Olfactory Function Assessment: Standardization of a New Quantitative Technique for the Indian Population

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Authors’ contributions

This work was carried out in collaboration of all authors. Author NJJ designed the study, wrote the protocol and wrote the first manuscript. Authors DR, MS and JKM supervised the study and managed the analyses of the study. Author VV assisted in data collection and managed literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aim: Olfactory function assessment is often neglected in clinical settings due to a lack of appropriate cost effective techniques. We therefore aimed to develop a cost effective, reliable and culturally appropriate tool for olfactory function assessment among the Indian population and to compare olfactory functions among 63 healthy controls and 32 idiopathic Parkinson’s disease patients.

Materials and Methods: Olfactory stimuli were applied to the nostrils of the participants using an olfactometer. Five different odoriferous substances common to Indian culture were used for the study in three different concentrations: ginger (1%, 2%, 3%), cardamom (0.4%, 2%, 3%), garlic (0.8%, 1.4%, 2%), coffee (1.6%, 2%, 4%), vanilla (2%, 3%, 4%). Olfactory recognition threshold,
olfactory identification score and olfactory discrimination score were observed among the control population and Parkinson’s disease population.

**Results:** The olfactory recognition threshold was significantly high among the Parkinson’s disease group compared to controls (Mann Whitney U test, p<0.001). Reliability was tested using the test-retest method among the control group and all olfactory variables in three different concentrations had either r value closer to 1 or 1, which shows an acceptable level of reliability. The correlation was found to be significant (p<0.001). A receiver operating characteristic (ROC) curve drawn for olfactory recognition thresholds at different concentrations for the five odouriferous substances and the area under the curve was found to be significant (p<0.001). A receiver operating characteristic (ROC) curve drawn for olfactory recognition thresholds at different concentrations for the five odouriferous substances and the area under the curve was found to be significant in classifying the cases and the control.

**Conclusion:** The newly developed olfactory assessment tool was found to be reliable and effective in assessing olfactory parameters like recognition threshold, identification score and discrimination score among the Indian population.

**Keywords:** Olfaction; Parkinson’s disease; Pressure-Olfactometer.

1. INTRODUCTION

Olfactory function has an important role in determining the quality of life as it is involved with emotions, memories and food preferences. Human beings are capable of differentiating multicomponent mixtures of odorants which exceeds much more than the visual and auditory resolution abilities [1,2]. But olfactory dysfunction is often neglected in clinical settings when compared to the visual defects and hearing defects. Nowadays, olfactory dysfunction has come under the spotlight as it is reported that it may precede many neurodegenerative disorders and can be used in the differential diagnosis of some diseases exhibiting common motor manifestations like idiopathic Parkinson’s disease and atypical Parkinson’s plus syndromes [3]. In Parkinson’s disease, olfactory dysfunction is reported much before the occurrence of motor symptoms [4,5,6]. Loss of smell and taste has also been widely reported as a clinical symptom of COVID-19 infection. Compromised olfactory function also serves as strong basis for identifying asymptomatic COVID-19 carriers [7].

New research suggests that marked olfactory dysfunction is now observed in Myasthenia Gravis, which was considered to be a peripheral disease of the cholinergic motor endplate, indicating a central nervous system component in disease pathology [8]. It is also reported that olfactory impairment can predict mortality in old age people, as mortality positively correlates with an increase in olfactory impairment [9].

The most commonly used tests for olfactory assessment worldwide are the University of Pennsylvania Smell Identification Test (UPSIT), the Sniffin’s sticks test and the 12-Odor brief smell identification test (B-test), which uses odours unfamiliar to Indian culture and makes the odour identification test more difficult for the Indian population. The use of these tests is also limited among Indians due to the high cost, unavailability of the test kits [10]. Thus there is a lack of structured, validated and culturally appropriate tools to assess olfactory functions among the Indian population.

The present study aims to evaluate the effectiveness of using a pressure-olfactometer, with five different odours that are common to Indian population: ginger, cardamom, garlic, coffee, and vanilla in three different concentrations. The proposed olfactory assessment method is cost-effective, reliable and comfortable to perform in out-patient departments.

