OLFACTION AS A DIFFERENTIATING TOOL IN IPD, FIRST DEGREE RELATIVES OF FAMILIAL PD AND PARKINSONISM

Abhishek Pathak2, Jeen Mathew3, Vinay Goyal1, Garima Shukla1, Madhuri Behari1*
1: Professor, Department of Neurology, A.I.I.M.S, New Delhi,
2: Assistant Professor, Department of Neurology, I.M.S, B.H.U
3: Nursing Research associate, Department of Neurology, A.I.I.M.S, New Delhi

ABSTRACT:
Olfactory dysfunction is considered a marker of synucleopathies and is reported in patients of Parkinson Disease (PD), multiple system atrophy (MSA) and Dementia with Lewy bodies (LBD) several years before motor symptoms make their appearance. It therefore, can be used as a biomarker to detect these ailments before motor symptoms make their appearance in the absence of more expensive tests. We undertook this case control study to investigate olfactory function in PD, Parkinsonism plus syndromes (PPS i.e. MSA and Progressive Supranuclear Palsy (PSP)), familial PD and first-degree relatives of familial PD patients and compared the results with age and gender matched healthy controls. Consecutive 197 PD patients, 26 familial PD (FPD) patients, 23 first degree relatives of familial PD patients (RPD), 58 patients of Parkinsonism plus syndrome (PPS) including 38 MSA and 20 PSP patients and 200 age and gender matched healthy controls were included in the study from movement disorder clinic of a tertiary care teaching hospital in India. Clinical details were recorded on a pre-tested format. All the subjects underwent a formal olfactory assessment with validated Indian Smell Identification Test battery (INSIT) and commercially available Sniffin stics Test® (SST). There was significant difference in smell scores between PD patients familial PD (FPD) patients and PPS patients on one hand and healthy controls on the other (all p<0.001) on both Indian smell Identification Test battery (INSIT) and commercially available Sniffin stics Test® (SST). There was significant difference in smell scores between PD patients familial PD (FPD) patients and PPS patients on one hand and healthy controls on the other (all p<0.001) on both Indian smell Identification Test battery (INSIT) and commercially available Sniffin stics Test® (SST). Both INSIT and SST showed similar results when tested on PD, PPS, FPD, RPD and healthy controls, showing an agreement of 83% between the two tests. Statistically significant difference between MSA and PSP and healthy controls were observed; but not between MSA and PD and between RPD and age matched healthy control. ROC curve showed sensitivity of 0.81 and specificity of 0.68 for INSIT at cut off score of 7 and of 6 on INSIT and SST respectively. The study confirms that INSIT, the indigenously prepared inexpensive Indian smell identification test using odorants, which are familiar to Indian population, is non-inferior to the SST and that there is a good agreement between the two. Olfactory dysfunction exists in PD, Parkinsonism plus syndromes patients, especially so in MSA and FPD patients in comparison to healthy controls. There was significant difference in the smell scores between MSA and PSP patients. We did not find significant difference between the smell scores of first-degree relatives of familial PD and healthy control.

KEY WORDS: Parkinson disease, Olfaction, Familial PD, Parkinsonism, First degree relatives

INTRODUCTION:
Parkinson’s Disease (PD) is so far considered to be predominantly a motor disorder characterized by rigidity, slowness and tremor. Non motor symptoms include: olfactory dysfunction, constipation, rapid eye movement (REM) sleep behavior disorder (RBD), depression, and others. Many studies have addressed and shown olfactory dysfunction in PD. Reported prevalence of olfactory impairment is 74.5% in PD patients when adjusted with age matched controls. Olfactory dysfunction predates motor symptoms and may be used as a screening tool for people at risk. Very few studies have used different regional based smell kits. Olfactory function is also impaired in familial forms of Parkinsonism in which the genetic defect is known. Naheed et al...
described olfactory dysfunction in familial PD patients with LRRK mutation. The ‘Dual Hit’ hypothesis suggests that the causative agent of PD enters the body through olfactory nerve via nasal mucosa and the alimentary canal through ingestion of the unidentified agent. Indeed Hummer et al observed impaired olfactory threshold, identification and discrimination in patients of sporadic ataxia and MSA and concluded that olfactory thresholds were abnormally high in 16% of the patients. Odor discrimination and odor identification tests were impaired in 44% and 74% of the patients, respectively. Additionally, Pouclette et al also reported presence of Lewy bodies in rectal biopsy of PD. However, olfactory testing being a non-invasive procedure as is preferred over rectal biopsy. Olfactory test therefore, provides a simple non-invasive tool in differentiating between PD from parkinsonism plus syndromes (PPS) and as a biomarker of PD.

