# Urinary Metabolites Signatures Reflect the Altered Host Metabolism in Severe Obstructive Sleep Apnea

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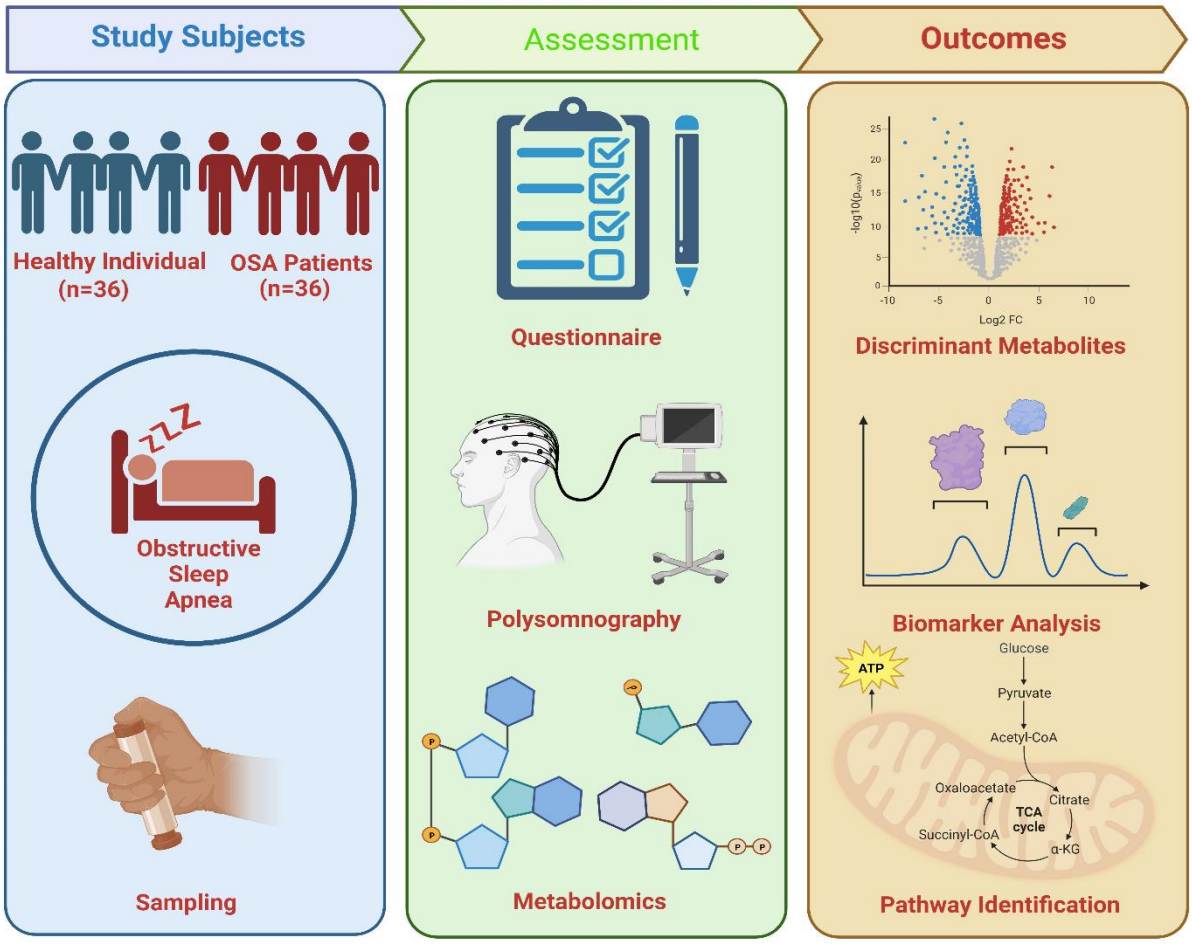


