NeuroSPECT Evaluation Using Neurochemical Stimulation Of The Patients Complaining Of Post-traumatic Anosmia

Authors

Mohammad Eftekharī a
Mohsen Saghari a
Majid Assadi a
Majid Kazemi b
Armaghan Fard Esfahani a
Babak Fallahi Sichani a
Ali Gholamrezanezhad a
Jalal Mehdizadeh b
Mohammad Sadeghi-Hasanabadi b
Mohsen Naraghi b

a. MD, Nuclear Medicine Research Center. Tehran University of medical sciences.
b. MD, Department of Otorhinolaryngology. Tehran University of medical sciences.
Address: Shariati hospital. Northern Kargar St. 14114 Tehran – Iran.

Corresponding author: Majid Assadi
Address: Research Institute for Nuclear Medicine, Tehran University of Medical Sciences, Shariati hospital, Northern Kargar St. 14114 Tehran- Iran.
Phone: ++98-21-8633333
Fax: ++98-21-8026905
E-mail address: assadipoya@yahoo.com
Abstract

Background: Most olfactory testings are subjective and since they depend upon the patients’ reactions, are prone to have false positive results. We decided to use quantitative brain perfusion SPECT in order to detect possible areas of brain activation in response to odorant stimulation in patients with post-traumatic anosmias and comparing them with a group of normal subjects. Patients and Methods: Fourteen patients with post-traumatic anosmia and ten healthy controls, were entered in this prospective study. All subjects underwent Brain SPECT after intravenous injection of 740-MBq $^{99m}$Tc-ECD and 48 hours later, the same procedure was repeated while vanilla powder stimulus was delivered. Results: In most of the seven regions of interest (Orbital Frontal Cortex, Inferior Frontal Pole, Superior Frontal Pole, Posterior Superior Frontal Lobe, Parasagittal Area, Occipital Pole, cerebellar area) the post-stimulation quantitative values show significantly higher increase of the pre-stimulation values in the normal volunteers than the anosmic patients (except cerebellar areas and the right occipital pole). Conclusion: Brain SPECT is a valuable imaging technique in the assessment of post-traumatic anosmias and could be considered as an alternative and competitive
for other imaging techniques. SPECT is specially a useful alternative when functional MRI is unavailable or unsuitable.
Key Words:

Brain SPECT, Post-traumatic Anosmia, $^{99m}$Technetium-ECD, Olfaction.


Background

Although olfaction is the primal sense in animals, it has also an important role in the human life. Loss of this unique sensation could be extremely unpleasant and also it can be associated with deterioration of communicational functions of the patients\textsuperscript{1, 2}. In fact, disorders of the sense of smell can be frustrating for both the patient and physician\textsuperscript{3}. Quality of life studies have shown a general decrease in the level of satisfaction with life among those patients with continuing olfactory impairment\textsuperscript{4}. Consequently, there is a growing interest into the investigation of smell disorders in both research and clinical practice and lots of efforts have been made to provide a noninvasive tool to elucidate the underlying pathology. However, it seems that the human works in describing and objectification of the smell disorders were not efficacious enough.

The ability to accurately measure loss of olfactory function is important not only for research purposes, but also to follow progression of the disease and also for appropriate management of the patients. The most powerful tools the clinician has in the diagnosis of olfactory disorders are only the patient's history and clinical assessment\textsuperscript{5}. However, this clinical definition and measurement of smell loss have been difficult to achieve, in
part because the symptom is dependent upon patients' subjective complaints and in part because techniques used to demonstrate smell loss are based upon psychophysical measurements.  

Most objective testings rely on measuring detection thresholds of a specific odorant and/or by measuring the ability to identify odorants by the patients. Although it has been noted that these tests are capable of estimating the various levels of decreased sense of smell and are also somewhat able to identify malingerers, but all of these methods have major limitations, which are widely acknowledged. One of the main problems that stand in the way of analyzing olfactory disorders at present is that the majority of the methods still current are largely subjective and depend upon the patients’ reactions. It is on this account that many of the tests that have been conducted in this field do not carry much conviction as they are not fit for quantification of these disorders. There is a strong likelihood that the patient is out to deceive the analyst by pretending malingering; this so in the case of the existence or nonexistence of post-traumatic anosmia, which is a frequent complication of head injury. This olfactory dysfunction following trauma is currently compensable according to existing American Medical Association guidelines and therefore either from the legal point of view or for planning an appropriate the medical management, differentiating real
anosmias from the affections of the patients is of unique importance. Unfortunately, up to now all methods which have been devised for differentiating the real anosmias from the affected ones have major limitations, were not completely reliable and in the case of electrical olfactory evoked potentials, olfactometers and electroencephalograms were restricted to research centers and are nonpractical for general use\textsuperscript{3,9}. As a natural consequence it becomes extremely difficult to hit upon a decision in such cases.

