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Serum metabolomics study in a group of Parkinson's disease patients from northern India

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ABSTRACT

Background: Parkinson's disease (PD) is the result of progressive degeneration of the nigrostriatal dopaminergic pathway and depletion of neurotransmitter dopamine in the striatum.
Methods: We included 17 patients with PD along with 7 patients of progressive supranuclear palsy (PSP), 6 patients of multiple system atrophy (MSA) and 22 age and sex-matched healthy controls. We analyzed metabolite profiles in the serum of these patients and controls using ^{1}H NMR spectroscopy.
Results: Isoleucine, valine, alanine, glutamine and histidine in PD, PSP and MSA were significantly ($P < 0.001$) higher than controls, whereas, glutamate and glucose were significantly increased in PD ($P < 0.001$), PSP and MSA ($P < 0.05$) vs. control. Citrate was increased in PD, PSP and MSA ($P < 0.05$) vs. control. While, acetone, lactate and formate were higher at $P < 0.001$, threonine is increased at $P < 0.05$. The 3D scattered score plot of OPLS-DA model revealed clear differentiation among the groups, $R2 = 0.92$ and $Q2 = 0.78$.
Conclusion: Significant differences in various metabolite levels were found between control and disease groups. Common amino acids that are significantly higher in all groups include branched chain amino acids, which could increase neuronal excitability.

1. Introduction

Parkinson's disease (PD) is an escalating movement disorder resulting from a progressive degeneration of the nigrostriatal dopaminergic cells and depletion of neurotransmitter dopamine in the striatum. Parkinson's disease has very wide clinical features, including motor and non-motor symptoms [1]. Because of this wide range of symptoms of PD, it overlaps the other neurodegenerative diseases. Progressive supranuclear palsy (PSP) has a pathologic diagnosis with neurodegeneration characterized by abnormal tau pathology in the form of globose neurofibrillary tangles, tufted astrocytes, coiled bodies, and threads, with a predominance of 4-repeat (4R) tau isoforms [2]. Multiple system atrophy (MSA) is a rare adult-onset rapidly progressive fatal neurodegenerative disorder characterized by the abnormal aggregation of misfolded α-synuclein primarily in oligodendrocytes [3–5]. To date, available data have not yet revealed any reliable biomarker to detect early neurodegeneration in PD and to monitor and detect the effects of drug candidates on the disease progression. Metabolomics study has the potential to provide valuable insights into the etiopathogenesis of PD, to discover novel molecular targets for the treatment of PD, and to identify reliable and sensitive PD biomarkers. Biomarkers may play an important role in monitoring the progression of PD, in the early diagnosis of PD, and in determining the efficacy of therapeutic intervention.

The branched-chain amino acids (BCAAs) valine, leucine and isoleucine are essential amino acids involved in several important brain functions. BCAAs are catabolized in tri-carboxylic acid cycle to produce reduced nicotinamide adenine dinucleotide for mitochondrial respiration. In addition, within the brain, BCAAs participate either directly or indirectly in the synthesis of neurotransmitters and in maintaining the nitrogen balance of the glutamate–glutamine cycle between astrocytes and neurons. BCAA homeostasis is therefore essential to normal brain physiology [6]. 2-Methyl-3-hydroxybutyryl-CoA dehydrogenase (MHBD) is a mitochondrial enzyme involved in the catabolism of isoleucine and branched-chain fatty acids. MHBD deficiency causes impaired catabolism of isoleucine presenting as neurodegenerative disease [7]. Glutamate metabolism changes take place in many neurodegenerative pathologies, and the disruptions may be related to changes of glutamate metabolism enzyme activities, alterations of the main energy formation reactions in mitochondria, and shifts of oxidation/redox

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balance in cells. Imidazole-containing free amino acids (histidine, methylhistidine), catecholamines (L-DOPA and dopamine), citrulline, ornithine, glycine and antioxidant dipeptides (carnosine and anserine) are accounted for significant differences between control and Alzheimer's disease (AD) subjects [8]. In many neurodegenerative diseases, including Huntington's disease (HD), AD, and Amyotrophic lateral sclerosis (ALS), multilayered evidence suggests that glutamatergic dysregulation is an important contributor to disease pathology [9]. Disruption of homeostasis of lipid and glucose metabolism affects production and clearance of β-amyloid and tau phosphorylation, and induces neurodegeneration [10].

NMR spectroscopy technique is used for rapid metabolite detection because it requires an uncomplicated preprocessing procedure, and is a feasible method to obtain essential information from complex and intact biological samples [11]. ^{1}H NMR study of serum may reveal abnormal metabolite patterns, which could have the potential to serve as surrogate markers for monitoring neurodegenerative disease progression. The goal of these efforts is to identify biomarkers that are uniquely correlated with PD in order to accurately diagnose and treat the ailment. The application of metabolomics to investigate PD will provide a systematic approach to understand the pathology of PD, to identify disease biomarkers, and it will complement the genomics, transcriptomics and proteomics studies.