**Graphical Abstract**

**Abstract**

Obstructive sleep apnea (OSA) is a very common sleep-related breathing disorder. The onset and progression of OSA are often linked with severe cardiovascular and metabolic comorbidities. At the same time, given the increasing prevalence of OSA, novel methods to screen OSA and its follow-up are needed. Untargeted metabolic profiling of OSA patients and healthy controls was planned to capture a snapshot of urinary metabolites and potential biomarkers using the gas chromatography-mass spectrometry (GC-MS) method. Polysomnography (PSG) confirmed severe OSA patients with AHI index ≥30 were considered for urine sample collection. The sample size was constituted of OSA (n=36) and healthy controls (n=36). Metabolite extraction and derivatization were performed and analysed by using GC-MS. The obtained data set was statistically analysed using univariate and multivariate analysis. The Orthogonal partial least-squares discriminant analysis (OPLS-DA) was performed to screen differential metabolites between OSA patients and healthy controls. The metabolomic analysis revealed a total of 143 significantly altered metabolites of interest. Biomarker analysis allows for the creation a list of putative urinary biomarkers, including GABA, malic acid, glutamic acid, epichoric acid etc., with an accuracy of 99.8% to 100% for OSA screening. Subsequently, pathway analysis revealed that related biochemical pathways like the tricarboxylic acid cycle (TCA), glutamate/glutamine, amino acid and fatty acid metabolism, that are significantly interlinked with these metabolic biomarkers, can play a crucial role in the pathogenesis of OSA. This study paves the way to undertake mass screening in a larger population to identify specific and reliable biomarkers.

**Keywords:** Sleep Disorder, Urinary Biomarkers, Metabolomics, Gas Chromatography-Mass Spectrometry.

**Abbreviations**

AHI; Apnea hypopnea index

GC-MS; Gas Chromatography-Mass Spectrometry

OSA; Obstructive Sleep Apnea

OPLS-DA; Orthogonal partial Least square discriminant analysis

PCA; Principal component analysis

PSG; Polysomnography

SVM; Support vector machine

TCA cycle; Tricarboxylic acid cycle

VIP; Variable importance in the projection

## Introduction

Obstructive Sleep Apnea (OSA) is a widespread sleep-related disorder which involves recurrent reduction or complete cessation of breathing activity during sleep due to intermittent collapse of the upper airway. The overall prevalence in the population ranges from 9-38%, affecting significantly more men than women [1]. There is an increased recognition that the prevalence of OSA is increasing globally [2]. OSA is well-recognized as an independent and confounding risk factor for several clinical disorders, including cardiovascular diseases, systemic hypertension, stroke and metabolic disorders [3,4,5,6]. A new study reported that OSA coexists in 30% of patients with insomnia [7,8]. Therefore, the rising OSA also contributes to the growing number of patients with various disorders. However, at the same time, OSA largely remains undiagnosed in many patients [9].

The diagnosis of OSA is based on the apnea-hypopnea index (AHI) i.e., basically a combined average number of apnea (cessation of breathing) and hypopnea (reduction of breathing) episodes occurring per hour of sleep. In general, the threshold value of AHI is categorized as mild (5–15), moderate (15–30), or severe (30 or higher) events/hr [10]. Keeping in view of the increasing global burden of OSA, a reliable and convenient method that can faithfully detect OSA expansively and could indicate the disease progression towards a particular metabolic syndrome would be very useful. The gold standard of OSA diagnosis is polysomnography (PSG) which dictates that the diagnosis and management of OSA require overnight hospital stay, complex data analysis and interpretations [11,12]. Despite its utility in the clinic there is an incessant need for newer methods which could be used to screen a larger population to estimate the precise prevalence of the disease so that the data could be utilized to provide the health care solution to the larger patient's cohort.

The OSA is a characteristic feature of metabolic syndrome and a risk factor for the number of associated metabolic complications. However, the contributory and causative factors leading to metabolic syndromes in OSA remained largely uncertain. Although, OSA has been known to be associated with

obesity and excess accumulation of triglycerides, the exact relationship between obesity with OSA is obscure. OSA is a secondary cause of hypertension and is independently associated with target-organ damage in hypertensive patients. However, OSA remains largely underdiagnosed and undertreated [13].

Performing metabolomics to find biomarkers for diseases is an upcoming field which has been utilised to profile metabolites in OSA earlier [14,15]. Innovative approaches like metabolomics are a new discipline, and there is a need to unearth metabolite-based biomarkers. In that way, We are the first of compare the urinary metabolome of severe OSA patients and control subjects to revealed the urinary based non-invasive biomarkers.

Here we report the usage of GC-MS for comprehensive analysis of urinary metabolites in OSA. We report different classes of metabolites, such as fatty acids, carbohydrates, amino acids and steroids. By performing univariate and multivariate analysis, we prepared a catalogue of metabolites that differ significantly in OSA with respect to healthy volunteers. We further delve into an analysis of potential biomarkers and significantly altered metabolic pathways, which could be potentially useful in understanding the underlying pathophysiology of OSA.