In a review of the literature it can be found that only one study has evaluated the brain single photon emmission tomography (SPECT) findings in patients affected by post-traumatic anosmia\textsuperscript{12} and still information about the efficacy of this technique is extremely scarce. The results of this only study is partially confirmed in another study using positron emmision tomography (PET) in patients complaining of post-traumatic anosmias, but it was emphasized that further work should be undertaken to evaluate the role of SPECT and PET functional imagings as screening tools for the evaluation of anosmias. Of the different and currently-available imaging techniques in the study of the olfactory system, functional magnetic resonance imaging (fMRI) has been more encouraging and is able to detect areas of brain activity in response to odorant presentation in more detail than older
methods\textsuperscript{3, 10, 11}. However, its use is still limited to research centers. Therefore, we decided to use a similar rational by using SPECT in order to detect possible areas of brain activity in response to odorant presentation and to evaluate the potential of SPECT imaging in the differentiating real anosmias from the malinjered ones by investigating the quantitative SPECT findings in patients with post-traumatic anosmias and also comparing them with a group of normal subjects.
**Patients and Methods**

Between January 2004 and January 2005 fourteen anosmic patients (8 men and 6 women) and ten healthy control volunteers (6 men and 4 women), all right handed, were entered in this prospective study. Each participant was healthy, not taking any medication, was able to breathe normally in each naris, and had no subjective nasal pathology. The patients were selected from a population of patients who were referred to our otolaryngology department for the evaluation and treatment of their post-traumatic anosmia. The study was explained and only fourteen patients accepted to participate. All the participants had a history of mild to moderate head injuries that resulted in post-traumatic anosmia. The time interval between the SPECT examination and the traumatic insult was 3 to 8 years (mean time interval, 4.6 years). None of the patients had history of olfactory diseases or invasive therapeutic interventions on brain or nose before or after the traumatic insult. The control volunteers had no history of mental disorders and head trauma.

The diagnosis of anosmia in the patients was confirmed based on the olfactory stimulation testing that indicated that the subjects were completely unable to identify common odors. Also the presence of normal olfactory perception in the control group was assessed by the same method. All
subjects were free of remarkable mental disorders, as it was confirmed by a psychiatric interview at the time of recruitment.

All patients gave informed consent to participate in this study, which was approved by the committee on ethics at the faculty of medicine, university of Tehran.

Brain scintigraphy

A commercial ECD preparation was used. The labeling and quality control procedures were performed according to the manufacturer’s instructions.

All subjects had an intravenous line established while they were lying down, with their eyes close and ears unplugged, in a quiet darkened room with low ambient sound and light. After approximately 30 min, each subject received a 740-MBq intravenous injection of tracer while they were still lying down in the same quiet darkened room.

One hour after IV injection of 750 MBq (20 mCi) $^{99m}$Tc –ECD in a room with low level of ambient light and minimal background noise, SPECT scanning was performed. Scans were performed on a dual head ADAC camera, equipped with a pair of low energy, high resolution collimators. The full-width at half maximum (FWHM) of this system, as measured in-house, was 12-mm for $^{99m}$Tc. Standard head positioning was based on uniform alignment of the external auditory meatus using
automated table positioning and camera-to-head-detector ratio values. The total acquisition time was 35 minutes for each Study. Images were acquired in a 64 x 64 X 64 three-dimensional pixel matrix at 64 steps, 30 s each step. Data were scatter corrected using the commercially available Triple Energy Window method. Then the data were processed by back projection and filtered by Butterworth filter, using a Nyquist frequency cutoff of 0.5 and order of 5. Images were reconstructed and displayed in all three orthogonal planes.

48 hour later, the same procedure with all of the above-mentioned steps was repeated while vanilla powder stimulus was delivered in both nostrils during normal breathing.

**Statistical Evaluation**

The method of SPECT images analysis was similar to that of Varney and Bushnell\(^\text{12}\). Using a sagittal cut that bisected the frontal lobes at approximately level of the olfactory nerve at the anterior end and the level of the occipital pole at the posterior end, seven regions of interest (ROI) were drawn for quantitative analysis:

1. Orbital Frontal Cortex.
2. Inferior Frontal Pole.
5. The Parasagittal Area.

6. The Occipital Pole.

7. The cerebellar area.

The mean activity in each of the seven ROIs of either hemispheres was calculated and therefor, fourteen uptake indexes were obtained for each person (Fig 1.). We evaluate the post-stimulation values of each segment as a fraction of the corresponding pre-stimulation values: 

\[
\frac{\text{post-stimulation count} - \text{pre-stimulation count}}{\text{pre-stimulation}} \times 100.
\]

The same method was applied for the post-stimulation images.