2. MATERIALS AND METHODS

A total of 63 healthy controls and 32 Parkinson’s disease patients were recruited for the study. All of the test procedures were explained and informed consent was obtained from the participants. For testing olfaction, ginger, cardamom, garlic, coffee and vanilla were selected as five common locally recognized, odouriferous substances common to the Indian population. Solutions of these substances were prepared in deionized water, each in three different concentrations. The concentrations selected on the basis of pilot study were as follows: ginger: 1%, 2%, 3%; cardamom: 0.4%, 2%, 3%; garlic: 0.8%, 1.4%, 2%; Coffee: 1.6%, 2%, 4% and vanilla: 2%, 3%, 4%. The test was done in a quiet closed room to prevent any other
sensory disturbances. In between each test, an interval of 30 seconds was provided to prevent olfactory desensitization.

**Pressure-olfactometer:** The olfactory stimuli were separately and individually applied to the nostrils of the participants using an olfactometer by the blast injection method [11]. The olfactometer consists of a bottle with a tight rubber stopper, equipped with a sterilizable inlet and outlet tubes. The test used 15 similar bottles with five different odoriferous solutions in three different concentrations. Both inlet and outlet tubes were closed using pinch clamps. The air in the bottle becomes saturated with vapour from the odorous liquid in the bottom of the bottle. A specific volume of air was injected through the inlet tube into the bottles by the examiner, which causes the release of a jet of odorous vapours through the outlet tube into the nostrils of the participants when the pinch clamp was released. The following parameters were observed.

**Olfactory recognition threshold:** This is designated as the minimum pressure of the odorous vapours, which is required by the participant to recognize the presence of an odour in the vapours released in to their nostrils at a particular concentration, which correlates with the volume of air injected in to the bottle through the inlet tube. It is expressed in pounds per square inch (psi). For a more accurate test, the olfactory recognition threshold was recorded at three different concentrations of a particular odoriferous solution.

**Olfactory identification score:** The participants were blindfolded and provided with five different odours at the highest concentration and maximum pressure and were asked to choose from the five choices provided. Each correct response scored one and for incorrect response or no response zero. The maximum score was five.

**Olfactory discrimination score:** Ten pairs of olfactory stimuli were presented, with five pairs of similar odours and five pairs of different odours. Each pair was then presented in random order and the participants were asked to state whether the odours were same or different. Each correct response scored one and incorrect response zero. The maximum score was ten.

### 2.1 Statistical Analysis

Statistical analysis was performed using IBM SPSS version 20.0 software. For comparative distribution of the demographic variables age and sex, the chi-square test was used. Age wise distribution and comparison of olfactory recognition thresholds at different concentrations among different age groups was done using the Kruskal wallis test. For gender-wise distribution and comparison of the olfactory recognition threshold at different concentrations among males and females, the Mann Whitney U test was used. For comparison of the olfactory recognition threshold at different concentrations, among the control group, the Friedman test was done. Reliability was assessed by the test retest method. The Pearson correlation was used for assessing correlation between two time points, where p<0.001 was considered as statistically significant. The Friedman test was used for comparison of the olfactory recognition threshold at different concentrations among the Parkinson’s disease group.

### 3. RESULTS

The age of control group varied from 23 to 75 years with a mean of 47.9±15.9 years and in the Parkinson’s disease group, the age ranged from 31 to 75 years with a mean of 57.9±10.8 years. Among total samples in the Parkinson’s disease group and control group, most of the patients were aged greater than 60 years. Twenty (58.8%) patients were males in the Parkinson’s disease group and thirty four (54.0%) patients were females in the control group (Table 1). Age was categorized into three class intervals (21-40 years, 41-60 years, 61-80 years) among the control group and olfactory recognition thresholds were assessed at three different concentrations. As the data was non-normal, the Kruskal Wallis test was used for comparison of olfactory recognition thresholds at different concentration levels, for different age class intervals. It was observed that all olfactory variables showed a trend that if age increases there will be an increase in the olfactory recognition threshold (p<0.001) (Table 2).

The distribution and comparison of olfactory recognition threshold based on gender shows an increase of this parameter in males compared to females except for cardamom (2% and 3%) and garlic (0.80% and 1.40%) (Table 3).