## Materials and Methods

**Reagents:** Alkane standard contains $C_{10}$ , and all even n-alkanes from $C_{20}$ to $C_{40,}$ 50 mg/l in n-heptane was procured from Supelco (Supelco inc., Bellefonte, PA, USA). Methoxamine and Pyridine were acquired from Sigma (Sigma Aldrich, St. Louis, MO, USA). N-Methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)+ 1% TMCS was purchased from Thermo (Thermo Fischer Scientific, MA, USA). Acetonitrile used as extraction solvent was obtained from Merck (Fairfield, OH, USA). Urease was purchased from Sigma (Sigma Aldrich, St. Louis, MO, USA).

**Samples:** The Case-control study was performed by collecting urine samples from OPD visiting patients at King Georges Medical University and Midland hospital, Lucknow, from February 2021 to

March 2022. The institutional ethical committee approved the study (ref. code:104th ECM II B-FS/P1) and consent forms are obtained from all the study subjects. After polysomnography and questionnaires based assessments, mid-stream urine was collected in the morning from all the subjects. After settling for approximately 10 min, the supernatant was collected in a 2 mL tube and placed at -80ºC until use. For the sample collection, there were no nutritional or any other limitations. The selection criteria are based on questionnaires (for both groups) and clinical diagnosis (polysomnography of OSA group only). A total of 36 cases of OSA patients with a mean age of 49.5±12.7 years were enrolled before starting any treatment. In addition, 36 Control were included with a mean age of 38.6±3.6 years in this study as volunteers for comparison of metabolomics profiling (Table 1).

**Metabolite extraction:** Before extraction, samples were stored at room temperature for thawing and 50µL of urine sample was mixed with 100µL of urease solution (10 mg/mL). After adding urease to urine samples, samples were incubated at 37ºC in a water bath for 30 minutes. After that samples were stored on ice for cooling then 500µL ACN was added. Each sample was vortexed for 30 seconds, the mixture was allowed to settle for 10 min and centrifuged at 8000×g at 4ºC for 10 min to allow proteins precipitation. Labconco CentriVap system (Labconco, Kansas City, MO, USA) was used to concentrate and dry the metabolite extracts. Then the supernatant was collected and lyophilized under vacuum using Labconco CentriVap system. Subsequently, dried samples were methoxymated in 30µL of metoxyamine solution (20mg/mL in pyridine) and then stored at 40ºC for 30 min. After that 70µL of MSTFA+1%TMCS was added and stored at 60ºC for 1 hour. Then derivatized samples were transferred to a vial for GC-MS analysis.

**Gas chromatography Mass Spectrometry:** Trace 1300/TSQ 3000 Gas Chromatography-Mass Spectrometer (Thermo Fischer Scientific, MA, USA) was used for metabolomic data acquisition. For GC-MS/MS analysis, 1µL of derivatized samples were injected into a Thermo TRACE 1300 series gas chromatography system coupled with TSQ 8000 series mass analyzer equipped with a TriPLUS 100 auto sampler. The separation was achieved using a Trace GOLD TG-5MS column (Thermo Scientific)

with diameter of 0.25 mm, stationary phase film thickness of 0.25 µm and length of 30 m. The initial temperature of the oven was 50 °C for 1 min; temperature was increased to 100°C at rate of 6 °C per min, then to 200 °C with the ramp of 4 °C/min and finally to 280 °C at the rate of 20 °C which was held constant for 3 min. The transfer line, ion source and injection port temperature were 250, 250, and 200 °C, respectively. Helium was used as carrier gas and Argon was used as collision gas. Samples were injected in splitless mode and carrier flow rate was set to 1 mL/min. All the samples were run on full scan mode (50-650 amu) and raw data obtained were collected for further analysis. The alkane standard was run at the beginning of the batch run, and at the end of the batch run as a retention index standard for RI calibration in GC MS analysis (Mohit et. al. frontiers).

