An investigation of retronasal testing of olfactory function in a Turkish population

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Background: The aim of this study was: (1) to perform a preliminary study for the validation of “retronasal olfactory testing” in the Turkish population to find the best way to evaluate smell and taste disorders in Turkey; (2) to determine if cultural differences make application of the test more difficult; and (3) to determine the flavors that participants had not yet tasted by using the survey method.

Material/Methods: The study included 330 volunteers. Orthonasal olfactory function was assessed psycho-physically using the “Sniffin' Sticks” olfactory test. Retronasal olfaction was assessed using a collection of 20 available food powders applied to the oral cavity. Also, all participants filled in a questionnaire of 50 items about the flavors they had not tasted before.

Results: The mean age of the participants was 26±7.3 years. Participants were divided into 3 groups according to the “Sniffin’ Sticks” test results: anosmia, hyposmia, and normosmia groups. Differences in retronasal olfaction scores were significant among the 3 groups.

Conclusions: The retronasal olfactory test appeared to perform well, but modifications of odorized powders or granules and distracters used in the retronasal olfactory test, taking into account Turkish cultural differences, is likely to improve its performance.

MeSH Keywords: Taste • Smell • Olfaction Disorders

Abbreviations: UPSIT – University of Pennsylvania Smell Identification Test; GATA – Gulhane Military Medicine Academy; T – odor threshold score; D – odor discrimination score; I – odor identification score; TDI – sum of the scores from the subtests of “Sniffin’ Sticks” olfactory test; odor threshold score, odor discrimination score, and odor identification score

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Background

Recent research has shown that odorants travel to the olfactory receptors by 2 routes: sniffing brings odorants through the nostrils into the nasal cavity (orthonasal olfaction), and chewing and swallowing force odorants emitted by foods upward behind the palate and into the nasal cavity from the rear of the mouth (retronasal olfaction) [1–6]. Thus, the flavor of the food is perceived via the sense of the smell [7]. As a result, taste perception is known to mainly consist of the interaction of retronasal olfaction and gustatory stimuli [8].

Approximately 5% of the general population is anosmic, but about 15% have a reduced sense of smell [9–11]. A significant number of patients complain of a reduction in their sense of smell, so it becomes important to reveal the factors that can influence both orthonasal olfaction and retronasal olfaction. Despite this fact, retronasal smelling has thus far received significantly less attention than its orthonasal counterpart. There are numerous ways of assessing nasal chemosensory performance (e.g., the University of Pennsylvania Smell Identification Test, UPSIT [12], “Sniffin’ Sticks” [13,14], or measurements of event-related potentials [15]), which have been validated for various countries and populations. However, only 2 test kits – retronasal olfactory testing [16] and the Candy smell test [17] – have been developed and validated on patients in Germany for the simple assessment of retronasal olfactory testing that resembles everyday challenges to retronasal olfactory identification abilities.

The aim of this study was (1) to perform a preliminary study for validation of “retronasal olfactory testing” in the Turkish population to find the best way to evaluate smell and taste disorders in Turkey; (2) to determine if cultural differences make application of the test more difficult; and (3) to determine the flavors that participants had not yet tasted by using the survey method.

Material and Methods

This study was approved by the Clinical Research Ethics Committee of Istanbul Cerrahpasa Medical Faculty. The study was conducted according to the Declaration of Helsinki on Biomedical Research Involving Human Subjects. Informed consent was obtained from all participating subjects. The study was conducted at the otorhinolaryngology clinics of Gulhane Military Medicine Academy (GATA) Haydarpaşa Training Hospital and Istanbul Surgery Hospital and included 330 volunteers.

The volunteers were evaluated using flexible nasopharyngeal endoscopy, and subjects with no signs of nasal polyposis, nasopharyngeal pathologies, or rhinosinusitis were included in the study. Exclusion criteria included patients with malignancy, head trauma, and neurologic or psychiatric disorders (e.g., Alzheimer disorder, Parkinson disease, epilepsy, schizophrenia).

The “Sniffin’ Sticks” test and retronasal olfactory tests were performed. In addition, all volunteers were required to fill out a questionnaire.

Psychophysical testing of olfactory function was performed with the validated “Sniffin’ Sticks” test. Odorants were presented using commercially available felt-tip pens (“Sniffin’ Sticks,” Burghart GmbH, Wedel, Germany) [4,13]. For odor presentation, the pen cap was removed by the experimenter for approximately 3 seconds, and the tip of the pen was placed approximately 1.0–2.0 cm in front of the participant’s nostrils. The test consisted of 1 threshold and 2 suprathreshold subtests: tests for olfactory thresholds of n-butanol, odor discrimination (16 triplets with 2 different odors), and odor identification (16 common odors presented in a 4-alternative forced-choice procedure). The maximum score for each subtest was 16, resulting in a maximum composite score of 48 (threshold, discrimination, and identification [TDI] score) [18]. Normal values for the TDI composite score are >30.5, with a cut-off between anosmia and hyposmia at 16.5 [19]. According to the TDI scores, the participants were diagnosed as anosmic, hyposmic, or normosmic.