**Statistical Analysis**

Statistical analysis was performed using Variance Analysis (ANOVA) and student’s \( t \)-test for paired data and comparison of demographic data. SPSS for Windows (Release 11.5.0) was used for statistical analysis. A probability of less than 0.05 was regarded significant.
**Results**

The groups were comparable with regard to demographic data. Mean age was 37 (18–56) yr in the anosmic patients and 33 (22–42) yr in the normal control group.

Quantitative SPECT data for each region of anosmic and control subjects are shown in table 1. In most of the regions the mean post-stimulation values, which is expressed as the percentage of the pre-stimulation values, are significantly higher in the normal controls than the anosmic patients ($P<0.05$). However, in both right and left cerebellar regions and in the right occipital region no statistically significant increase in the post-stimulation values is noted ($P=0.05$). No statistically significant left versus right difference and no gender difference in the pattern of cerebral activation were identified ($P>0.05$).
Conclusion

Availability of an objective and noninvasive technique by which smell function can be readily demonstrated and quantitated is of significant medical and medicolegal importance. Efforts to establish objective techniques to measure hyposmia and to determine the existence or nonexistence of post-traumatic anosmia have included EEG, olfactory evoked responses, and magnetoencephalography, but they were without particular success. With use of brain CT and MRI, some details of CNS pathology were obtained and measurements of olfactory bulb size and other anatomical structures in the CNS olfactory system have been made in normal subjects and in patients with hyposmias and anosmias of various causes. However, these methods provided no information about functional olfactory performance. In fact, up to now, the main available method for obtaining functional information about the olfactory system is functional MRI (fMRI). Since the introduction of functional MRI (fMRI) showed promise in defining brain activation in response to visual, auditory, and somatosensory stimuli, this technique has been applied to normal and anosmic subjects using olfactory stimuli to obtain quantitative data. Although only limited functional imaging studies have involved provocation paradigms, fortunately these efforts have been promising and successful results have been reported. In a recent study done by Henkin and Levy the role of fMRI for the
defining of brain activation in response to odors in patients who never recognized odors (congenital hyposmia) was evaluated. Brain activation in response to odors was present in patients with congenital hyposmia, but the activation was significantly lower than in normal subjects and patients with acquired hyposmia. One of the most widely referenced studies is that of Levy LM et al\(^6\), in which the authors found that brain activation to three different olfactory stimuli (pyridine, menthone, amyl acetate) was lower in all nine brain sections in anosmic patients compared with normal subjects and reached statistical significance for mean activation for each odor and in six of the nine individual sections studied. Activation in patients was found in regions associated with CNS processing of olfactory stimuli in normal subjects, but activation in patients was much less, particularly in inferior frontal and cingulate gyral regions of frontal cortex and in regions of medial and posterior temporal cortex. The authors concluded that quantitative CNS changes in smell function in response to olfactory stimuli in patients with hyposmia, demonstrate a novel, objective method by which these patients can be identified. In another study done by Levy LM et al\(^10\), fMRI was obtained in 21 patients with Type I and II hyposmias. Patients with Type I hyposmia (who could detect but not recognize odors) had less activation than patients with Type II hyposmia (who could both detect and recognize odors, albeit with less than normal acuity). Both patient groups had less activation than normal volunteers. The authors described fMRI as a
simple, rapid technique that can be used in a practical clinical setting to identify patients with hyposmias and to differentiate patients with different types of olfactory loss. However, it should be noted that fMRI has some important drawbacks, which prompt us to use quantitative brain perfusion SPECT as another objective, noninvasive technique: In most regions fMRI is more expensive than SPECT and has more contraindications and scheduling difficulties.