We conducted a study to assess olfactory dysfunction in idiopathic PD, Parkinson Plus Syndromes (PPS) including MSA and PSP patients, familial PD (FPD), first degree relatives of familial PD (RPD) patients and age matched healthy controls, using indigenous Indian Smell Identification Test (INSIT) and compared the result with a commercially available smell identification test i.e. SST (US NEUROLOGICALS). All subjects were recruited from movement disorders clinic of a tertiary care teaching hospital in New Delhi, India after obtaining IRB/EC approval and written informed consent of participants.

MATERIALS AND METHODS:

The study was conducted between October 2012 and June 2014. Consecutive Parkinson’s Disease Patients (PD), diagnosed on the basis of UKPDS Brain Bank Clinical Diagnostic Criteria of all ages and stages and both genders, Parkinsonism plus syndromes (PPS) patients consisting of MSA and PSP diagnosed on the basis of Consensus criteria for MSA and NINDS criteria for PSP of both genders, all age groups and all stages of disease, Familial PD patients (FPD) diagnosed in similar manner as PD patients having at least another family member affected with PD were recruited in the study. First-degree relatives of Familial PD patients were those first-degree relatives of FPD patients who did not have symptoms and signs of PD at the time of recruitment. Age and gender matched healthy controls had to meet all the exclusion criteria. Age and gender matched healthy controls were unrelated spouses of patients and attendants of other patients attending the clinic, meeting the exclusion criteria. Participants with following exclusion criteria were not included in the study: presence of dementia (MMSE <24), moderate or severe depression as measured on HAM-D scale, history of head injury, previous cranial or nasal surgery, known nasal pathology, viral infection or upper respiratory infection within past two weeks, use of nasal stuff, cigarette smoking, use of drugs known to reduce olfaction and individuals not willing to participate.

Demographic and clinical details were recorded on pre-tested format. It captured clinical variables, including age of onset of symptoms, duration of symptoms, symptoms, stage of the disease. For PD patients, UPDRS was evaluated during their “on” state in the hospital to allow ease of examination. PD patients were divided as tremor dominant or predominantly akinetic rigid type according to Stern et al. Levodopa equivalent daily dose (LED) was calculated as described by Claire et. al and staging was done using Modified Hoehn and Yahr stage. Olfactory testing were carried in out in drug ‘on’ state in a quiet, well ventilated room after explaining about the test procedure to the participants. SST consists of 12 odors namely, orange, leather, cinnamon, peppermint, banana, lemon, liquorice, coffee, cloves, pineapple, rose, and fish and INSIT consists of: cardamom (Elleteria Cardamomum), kewra (Pandanus odorifer), khus (Vetiveria Zizanoides), lemon (Citrus limone), mango (Mangifera indica), orange (Citrus Cinensis), pineapple (Ananas comosus), rose (Rosa Santana) and thinner (Pistacia terebinthus) and vanilla. The stics/ cotton buds dipped in the odorant were placed 2 cm from each nostril alternately with the other nostril being pinched. The subjects were instructed to inhale with the other
nostril and identify the smell by pointing to the correct answer from a group of four options written on a card (forced choice response). The first response was recorded and scored 1 for correct and 0 for a wrong answer. SST was done according to the instruction provided with the SST kit. Results were analyzed using Student's "t" test for comparison of mean scores in the patient group with the control group. Since the relatives of FPD were usually children of patients with FPD, they were much younger than other patients. Hence to compare this group we randomly selected a group from within the healthy controls to match RPD subjects in age and gender to compare the smell scores. Pearson's correlation test was used to determine the agreement between the tests.

The effect of stage, LEDD, duration of disease, motor UPDRS score, type of Parkinson disease (tremor dominant PD or akinetic rigid dominant) and presence of sleep abnormality in the form of RBD on the smell test scores were evaluated using linear regression analysis.

RESULTS:
One hundred and ninety seven PD, 26 Familial PD patients (FPD), 23 first degree relatives of PD patients (RPD), 58 Parkinson Plus Patients (38 MSA and 20 PSP) patients and 200 age and gender matched healthy controls were included in the study. Demographic and clinical details of all participants are shown in table 1. Mean ± SD age of PD and control group was 58.5 ± 13.1 years and 55.8 ± 7.4 years, respectively (NS), whereas mean ± SD age of the RPD, FPD and PPS were 48.36 ± 14.21, 58.34 ± 9.71 and 60.12 ± 9.72 respectively. There was no statistically significant difference in the mean ages between FPD and PPS, however, RPD were significantly younger. The mean ± SD age of controls with which RPD was compared was 42.4 ± 8.47 years (NS). Most PD patients were in H&Y stage 2.5 and 3 whereas FPD were in 2 and 3 stages. Male gender was similarly distributed in all the groups except FPD in which group more than 90% were males. There was no statistically significant difference in the ages or LEDD of participants in PD, FPD and PPS groups.