For retronasal olfactory testing, a standardized, validated test was used [16]. The test includes 20 items and is based on the identification of odorized powders or granules presented to the oral cavity (Table 1). The substances were applied to the midline of the tongue with fenestrated plastic sticks. Participants were free to sample as much stimulant as necessary for identification. This approach also minimized the problem of standardizing the area of stimulation and differences in tongue or oral cavity sizes. In a typical trial, the experimenter placed approximately 0.05 g on the middle of the tongue inside the oral cavity. After the administration of each powder, participants rinsed their mouths with tap water. The procedure was self-paced. Each substance was identified by means of a closed set with 4 verbal items using a forced-choice procedure. The test result was a sum score of the correctly identified stimuli.

All participants completed a questionnaire of 50 items about the flavors they had not tasted before. The questionnaire included all flavors used in the retronasal olfactory testing as target and distractor items (Table 1).

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS), version 21.0 (SPSS Inc., Chicago, IL). The normal distribution of considered variables was first evaluated using the Shapiro-Wilk test. Data are shown as mean ± standard deviation for continuous variables and the number
of cases was used for categorical ones. Subjects were divided into 3 groups according to the Sniffin’ Sticks score (normosmia, hyposmia, and anosmia). In addition, to explore the retronasal olfactory sensitivity in relation to groups, age, gender, cigarette smoking, and alcohol consumption, data were submitted to variance of analysis using the general linear model with Bonferroni post hoc tests. Correlational analyses were calculated according to Pearson. The level of significance was set at 0.05.

Results

The study was carried out on 330 subjects, and the mean age of the participants was 26±7.3 years, ranging from 19 to 53 years. The participants were divided into 3 groups according to “Sniffin’ Sticks” scores: normosmia, hyposmia, and anosmia. There were no differences between the groups in terms of age, gender, and alcohol consumption. There were statistically

| Target item | Distracter items | Brand name (distributor name and city location) |
|-------------|-----------------|-----------------------------------------------|
| Ginger      | Mustard, paprika, curry | Zencefil (Doğasal®, Ankara) |
| Grapefruit  | Lemon, sour cherry, red currant | Grapefruit (Firmenich®, İstanbul) |
| Bread       | Sauerkrout, pizza, garlic | Rusk bread (Etimek®, Bilecik) |
| Milk        | Vanilla, banana, coconut | Süttözu (Pınar®, İstanbul) |
| Strawberry  | Apple, red currant, tangerine | Çilek (Ori®, İstanbul) |
| Vanilla     | Cherry, banana, honey | Vanilin (Dr.Oetker®, İzmir) |
| Orange      | Raspberry, strawberry, cherry | Portakal (Ori®, İstanbul) |
| Onion       | Chives, salami, smoked ham | Soğan (Arifoğlu®, İstanbul) |
| Cocoa       | Caramel, muscat, juniper | Kakao (Dr.Oetker®, İzmir) |
| Celery      | Chives, parsley, carrots | Kereviz kök granül (Kurucum®, Isparta) |
| Mushrooms   | Bread, fish, white wine | Mushrooms (Firmenich®, İstanbul) |
| Paprika     | Ginger, curry, mustard | Karabiber (Bağdat®, Ankara) |
| Coffee      | Cinnamon, muscat, cocoa | Türk Kahvesi (Ülker®, İzmir) |
| Smoked ham  | Fish, bread, chives | Smoked ham (Firmenich®, İstanbul) |
| Cloves      | Anise, caraway, dill | Karanfil (Arifoğlu®, İstanbul) |
| Garlic      | Ham, chives, celery | Sanmsak Granül (Bağdat®, Ankara) |
| Muscat      | Cinnamon, coffee, cocoa | Muscat (Firmenich®, İstanbul) |
| Curry       | Mustard, cheese, cucumber | Küri (Bağdat®, Ankara) |
| Cinnamon    | Honey, caramel, cocoa | Tarçın (Doğasal®, Ankara) |
| Raspberry   | Peach, pineapple, white grapes | Raspberry (Firmenich®, İstanbul) |

Table 1. Odorized powder or granules used for retronasal olfactory testing.

| Characteristics | Normosmia | Hyposmia | Anosmia | p value |
|----------------|-----------|----------|---------|---------|
| Age            | 26.4±6.9  | 26.6±8.7 | 25.8±8.6 | 0.88    |
| Male gender/N  | 216/261   | 27/33    | 33/36   | 0.38    |
| Smoking (%)    | 107 (41%) | 18 (55%) | 30 (83%) | <0.001  |
| Alcohol usage (%) | 46 (18%) | 5 (15%) | 9 (25%) | 0.51    |
| Retronasal olfactory test scores (mean ±SD) | 16.8±1.7 | 12.8±1.8 | 9.3±1.7 | <0.001 |

Table 2. Characteristics of three groups.
significant differences in terms of cigarette smoking between the normosmia and anosmia groups.

