernible changes in olfactory function occur following olfactory tissue sampling. No changes were found. Small differences seen in the psychophysical test results may be related to numerous factors, such as normal test variability or change in olfactory ability as a function of progression of aging or disease (i.e., Alzheimer's and Parkinson's disease).

Daily variation in the ability to smell results from a number of factors (e.g., humidity, nasal cycle, cigarette smoking, and feeding). Thus, individual test scores which are depressed on one occasion might reflect these features and not the study subject's true baseline olfactory function. Olfactory testing with the UPSIT, a microencapsulated odor identification test,
uses a forced choice system. This means that regardless of whether a subject is able to identify an odor, he/she is asked to choose one of four choices for each odor presented. Patients with poorer olfaction ultimately resort to guessing answers on the UPSIT. The variation in performance on the UPSIT for a given individual is increased with worsening scores. Thus, subjects with higher scores on the UPSIT will tend to have less variability in their scores on repeat testing than those who are tested with lower test scores. Therefore, control subjects were observed to have the least variability of this test score.

Another factor which could impart an effect on data interpretation is the temporal relationship between the biopsy date and postbiopsy olfactory evaluations. Psychophysical testing performed prior to 6 weeks after the tissue sampling procedure could reflect local mucosal edema and wound healing within the olfactory cleft. Moreover, elderly study subjects or those in this study with neurodegenerative disorders might be expected to have a decrement in their UPSIT or PEA scores as a function of the time interval between psychophysical tests. In some cases, over 1½ years had lapsed from the biopsy date to the time of repeat testing.

An adverse effect on olfaction which might develop as a direct result of tissue removal can potentially occur through at least four mechanisms: 1. decreasing the number of neuroepithelial cells/unit area; 2. altering the physiology associated with the microenvironment of the olfactory tissue; 3. scarring across the aperture to the olfactory cleft, resulting in diminished odorant access; 4. direct injury to the cribriform plate.

Since human olfactory neuroepithelium is believed to have a regenerative capacity, diminished olfaction secondary to a decreased olfactory receptor cell density after tissue sampling is not a likely occurrence. Moreover, the actual size of a unilateral olfactory neuroepithelial bed is believed to range from 1 cm² to 2.57 cm². Therefore, even if the epithelial bed did not exhibit periodic cell turnover, and if 10 mm² of olfactory neuroepithelium were removed (equivalent to approximately 5 biopsy specimens containing neuroepithelium), less than 5% of the total (bilateral) receptor cells would be disrupted, hypothetically.

Damage to the olfactory bed could also theoretically result during the healing process if respiratory epithelial metaplasia occurs, as has been described. The respiratory metaplasia which might appear could be associated with physiological disruption of the microenvironment and result in aberrant or diminished odorant processing. Such alterations might affect more of the receptor cells than the actual number of those removed.

Scarring which spans the olfactory cleft can occur if trauma is inflicted on two opposing surfaces of the region. Therefore, regardless of whether or not neuroepithelium is removed, odorant access to the olfactory cleft could be blocked. The likelihood of this is small since most techniques used for tissue removal do not promote this kind of disruption. The least likely event leading to decrement in olfaction would be direct trauma to the cribriform plate.

CONCLUSION

Histological analysis of olfactory neuroepithelium from living subjects continues to advance our understanding of human olfaction in health and disease. Background information suggests that decrement in olfaction after olfactory cleft tissue sampling is possible, but unlikely. Analysis of these data finds no discernible changes which occur in human olfactory function after tissue sampling in either healthy individuals or in those with a preexisting diminished sense of smell. However, additional studies are needed with larger sample sizes and unilateral testing to definitively address this issue.

BIBLIOGRAPHY


Lanza, et al.: Human Olfactory Biopsy