Nuclear medicine also has been previously involved in the evaluation of olfactory perception and anosmic states, particularly post-traumatic anosmias. Nuclear medicine procedures (SPECT and PET) has been considered as functional imaging modalities by which patients with smell loss can be identified, their abnormalities quantitated, and their results compared with findings in normal subjects. In the study of Varney and Bushnell, neuroSPECT findings in patients rendered totally anosmic from head injury were investigated. The authors underscore the importance of orbital frontal hypoperfusion as a paraclinical sign of post-traumatic anosmia, particularly in patients with mild head injury who have normal computed tomography and magnetic resonance imaging scans. In the study of Varney et al, eleven patients with head injury resulting in severe anosmia and 11 controls matched for age were investigated using quantitative positron emission tomography. The study showed that posttraumatic anosmia is closely associated with cerebral perfusion abnormalities evident in cerebral PET images.
As to our knowledge, there is only a single similar study that detects possible areas of brain activity in response to odorant presentation and evaluates the potential of SPECT imaging in the determining real anosmias\textsuperscript{16}. In the study of Di Nardo et al, 15 volunteers (including 10 healthy adults and 5 patients with post-traumatic anosmia) underwent brain SPECT by HMPAO, before and after olfactory stimulation with lavender water. As to our results, variable degree of cortical activation was detected in participants. Gyrus rectus (+24.5%), orbito-frontal cortex (right +26.6%, left +25.6%), and superior temporal (right +9.9%, left +5.5%) cortical areas were remarkably activated, while only a slight perfusion increase was present in middle temporal (right +3.2%, left +2.1%) and parieto-occipital (right +0.4%, left +2%) regions. Those patients affected by posttraumatic anosmia showed a perfusion increment markedly inferior to 0.5% in every olfactory area. Similarly, our results demonstrate that patients with anosmia exhibit decreased brain activation compared with normal subjects following olfactory stimulation. These results might be expected based upon patient complaints of lack of smell\textsuperscript{6}, but they have heretofore not been widely documented by measurements that do not involve significant patient participation in the process. The results show that all regions of cerebral cortex have blood flow increment after pure first nerve (CN1) stimulation. This technique allows identification and definition of olfactory involving areas, and this makes it potentially of value to the clinician. Although the findings of Di
Namdo wee confirmed by our larger series of patients, however few minor differences between two studies are present: In our study more activation in the parietal and occipital regions was observed, that could be explained by the different radiotracer (HMPAO vs ECD) used in our study. In fact this difference could be presumably due to differences in pharmacokinetics of these two radiopharmaceuticals. As it was previously reported by Patterson et al, radiotracer activity of the parietal, occipital and superior temporal cortices were significantly lower with $^{99m}$Tc-HMPAO than $^{99m}$Tc-ECD. Although both tracers have good results in depicting the cerebral blood flow (rCBF), however as it was previously shown by Pupi et al, we believe that $^{99m}$Tc-ECD uptake is significantly more linear with regard to rCBF and thus it has less back-diffusion and better correlation to flow.

Depending on the method of analysis (fMRI, PET or SPECT), the nature and the intensity of the stimulatory odorant and the previous experience of the subjects with the odorant tested, there are different patterns of activation in different cerebral regions involving in the process of olfaction sensation. However, it seems that orbital and frontal regions are almost always activated. Behavioral evidence and imaging findings (PET and fMRI) have suggested that laterally specialized mechanisms for odor processing exist and showed that right orbitofrontal region has a main role in the olfaction processing. However, the results of this study and
that of the Di Nardo et al using SPECT did not show any statistically significant perfusion lateralization within the olfactory areas, which could be interpreted by the differences between the method of analysis\textsuperscript{16,19}.

Although it was previously researches found that women outperform men during odor identification\textsuperscript{22,23,24}, but our study reveals that the male and female have similar pattern of cerebral activation and we found no significant difference with respect to the extent and amount of the activated regions between men and women. Further investigation concerning the issue of gender effects on olfactory function seems warranted.

Similar to the fMRI technique, brain perfusion SPECT offers an objective approach, by which smell function can be assessed quantitatively without significant patient participation. Similarly, this technique offers another objective method by which differences between groups can be easily quantitated.

In conclusion it should be noted that brain SPECT is a valuable imaging technique and could be considered as an alternative and competitive for other imaging techniques (particularly fMRI) in the diagnostic management of patients complaining of post-traumatic anosmia. SPECT is specially a useful alternative when fMRI is unavailable or unsuitable and it is beneficial when more possible accuracy is desired (such as when fMRI results are either inconclusive or conflict with other clinical data). However, this approach must be addressed in larger series
of patients. Also SPECT has the ability to be even a more practical, accurate and informative modality than fMRI and therefore a direct comparison between two modalities should be performed.

**Competing Interests**

The authors(s) declare that have no competing interests.

**Author’s contributions**

ME and MS participated in the design of the study in the interpretation of the scintigraphic results. MA participated in its design and coordination, supervised the acquisition process and participated in the interpretation of the scintigraphic results and performed the statistical analysis. MK carried out the olfactory test. AFE, BFS and AG supervised the acquisition process and interpreted the scintigraphic results. JM, MSH and MN participated in the olfactory test and physical examination of patients. All authors read and approved the final manuscript.
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References


Legend

Fig 1. The regions of interest at the sagittal cut used for the quantitative analysis: 1=orbital frontal cortex, 2=inferior frontal pole, 3=superior frontal pole, 4=posteriorsuperior frontal lobe, 5=parasagittal region, 6=occipital pole, 7=cerebellum region.
Table 1. Quantitative SPECT data for each region of anosmic and control subjects.

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<th>ROI</th>
<th>Anosmic patients</th>
<th>controls</th>
<th>$P$ value</th>
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<tr>
<td>Orbitofrontal</td>
<td></td>
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<td>Right</td>
<td>7.2±38.46</td>
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<td>9.14±36.27</td>
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<td>24.06±42.98</td>
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Additional files provided with this submission:

Additional file 1: Cover letter.doc: 28Kb
http://www.biomedcentral.com/imedia/1255262962751948/sup1.DOC