**Reliability Test:** The reliability test was carried out among 40 subjects. The test-retest method was used for the assessment of reliability. The Pearson correlation was used for assessing
correlation between two time points in a two week time interval. All olfactory variables in three different concentrations, had either r value closer to 1 or 1, which shows an acceptable level of reliability. The correlation was significant (p<0.001) (Table 4).

The results of the comparison of olfactory variables at different concentrations within the Parkinson’s disease group was found to be statistically significant (p value <0.001). The mean and median value of ginger was high at 1%, cardamom at 0.4%, garlic at 0.8%, coffee at 1.6% and vanilla at 2%, which indicates that as the concentration increases, the olfactory recognition threshold decreases, which proves the sensitivity of the instrument (Table 5).

The olfactory recognition threshold was significantly high among the Parkinson’s disease group compared to controls (Mann Whitney U test, p<0.001) (Fig. 1). The mean value of the olfactory identification score among the Parkinson’s disease group was 4.87±0.34 and for the control group it was 3.27±1.04. The difference was statistically significant (p<0.001).

### Table 1. Comparative distribution of demographic variables

| Variable | Group | Total | \( \chi^2 \) Value | p value |
|----------|-------|-------|---------------------|---------|
| **Age** |       |       |                     |         |
| Range    |       |       |                     |         |
| Mean± SD | Disease, n (%) | Control, n (%) | 13 | 0.017* |
| <40      | 47.9±15.9 | 57.9±10.8 | 8.057 | 0.017* |
| 40-60    | 2(6.26%) | (30.2%) | 25 |         |
| 60-80    | 15(46.87%) | 17(26.47%) | 19 |         |
| **Gender** |       |       |                     |         |
| Female   | 14(43.75%) | 34(54%) | 48 | 0.346 |
| Male     | 18(56.25%) | 29(46%) | 49 |         |

*Chi-square test, *p<0.05

### Table 2. Age wise distribution and comparison of smell assessment parameters at different pressure levels

| Variable | Concentration | Olfactory Recognition Threshold(psi), age wise distribution (Mean±sd) | Test statistic (\( \chi^2 \)) | p value |
|----------|--------------|---------------------------------------------------------------------|-------------------------------|---------|
|          | 21-40 | 41-60 | 61-80 |          |          |          |
| Ginger   | 1%    | 2.81±2.09 | 10.34±7.13 | 12.23±7.17 | 23.24 | <0.001*** |
|          | 2%    | 1.53±0.29 | 3.14±1.84 | 4.25±4.48 | 24.58 | <0.001*** |
|          | 3%    | 1.21±0.23 | 1.70±0.99 | 3.55±4.44 | 19.28 | <0.001*** |
| Cardamom | 0.4%  | 1.36±0.19 | 1.75±0.41 | 4.49±2.48 | 29.25 | <0.001*** |
|          | 2%    | 1.21±0.11 | 1.41±0.28 | 2.32±2.04 | 20.72 | <0.001*** |
|          | 3%    | 1.08±0.11 | 1.24±0.16 | 1.65±0.918 | 23.58 | <0.001*** |
| Garlic   | 0.80% | 1.78±2.01 | 1.72±0.76 | 2.36±0.70 | 17.98 | <0.001*** |
|          | 1.4%  | 1.18±0.29 | 1.48±0.78 | 1.54±0.27 | 19.48 | <0.001*** |
|          | 2%    | 1.06±0.08 | 1.22±0.25 | 1.38±0.19 | 23.58 | <0.001*** |
| Coffee   | 1.6%  | 1.27±0.16 | 1.99±1.38 | 3.46±2.04 | 34.36 | <0.001*** |
|          | 2%    | 1.12±0.12 | 1.37±0.24 | 1.82±0.88 | 32.86 | <0.001*** |
|          | 4%    | 1.06±0.09 | 1.52±1.70 | 1.39±0.32 | 28.22 | <0.001*** |
| Vanilla  | 2%    | 1.43±0.28 | 2.08±1.20 | 4.35±2.73 | 27.61 | <0.001*** |
|          | 3%    | 1.22±0.16 | 1.42±0.23 | 1.74±0.44 | 21.77 | <0.001*** |
|          | 4%    | 1.11±0.11 | 1.27±0.25 | 1.44±0.31 | 18.16 | <0.001*** |