**Data Processing and metabolite identification** MS Dial version 4.80 was employed for peaks analysis, intensities extraction and annotation as previously reported by Misra et al. [16]. Peaks annotation was achieved through MS-DIAL using an in-house library. In analysis parameters, we have used MS-Dial parameters such as Mass range scan (50-650 Da), peak detection (mass peak height 1000 amplitude), deconvolution parameters (sigma window value 0.5 and EI spectra cut off 10 amplitude). Identification used retention time setting (used retention index file present in supplementary data and alkane index type) and in identification setting use Fiehn library and retention index tolerance 20 and retention time tolerance of 0.5 min. The m/z tolerance 0.5 Da and the EI similarity and identification score cut off was 70%. The metabolite annotation and assignment of the EI-MS spectra followed the metabolomics standards initiative guidelines (Sansone et al., 2007) [17]. Metabolites were annotated at Level 2, and the identification was based on the spectral database using a match factor > 70%. Furthermore, the identified metabolites were also referred to the Human Metabolome Database (HMDB) (http://www.Hmdb.ca) and previous references published. All annotated data were normalized through sum, cube root transformed and pareto scaled to get best normal distribution. After that statistically analyzed through multivariate and univariate approaches using the open-source software Metaboanalyst 5.0 (Pang et al., 2021) [18].

In particular, the Volcano plot was used for univariate analysis using a fold change of 1.5 and a P value ≤ 0.05, whereas the Principal component analysis (PCA) and the Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) were used for multivariate analysis. The OPLS-DA model was validated through the permutation test setting 20 as permutation.

**Results:**

**Differentially accumulated metabolites in the urine of OSA patients reflect changes in host metabolism**: A case-control untargeted metabolomics study was carried out in an equal number of OSA patients and healthy controls. All the patients included in this study were classified as severe OSA patients based on PSG report (AHI≥30 events/hour) combined with standard questionnaire-based assessment (Table 1).

Untargeted Metabolomics using MS-Dial revealed a total of 198 common annotated metabolites in the urine samples, mainly belonging to the classes of the carbohydrates, amino acids, organic acids and nucleosides.

Univariate analysis, we performed T-test and volcano plot between control and OSA patients. Total 142 significantly altered metabolites were identified (p-value <0.05), and the top 75 metabolites were represented as a Heat map (Figure 1 A). The t-stat value revealed 79 out of 142 were significantly up-accumulated (negative t-stat), whereas the remaining 63 metabolites were down-accumulated (positive t stat value) in OSA patients (Table 2). Further, we performed volcano plot analysis to identify most altered metabolites in OSA patients (p-value <0.05 and Fold change 2.0). Results of volcano plot analysis identified total 71 metabolites have significantly higher whereas 61 metabolites have lower accumulation in OSA patients (Figure 1B) (Supplementary data).

**Urinary metabolic signature in severe OSA patients:** The PCA analysis carried out on control and OSA patients pointed out a clear separation between the two groups. The separation, achieved by virtue of the first two PCs (PC1 vs PC2) highlighted that PC-1 explained the 29.6% of the total variance,

whereas PC2 has 13.4% (Fig. 2A). In addition, the loadings plot highlighted that PC1 was mainly dominated by 2-deoxy D-glucose, ribonic acid, GABA, D-malic acid whereas PC2 by putrescine, uracil, itaconic acid and glycine (Supplementary data).

Further, we used OPLS-DA analysis to determine the metabolites contributing to the discrimination between control and OSA groups. The OPLS-DA analysis also showed discriminatory clustering among controls and OSA. (Figure 2B). The OPLS-DA derived loadings variable importance in the projection (VIP) scores revealed that more than 75 metabolites with a VIP score higher than 1 that contributed to group separation. We identified representative top 30 metabolites with highest VIP scores contributing in discriminating between control and OSA groups (Figure 2C). Further, we performed OPLS-DA model overview and permutation test to check the predictive accuracy of study model. The value of R2Y is 0.991, and Q2 is 0.981 showing the goodness of fit and prediction (p-value <0.01). Additionally, R2Y and Q2 scores indicate significant urinary metabolite level alteration between control and OSA groups (Figure 2D).