Table 2 gives the summary of characteristics of each of the variables of interest by disease status. Significant differences in retronasal olfaction scores were detected among the anosmia, hyposmia, and normosmia groups with Bonferroni post hoc tests (p<0.002). There was a strong significant correlation between the TDI scores and retronasal olfactory test scores (p<0.001, r=0.82) (Figure 1). In addition, subtests of “Sniffin’ Sticks” (odor threshold, odor discrimination, and odor identification) had a significant correlation with retronasal olfactory testing (p<0.001, r=0.7; p<0.001, r=0.8; and p<0.001, r=0.78, respectively).

A significant effect of cigarette smoking on TDI and retronasal olfactory scores was found by the linear regression model, which was an inverse correlation between smoking with TDI and retronasal olfactory scores (p<0.001, r=0.25; p<0.001, r=0.27, respectively).

Retronasal olfactory testing allowed for discriminating between normosmic, hyposmic, and anosmic subjects. Specifically, there was almost no overlap between scores of anosmic (score of 11 at 95th percentile) and normosmic subjects (score of 14 at 5th percentile), whereas scores of hyposmic subjects exhibited overlap between both normosmic and anosmic subjects (Table 3).

In the normosmia group, some odorized powders or granules were consistently correctly identified while others less so (Table 4). Bread, strawberry, orange, onion, cocoa, paprika, coffee, and garlic were the odorized granules most reliably correctly identified, while smoked ham, ginger, curry, raspberry, and celery were most commonly mistaken. In the anosmia and hyposmia groups, as expected, a larger proportion of subjects wrongly identified odorants. Looking at the 2 groups together, smoked ham, ginger, celery, curry, and raspberry were the least well-recognized odorized granules from the 20 presented.

In addition, when the results of the “Sniffin’ Sticks” test were evaluated, it was found that turpentine, apple, leather, lemon,

Table 3. Descriptive statistics of results for Retronasal olfactory testing.

| Percentile | Normosmia | Hyposmia | Anosmia |
|------------|-----------|----------|---------|
| Mean ±SD   | 16.8±1.7  | 12.8±1.8 | 9.3±1.7 |
| Minimum score | 12        | 3        | 2       |
| Maximum score | 20        | 15       | 11      |

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and liquorice were the most commonly mistaken odorants in the normosmia group. In the hyposmia and anosmia groups, as expected, a larger proportion of subjects wrongly identified odorants. Considering the 2 groups together, apple and turpentine were these least well-recognized odors from the 16 presented.

Considering the results of the survey, we found that at least half of our participants had no idea about the tastes of smoked ham, juniper, curry, ham, anise, and white wine (Figure 2).

**Discussion**

Retronasal olfactory testing is an important tool in the clinical assessment of taste and smell disorders. However, cultural differences make the use of odor or flavor identification tests more difficult in different countries, as was seen during validation studies of the "Sniffin’ Sticks" tests before, because it was strongly dependent on familiarity with these specific odors and flavors [20–22]. When applied to our population, the retronasal olfactory test appeared to perform well and there was a strong significant correlation between “Sniffin’ Sticks” olfactory test results and retronasal olfactory test results. However, when evaluating the descriptive statistics of the retronasal testing, we detected that the scores of hyposmic subjects exhibited overlap between both normosmic and anosmic subjects. Thus, we thought that retronasal olfactory test required some adaptations, especially in using different odorized powders or granules, to improve the performance of the test in differentiating between the patients with normosmia, hyposmia, and anosmia.

In our population, the odorized granules or powders most commonly mistaken by subjects with normal olfactory function were ginger (66%), smoked ham (53%), curry (41%), raspberry (38%), and celery (32%). For the subjects with anosmia and hyposmia, the most commonly mistaken odorized granules or powders were raspberry (98%), curry (81%), celery (80%), ginger (90%), smoked ham (73%), grapefruit, and

| Target item | Normosmia | Hyposmia | Anosmia |
|-------------|-----------|----------|---------|
| Ginger      | 34        | 21       | 0       |
| Grapefruit  | 72        | 39       | 19      |
| Bread       | 98        | 94       | 78      |
| Milk        | 86        | 42       | 44      |
| Strawberry  | 98        | 82       | 47      |
| Vanilla     | 83        | 82       | 47      |
| Orange      | 100       | 100      | 64      |
| Onion       | 96        | 91       | 72      |
| Cocoa       | 99        | 91       | 80      |
| Celery      | 68        | 33       | 6       |
| Mushrooms   | 85        | 42       | 14      |
| Paprika     | 100       | 100      | 94      |
| Coffee      | 100       | 100      | 78      |
| Smoked ham  | 47        | 39       | 14      |
| Cloves      | 93        | 79       | 61      |
| Garlic      | 97        | 100      | 86      |
| Muscat      | 100       | 94       | 55      |
| Curry       | 59        | 24       | 14      |
| Cinnamon    | 95        | 67       | 59      |
| Raspberry   | 62        | 0        | 6       |