SST and INSIT scores showed statistically significant difference between PD and healthy controls, PD and RPD, PD and FPD; PSP and healthy controls (all p<0.001; fig. 1 and 2).

To test which among the INSIT odorant was best to discriminate between healthy controls and PD participants we looked at scores on different odorant (fig. 3). Best discriminative power was seen with khus (Vetiveria Zizanoides) and vanilla on INSIT to differentiate PD from healthy controls. Among the Indian smells, mango had the highest correct response in all the groups, including controls. It was 82% in PD group and 91.58% in control group. Low response was with rose and orange among controls and PD, respectively, whereas on SST it was with liquorice. On SST best discriminative effect was seen with liquorice to differentiate PD from controls.

Multivariate regression analysis did not show any correlation between stage of disease, duration of illness, motor UPDRS score, type of PD and LEDD with smell score either on INSIT or SST (Table 1) in all the groups of participants.

Correlation between stage and duration of illness, motor UPDRS score, LEDD and presence of RBD with smell score were tested using linear regression. These parameters showed similar result on INSIT and SST and did not show any significant correlation with either SST score or INSIT score in any of the participant groups. The ROC curve showed sensitivity of 0.814 and specificity of 0.68 for INSIT at cut off score of 7 and showed very good agreement between SST and INSIT (83% ; fig. 4).

DISCUSSION:
In the present study olfaction was studied in 197 PD, 26 FPD, 58 PPS (38 MSA and 20 PSP) 23 RPD and 200 age and gender matched healthy controls. For smell identification we used both commercially available SST as well as indigenously prepared previously validated INSIT in a small number of PD and control group of subjects and showed it to be non-inferior to SST 6. The present study has intrigued into the olfactory dysfunction in familial
PD patients and their first-degree relatives along with other parkinsonism plus patients (MSA and PSP). The mean smell identification scores using INSIT and SST were able to ascertain olfactory impairment in PD, FPD and MSA patients. Olfactory dysfunction in PSP patients was insignificantly lower than healthy control subjects but was higher than PD and MSA. Olfactory abilities were well preserved in first-degree relatives of familial PD patients.

No correlation was observed between H & Y stage, duration of disease, LEDD, motor UPDRS score and type of PD either on SST or INSIT scores on regression analysis. These results are in agreement with results of previous studies on regional olfactory test batteries. These findings indicate that olfactory dysfunction starts very early in the course of the disease and reaches almost maximum level when motor symptoms appear and due to ceiling effect no further worsening is observed with the progression of motor symptoms. This hypothesis is in agreement with Braak's hypothesis according to which loss of olfactory neuronal early in the course much before the involvement of various motor areas of brain. To further ascertain this hypothesis one needs to do longitudinal study on the same group of individuals.

We also observed that INSIT is non-inferior to SST. The scores obtained using INSIT were in agreement with those obtained using SST ($r = 0.81$, $P < 0.001$), indicating that INSIT is non-inferior to SST and can be used as a validated tool for testing olfactory dysfunction in PD and related disorders. Area under ROC curves for SST (0.814) and INSIT (0.7899) were also not significantly different ($P = 0.148$) (fig. 3). Maximum specificity and sensitivity for INSIT was obtained when a cut off value of 7 was used, that is, INSIT score values $\leq 7$ indicating disease. Using a cut off value of 7, we obtained a sensitivity of 81.68% and specificity of 68.47% in our study. Whereas for SST the cut off was $\leq 6$.

Limitations in this study were that the relatives of FPD patients were significantly younger as compared to FPD and PD patients as we could only recruit children of FPD patients due to residence of older relatives at far off places. We overcame this shortcoming by comparing olfactory score of this group with a subgroup of gender and age matched controls randomly chosen from the whole control group. The smell score in the RPD patients was significantly higher than PD, FPD and the PPS groups but did not attain significant difference when compared to age matched healthy controls. Absence of significantly low score on olfactory function in RPD as compared to matched controls may be due to several reasons: a) these participants do not have PD and may never develop it as not all of RPD subjects would go on to have PD, as this group was not tested for genetic mutations or dopamine transporter deficit, b) the test we used i.e. odor identification test is probably not very sensitive to detect early abnormality of olfactory function. Had we used odor threshold detection or odor discrimination tests, it is possible we could have detected significant abnormality in this group. However, a rigorous follow up especially using FDG-Dopa PET or TRODAT SPECT scans and clinical examination of those among this group who scored low on olfactory function may reveal that some of them may indeed progress to develop PD. Secondly, we did not have genetic analysis of our FPD patients. Also, we could not recruit siblings of FPD patients in the study due to the limitation of distant places of their residence. We also had rather low numbers of participants in each group.