**Analysis of potential biomarkers:** We further performed automated multivariate ROC curve based exploratory analysis for important feature identification and performance evaluation. Support vector machines (SVMs) with 2 latent variables of PLSDA were utilized in ROC curves analysis. In this analysis, as the number of metabolites were increased AUROC also reached near perfect. SVM with 5 metabolites has good AUROC (0.996, 95% CI= 0.938-1) and increasing number of metabolites up to 10 increased AUROC (0.999, 95%CI=0.988-1). Further increasing the number of metabolites from 15 to 100 AUROC achieved on their perfect value (1, 95% CI=1-1) (Figure 3A). In predictive accuracy analysis, increasing number of metabolites correlates with predictive accuracy of the model. In SVM model, predictive accuracy reaches at its perfect on selecting 50 features (Figure 3B). By performing these analyses, we were able to mark top 25 predictive biomarker in OSA with their selected frequency. All 25 predictive biomarker of OSA were reported identified as significantly altered in T-test and log 2-fold change analysis (p-value <0.05) (Figure 3C).

**Metabolite enrichment and Pathway analysis:** Metabolic pathway analysis (MetPA) was used to understand the significantly activated metabolic pathways. In pathway analysis, we identified 10 most impacted metabolic pathways in OSA patients (Impact >0.3 and p-value <0.05). The results of pathway analysis were presented through bubble plot, where each bubble corresponds to one metabolic pathway, whereas the bubble size and colour are representative of enrichment and more significant alterations, respectively (Figure 4). Five out of the 10 most impacted pathways were upregulated, whereas 5 were downregulated. In particular, in OSA patients the tyrosine metabolism, histidine metabolism, pentose and glucuronate interconversion, cysteine and methionine metabolism, starch and sucrose metabolism were up accumulated. On the contrary; TCA cycle, amino acid (glycine, serine, threonine, Alanine, aspartate glutamine and glutamate) metabolism, aromatic amino acid biosynthesis were downregulated.

## Discussion

Questionnaire-based assessment combined with PSG remains the gold standard as a diagnostic tool for diagnosing OSA. There has been a steady rise in the incidence of OSA, resulting in the need for a robust biochemical assay that could be utilized for its mass screening (R). There is also an unmet need for a biochemical and non-invasive marker that can indicate the severity of OSA. In recent times, the advent of metabolomics is becoming a method of choice that can potentially provide the underlying metabolite status in patients compared to controls [15,19]. The approach to unearthing diagnostic biomarkers using Metabolomics is fast becoming a promising strategy to identify specific metabolites as sensitive, specific and predictive biomarkers [20,21].

Healthy controls and severe OSA patients exhibit marked physiological differences [22]. In an early study from our group has also shown significant changes in plasma samples of OSA patients that are validated statistically [23]. The present study offers a comparative snapshot of healthy controls'

metabolic profiles compared to severe OSA patients. Analysing the host's urinary metabolites, which reflect the metabolic alteration, can be used as a surrogate for pathophysiological changes.

Targeted Metabolomics has been employed to investigate urinary metabolic signatures in OSA. However, the present study allows us to fully scan urine samples and develop a novel biomarker panel. Multivariate PCA and OPLS-DA are used to identify the discriminant metabolites between the case and control groups. As we screened severe OSA patients in this study, we identified several metabolites linked to metabolic comorbidity and mental health. Another pathway revealed by analysis for top enriched and impacted metabolic mechanisms was glutamine and glutamate metabolism. Collectively, it is well established that glutamate and γ-aminobutyric acid (GABA) is a major excitatory and inhibitory neurotransmitter that acts as crucial intermediate in the brain's cellular metabolism, influencing the circadian rhythm cycle in sleep-wake pattern [24]. Glutamate is the metabolic precursor of GABA; alteration in glutamate and GABA metabolism may play important roles in controlling cortical excitability [25]. Low GABA and high glutamate in the insular cortex have been correlated with cardiovascular regulation in patients with OSA [26]. In the present study, the glutamate metabolic pathway was positively associated with the severity of OSA subjects, validating the role of glutamate and GABA metabolism in OSA. Alterations in GABA and glutamate levels trigger the regulation of one another and brain activity, further significantly associated with depression and anxiety in OSA patients.

It has been known that alterations in the levels of certain metabolites related to fatty acid, carbohydrate and amino acid metabolism were associated with the incidence of OSA [27]. Again, the alterations in several amino acid metabolism in our study reflect the expected pathophysiology of severe OSA and other OSA-induced metabolic disorders. Several conditions are associated with OSA, such as insulin resistance, visceral fat deposition, and dyslipidaemia, and other conditions closely related to OSA, such as obesity and reduced sleep duration [28]. Excessive lipid storage is usually linked to obesity, and fatty acids become an alternative energy substrate in place of glucose, resulting in reduced synthesis action.