2. Material and methods

2.1. Patients and serum samples

UK PDS Brain Bank criteria for the diagnosis of PD were applied to select the patients [12]. For disease controls blood samples were collected from patients with PSP and MSA. The diagnosis of PSP and MSA was carried out according to the criteria of NINDS-SPSP. Seventeen PD, 7 PSP, and 6 MSA patients were diagnosed. All these patients were referred to our tertiary care hospital from primary and secondary care hospitals and diagnosed for the first time with respective ailments.

The patients and control subjects gave written informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by Institutional ethics committee.

The laboratory personnel were maintained masked to the clinical diagnosis and group of the subjects, matching each blood sample by letter coding, and so were the clinicians to subsequent concentrations until the end of the study. Antecubital whole-blood samples were drawn from a peripheral vein using a 25-gauge needle in the morning hours (08:30–10:30) after an overnight fast and 15 min of supine rest. Blood collected in a serum separator tube was kept for 30 min. Serum from blood after clotting, was separated out and collected in a clean tube and again centrifuged for 10 min at 3000 rpm and frozen at −80 °C until NMR analysis was performed. We have not obtained cerebrospinal fluid from patients and controls due to ethical considerations. Analysis of serum was performed in a controlled manner so that the patient and control characteristics, e.g., age, gender, smoking and also their food habits shall not influence the metabolite analysis. In the questionnaires, the information regarding their diet was based on at least one week retrospective recall, because, certain diets can influence the concentrations of individual amino acids after a prolonged intake. None of the selected patients were taking any medication. Twenty-two age- and sex-matched healthy volunteers without any neurological problems were used as controls and they were also free from any medication. Patient characteristics are presented in Table 1.

2.2. NMR experimental setup

The NMR spectroscopic study was performed, to observe the variations if any, in the metabolic profile of the patients and control group.

Table 1
Patient characteristics in PD, MSA and PSP.

S. N.	Parameter	PD n = 17	MSA n = 6	PSP n = 7	Control n = 22
1	Age in y median (range)	58(45–75)	55(45–66)	68(50–78)	54(44–70)
2	Male	12	4	5	18
3	Female	5	2	2	4
4	Non-smokers (%)	88	83	86	82
5	Vegetarian (%)	70	68	65	64
6	Location	Northern India			

The data set composed of healthy Controls (n = 22), PD (n = 17), PSP (n = 7) and MSA (n = 6). The collected serum samples were subjected to NMR acquisition. The serum samples were recorded in native form using TSP as an internal standard and as a coaxial insert. The NMR experiments were performed using a Bruker Biospin Avance III 800 MHz NMR (Bruker GmBH) spectrometer equipped with a 5 mm triple resonance inverse (TCI) 1H/13C/15N cryoprobe with a *Z*-shielded gradient and standard vertical bore, operating at a proton frequency of 800.21 MHz (18.8 T). ^{1}H NMR spectra of serum samples were acquired with water pre-saturation at 300 K with 128 scans and 4 dummy scans. The spectra were acquired using 1D single pulse and Carr-purcell-meiboom-gill (CPMG) pulse sequence with the following experimental parameters: spectral width of 12,820.5 Hz, time domain data points of 64 K, a total relaxation delay of 6.55 s, with a total recording time of approximately 17 min. CPMG pulse sequence (PRESET–90- (d - 180 - d) n–Aq) with an echo time of 100 ms for samples were performed. All the spectra were processed by applying a line broadening of 0.3 Hz to the FID prior to Fourier Transformation.

The recorded spectral data of the samples were pre-processed through manual phase correction followed by absolute baseline correction. Further, they were then characterized as reported in literature [13] and compared with standard NMR spectra of metabolites available in the biological magnetic resonance bank (BMRB, www.bmrb.wisc.edu), Human metabolome data base (HMDB, www.hmdb.ca) and through NMR suite 8.1 (Chenomx) software.

2.3. Statistical analysis

In order to observe the perturbation and significance of the metabolites, for differentiating the groups, the quantification was performed using Quantas (Quantification by Artificial Signal) [14] which is a software based protocol for concentration measurement by externally generating an artificial signal at −1 ppm chemical shift in the ^{1}H CPMG NMR spectra. Well-resolved metabolite signals were integrated manually, and the concentration of the metabolites was calculated in mmol/l, with respect to known concentration of TSP from their respective integral values. Further, in order to classify the groups among cases, the ^{1}H CPMG NMR spectral regions were reduced to discrete binned regions between 0.7 and 8.5 (excluding the region responsible for water i.e. 4.65–5.2 ppm) of 0.01 ppm bucket size using Bruker AMIX software (ver 3.8.7, Bruker Biospin). The data normalization was performed using sum of absolute intensities in integration mode scaled to total intensity. These binned regions underwent orthogonal signal correction (OSC) using two components and followed by OPLS-DA with full cross validation using 'The Unscrambler X' Software package (ver 10.0.1, Camo ASA).

Statistical differences between PD, PSP, MSA and control serum metabolites were analyzed using the Mann–Whitney *U* test. Mean significant differences in > 2 groups were analyzed using 1-way analysis of variance followed by Tukey's multiple comparison test. A $P < 0.05$ was considered to be statistically significant. These analyses were done using the SPSS statistical software, ver 16.0.