*Kruskal Wallis test, ***p<0.001 considered as statistically significant*
Table 3. Gender wise distribution and comparison of smell assessment parameters at different pressure levels

| Variable | Concentration | Olfactory Recognition Threshold (psi) | U Statistic | p value |
|----------|---------------|---------------------------------------|-------------|---------|
|          |               | Gender wise distribution               |             |         |
|          |               | **Means±sd**                            |             |         |
|          |               | Male                                   | Female      |         |
| Ginger   | 1%            | 9.75±7.98                              | 7.59±6.31   | 407.5   | 0.231  |
|          | 2%            | 3.34±3.59                              | 2.63±1.83   | 386.0   | 0.139  |
|          | 3%            | 2.49±3.55                              | 1.68±1.01   | 352.5   | 0.051  |
| Cardamom | 0.40%         | 2.66±1.99                              | 2.13±1.69   | 367.0   | 0.081  |
|          | 2%            | 1.54±0.42                              | 1.64±1.53   | 364.0   | 0.072  |
|          | 3%            | 1.29±0.24                              | 1.31±0.69   | 320.5   | 0.016* |
| Garlic   | 0.80%         | 1.71±0.48                              | 2.09±1.67   | 470.0   | 0.750  |
|          | 1.40%         | 1.38±0.26                              | 1.43±0.74   | 364.5   | 0.075  |
|          | 2%            | 1.24±0.19                              | 1.2±0.26    | 339.5   | 0.032* |
| Coffee   | 1.6%          | 2.4±1.6                                | 1.98±1.61   | 378.0   | 0.110  |
|          | 2%            | 1.45±0.34                              | 1.38±0.68   | 340.5   | 0.034* |
|          | 4%            | 1.56±1.63                              | 1.17±0.27   | 263.5   | 0.001**|
| Vanilla  | 2%            | 2.57±1.98                              | 2.44±2.01   | 431.5   | 0.394  |
|          | 3%            | 1.48±0.37                              | 1.43±0.33   | 439.0   | 0.453  |
|          | 4%            | 1.35±0.33                              | 1.2±0.18    | 379.0   | 0.113  |

Mann Whitney U test, *p<0.05, **p<0.01, considered as statistically significant

Table 4. Reliability assessment

| Olfactory Variables | Concentration | r value | p value |
|---------------------|---------------|---------|---------|
| Ginger              | 0.01          | 0.927   | <0.001***|
|                     | 0.02          | 0.899   | <0.001***|
|                     | 0.03          | 0.935   | <0.001***|
| Cardamom            | 0.004         | 0.906   | <0.001***|
|                     | 0.02          | 1       | <0.001***|
|                     | 0.03          | 1       | <0.001***|
| Garlic              | 0.008         | 0.999   | <0.001***|
|                     | 0.014         | 0.53    | <0.001***|
|                     | 0.002         | 1       | <0.001***|
| Coffee              | 0.016         | 0.965   | <0.001***|
|                     | 0.02          | 0.999   | <0.001***|
|                     | 0.04          | 1       | <0.001***|
| Vanilla             | 0.02          | 0.995   | <0.001***|
|                     | 0.03          | 0.99    | <0.001***|
|                     | 0.04          | 1       | <0.001***|

Test retest method, Karl Pearson correlation, ***p<0.001 considered as statistically significant

A ROC curve was drawn for olfactory recognition thresholds and the area was determined to classify cases and controls. In the case of ginger, an olfactory recognition threshold of >2.110 psi, shows the chance of cases with an area under the curve as 0.928, which is significant along with 82.35% sensitivity and 84.12% specificity. Cardamom showed a significant cut off value of 1.595, with an area under the curve of 0.955 and the sensitivity was reported as 91.17% and specificity as 84.12% to predict cases. Garlic also showed 1.395 as the cut off value for Parkinson’s disease, for which the area under the curve was 0.921 and was significant. Sensitivity was 79.41% and specificity 77.78%. Coffee showed a significant cut off value of 1.475, with an area under curve of 0.950 and the sensitivity was reported as 91.17% and specificity as 84.12% to predict cases. Vanilla also showed 1.475 as the cut off value for cases, for which the area under the curve was 0.950 and was significant. Sensitivity was 91.17% and specificity 90.47% (Fig. 2). These data suggest that the olfactory recognition threshold was appropriate for classifying cases and controls.
Table 5. Comparison of olfactory recognition threshold at different concentrations, in the Parkinson’s disease group