The present study examines smell dysfunction in a group of patients with parkinsonism including PD, MSA and PSP patients and compared them individually with healthy controls along with FPD and PD patients. Our study found that there was significant difference between MSA, PD and FPD on one hand and PSP and healthy control subjects on the other and non significant difference between MSA and PD and FPD. Wenning et al have described moderate smell dysfunction in 29 MSA patients in comparison to healthy controls using UPSIT. However, no difference was seen between MSA-C and MSA-P subtypes. Other studies using UPSIT on 8 patients with primary autonomic failure (PAP), 23 with PD, and 20 with MSA, found significant difference of olfactory
dysfunction between patients of PAF and PD and moderate degree of dysfunction between MSA and PD. Several other studies using small number of subjects comparing MSA, PAF, PD and PSP found moderate difference between the groups. In contrast, to our study, another study found no significant difference between 21 PSP and age matched healthy controls. However, results of study conducted by Silverira-Moriyama et al using UPSIT are similar to those of ours who found that 36 PSP patients had significantly lower smell score as compared to the healthy control subjects (P < 0.001), though not as much as PD patients. In yet another study, 23 first-degree relatives of PSP patients exhibited lower UPSIT score than 23 age matched controls. Olfactory dysfunction has been shown in several neurodegenerative disorders including Alzheimer’s disease (AD). It is believed that as compared to tauopathies (PSP, AD), synucleinopathies (PD, MSA, DLB) have higher disturbance of olfaction as shown in several studies. The reason for this can be attributed to previously referred “Dual Hit” pathophysiology of these disorders. Indeed we also observed higher olfactory dysfunction in PD, FPD and MSA as compared to PSP and controls.

In conclusion, our study has confirmed reduced olfactory function in PD, FPD, and MSA patients and has shown good agreement between commercially available SST and indigenously prepared INIST tests. It can also differentiate PD and FPD from controls. It also showed insignificantly low odor scores in PSP patients and RPD subjects. INIST also has shown acceptance by Indian patients, as the odorants used are familiar to Indian patients and does not have fish as one of the odorants. Many Indians are vegetarians and feel offended when exposed to smell of fish or meat. The present study has few limitations. The study while investigating olfactory dysfunction investigated only odor identification without assessing for odor threshold and odor discrimination, which can be assessed by olfactometer, requiring expensive tools and smell kits. The differential loss of these components contributes differentially to the olfactory loss and is more sensitive to identify early olfactory dysfunction. Odor threshold and discrimination test gain importance especially while detecting low performers among first-degree relatives of familial PD patients to identify those who may develop PD later on. As SST has fish as one of the odorants, many of our patients who are vegetarians felt offended by it, so we excluded fish odor from INIST, keeping in mind the sentiments and familiarity of odors by Indian people. In future it may be possible to identify those who INIST score ≤ 7, look for presence of RBD and low uptake on F18-Dopa PET and follow them up to see if they indeed develop clinical symptoms and signs of PD.

CONCLUSION:
In conclusion, we would like to state that; PD, familial PD and MSA patients have significant olfactory dysfunction. Patients with PSP also have some degree of olfactory dysfunction, but not as significant as PD patients. However, there is significant difference between PSP and MSA. We also observed that at a cut off of INIST score of ≤ 7 there was high sensitivity and specificity for picking up PD patients. This study confirmed the findings of our earlier study in which we found that INIST, the indigenously created battery to test olfactory dysfunction is equally good in detecting olfactory dysfunction as compared to commercially available SST. This battery consists of odorants to which Indian patients are familiar with and it is economical for use in clinical practice in India. In the group of un-affected first-degree relatives of familial PD patients, we did not find significant difference from age and gender matched healthy controls. We believe that long term follow up of low performers among these individuals may detect cases of PD among these; and if this proves to be correct they would benefit from a neuro-protective therapy, when available. The study suffers from some flaws, such as small numbers, absence of genetic test results in familial cases of PD and smell detection being a rather crude test, detecting only low performers on odor detection, as compared to odor threshold or odor discrimination.
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