Table 4. Percentage (%) of correctly identified items used for retronasal olfactory testing.
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Table 5. Modified list of odorized granules and distracters proposed for the identification component of retronasal olfactory test (The proposed flavours and distracters are in italic, the odorant is in bold).

| 1 | Ginger (thyme) | Mustard | Paprika | Curry |
| 2 | Grapefruit | Lemon (peach) | Sour cherry | Red currant |
| 3 | Bread | Sauerkraut | Pizza | Garlic |
| 4 | Milk | Vanilla | Banana | Coconut |
| 5 | Strawberry | Apple | Red currant | Tangerine |
| 6 | Vanilla | Cherry | Banana | Honey |
| 7 | Orange | Raspberry | Strawberry | Cherry |
| 8 | Onion | Chives | Salami | Smoked Ham (ham) |
| 9 | Cocoa | Caramel | Muscat | Juniper (vanilla) |
| 10 | Celery (cumin) | Chives | Parsley | Carrot |
| 11 | Mushroom | Bread | Fish | White wine |
| 12 | Paprika | Ginger | Curry | Mustard |
| 13 | Coffee | Cinnamon | Muscat | Cocoa |
| 14 | Smoked ham (sausage) | Fish | Bread | Chives |
| 15 | Cloves | Anise | Caraway | Dill |
| 16 | Garlic | Ham | Chives | Celery |
| 17 | Muscat | Cinnamon | Coffee | Cocoa |
| 18 | Curry (sesame) | Mustard | Cheese | Cucumber |
| 19 | Cinnamon | Honey | Caramel | Cocoa |
| 20 | Raspberry (banana) | Peach | Pine apple | White grape |

mushroom (both 70%). A possible explanation for these results could be the similarity of distracters with the odorized powders or granules, as in the case of grapefruit (distracters are lemon, cherry, and grapes) and mushroom (distracters are bread, fish, and white wine).

Also, according to our survey, at least half of our population was unfamiliar with odorized powders or granules that are used in the retronasal olfactory testing, such as raspberry, curry, celery, ginger, and smoked ham. Ginger is widely used with honey because of ginger’s bitter taste, and that is why...
our population was not accustomed to its original taste. The participants confused its taste with paprika. Raspberry, curry, and celery are not used widely in any regions of Turkey. Raspberry was most often was confused with white grapes, curry with mustard, and celery with chives. In addition, smoked ham is not a product used in traditional Turkish kitchens. Most of the participants might have understood that it was a taste of meat, but the options offered in the test were not familiar, so they answered that they had no idea about the smoked ham item.

To overcome these problems, we propose a modification of the original list of flavors. According to Hummel et al., one of the criteria for the selection of odorants in healthy subjects was the >75% successful identification of individual odorants from a list of 4 descriptors [13]. In our study, we found that ginger, smoked ham, curry, raspberry, and celery were not sufficiently well-known in healthy Turkish subjects (Table 4). Therefore, we will use thyme, cumin, sausage, sesame, and banana, which are items frequently consumed in our society, in place of ginger, celery, smoked ham, curry, and raspberry, respectively (Table 5). Also, we propose a modification of the original list of distracters by excluding the unfamiliar ones to achieve more accurate results. We plan to evaluate the results of these modifications (Table 5) in the test in a further study testing retronasal olfactory function in both normal subjects and patients with an impaired sense of smell.

Our study has several limitations. One limitation is obviously the low number of female participants. It should be better to test both genders in a more representative numbers. Also, it would be better to show the test-re-test quality of the retronasal olfactory testing and the lack of test-re-test reliability is another limitation of the study. Thus, further studies with more participants and including both genders and all age groups are needed for presenting the retronasal olfactory function and flavor familiarity of the whole Turkish population.

Conclusions

These results provide the basis for routine clinical evaluation of patients with olfactory disorders using retronasal olfactory testing, which is a simple and easy-to-perform test of the retronasal olfaction in our sample of the Turkish population. Modifications of odorized powders or granules and distracters of the test, taking into account cultural differences, are likely to improve the performance of the test.

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