| Variables  | Mean±SD (psi) | X ² Statistic | p value        |
|------------|--------------|---------------|----------------|
| Olfactory variables                      |              |               |                |
| Ginger     |              |               |                |
| 1%         | 20±1.4       | 39.28         | <0.001***      |
| 2%         | 14.3±6.3     |               |                |
| 3%         | 10.8±7       |               |                |
| Cardamom   |              |               |                |
| 0.40%      | 19.7±1.8     | 44.93         | <0.001***      |
| 2%         | 12.2±7.7     |               |                |
| 3%         | 8.9±7.0      |               |                |
| Garlic     |              |               |                |
| 0.80%      | 17.7±6.0     | 47.65         | <0.001***      |
| 1%         | 8.8±6.9      |               |                |
| 2%         | 6.5±6.5      |               |                |
| Coffee     |              |               |                |
| 1.60%      | 19.7±3.2     | 48.79         | <0.001***      |
| 2%         | 10.1±6.9     |               |                |
| 4%         | 7.8±6.8      |               |                |
| Vanilla    |              |               |                |
| 2%         | 19.7±3.2     | 51.23         | <0.001***      |
| 3%         | 10.7±7.7     |               |                |
| 4%         | 6.9±6.5      |               |                |

Friedman test used for comparison, ***p<0.001

Fig. 1. Boxplot of olfactory recognition thresholds at different concentrations among Parkinson’s disease group

Fig. 2. ROC-curve for olfactory recognition thresholds
4. DISCUSSION

The present study was aimed at developing a cost effective, culturally appropriate olfactory assessment tool for quantitative analysis of parameters such as olfactory recognition threshold, olfactory identification score and olfactory discrimination score among the Indian population. The use of odours that are familiar to a population will increase the identification score and prevent bias.

On analysing data regarding age and olfactory function, it was revealed that as the age increases the olfactory recognition threshold also increases, which agree with the findings of other studies. Previous studies have reported that the human olfactory epithelium shows age related changes in nature, cellular patterns, number of receptors and vascularity of the epithelium. Along with that, the size of the olfactory bulb and number of its laminae also declines with age [12], which was also proved in MRI studies [13]. Kishikawa et al reports that neurofibrillary tangles also increase in the olfactory bulb as a function of age [14]. Our data correlates with these findings.

Compared to women, men had a high olfactory recognition threshold, but the difference was statistically significant only in garlic at 2%, and in coffee at 1.6% and 2%. But for cardamom at 3% concentration, women had a high olfactory recognition threshold. The study conducted by Sorokowski et al. [15], reports that women outperform men in olfactory abilities. The reason for this female superiority might be the result of interaction between early endocrine related influences on regions of the human brain involved in odour perception and the hormonal mechanism involved in later life [15,16].

Among Parkinson’s disease patients, the olfactory recognition threshold was significantly high compared to the control group (p<0.001). The olfactory identification score and discrimination score among idiopathic Parkinson’s disease patients were significantly low compared to the control group (p<0.001). This observation is consistent with previous studies [17,18,19].

5. LIMITATIONS

In our study, the electrophysiological aspects of olfaction were not observed and also we focussed in the olfactory dysfunction among idiopathic Parkinson’s disease patients only.

Olfactory assessment in other disease conditions using the newly developed technique will be done in the next phase of the study.

6. CONCLUSION

The olfactory test developed in our study is cost effective, easily administered and reliable. It can be used in the quantitative assessment of olfactory function among the Indian population. The test can be used to identify olfactory dysfunction among the disease population in the out-patient setting.

CONSENT

Informed consent was collected from the participants involved in the study.

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee of the Little Flower Hospital and Research Centre (EC/25/2018), Angamaly, Kerala, India.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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