In this study, levels of several fatty acids were found to be changed palmitic acid, and epicholic acid were highly increased in the urine sample of severe OSA patients, indicating that these fatty acids were the primary source of energy and may be associated with fat deposition in OSA induced obese condition.

Indeed, low levels of physical activity are known to be associated with an increased occurrence of OSA and cardiometabolic risk [29]. The physical idleness leads to obesity which is a common factor in the pathogenesis of metabolic syndrome and OSA. There is a profound significance of metabolic alterations and perturbations in obese subjects making them more prone to develop OSA [30]. Although, advanced LC-MS techniques were used to identify lipid biomarkers, the characterization of other metabolites remained under exploration. Our study revealed the metabolic changes resulting in the modulation of pathways such as galactose metabolism, amino acid metabolism, pentose and gluconate interconversions and TCA cycles.

The involvement of integrated metabolomics with TCA cycle in OSA's pathogenesis has not been studied much. The intermittent hypoxia condition in OSA leads to the impairment in TCA cycle, and oxidative phosphorylation flowed by the overproduction of reactive oxygen species (ROS) leads to dysfunctional immunometabolism [31]. Hypoxia-induced activation of a reverse tricarboxylic acid cycle (rTCA) with reductive glutamine metabolism provides precursor molecules for *de novo* lipogenesis [32]. It is hypothesized that OSA causes the feeding of electrons into an inefficient respiratory chain, making the physiology susceptible to increase ROS by providing mitochondria-derived substrate for gluconeogenesis [33].

The biomarker analysis results identified metabolites potentially involved in the altered metabolic pathways. Hence, key metabolites of these altered pathways could be exploited as a biomarker for OSA. Our study also revealed the use of GC-MS/MS-based untargeted metabolomic profiling as an effective analytical tool to illustrate metabolomic profiles in OSA. Biomarker identification and ROC curve analysis showed that the combination of amino acid and glycerophospholipids could effectively

discriminate OSA patients from healthy controls. These metabolites showed promising sensitivity and/or specificity, which indicates that these metabolites might be useful biomarkers in monitoring and detecting OSA.

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**Author Contribution:** PC, AS and M conceptualized the idea of manuscript. M and MST performed all the experiment. M, MST, BPS and FA analysed the data. M, MST, AS, FA and PC wrote and evaluated the manuscript. All authors concur with the final version of the manuscript.

**Conflict of Interest:** The authors declare no conflict of interest.

**References:**


[1] C.V. Senaratna, J.L. Perret, C.J. Lodge, A.J. Lowe, B.E. Campbell, M.C. Matheson, G.S. Hamilton, S.C. Dharmage, Prevalence of obstructive sleep apnea in the general population: A systematic review, Sleep Med Rev 34 (2017) 70-81.

[2] A.V. Benjafield, N.T. Ayas, P.R. Eastwood, R. Heinzer, M.S.M. Ip, M.J. Morrell, C.M. Nunez, S.R. Patel, T. Penzel, J.L. Pépin, P.E. Peppard, S. Sinha, S. Tufik, K. Valentine, A. Malhotra, Estimation of the global prevalence and burden of obstructive sleep apnoea: a literature-based analysis, Lancet Respir Med 7 (2019) 687-698.

[3] Y. Yeghiazarians, H. Jneid, J.R. Tietjens, S. Redline, D.L. Brown, N. El-Sherif, R. Mehra, B. Bozkurt, C.E. Ndumele, V.K. Somers, Obstructive Sleep Apnea and Cardiovascular Disease: A Scientific Statement From the American Heart Association, Circulation 144 (2021) e56-e67.

[4] C. Lombardi, M.F. Pengo, G. Parati, Systemic hypertension in obstructive sleep apnea, J Thorac Dis 10 (2018) S4231-s4243.

[5] S. Jehan, M. Farag, F. Zizi, S.R. Pandi-Perumal, A. Chung, A. Truong, G. Jean-Louis, D. Tello, S.I. McFarlane, Obstructive sleep apnea and stroke, Sleep Med Disord 2 (2018) 120-125.

[6] T. Kim, J. Kang, Relationship between obstructive sleep apnea, insulin resistance, and metabolic syndrome: a nationwide population-based survey, Endocr J 70 (2023) 107-119.

[7] H. Janssen, L.N. Venekamp, G.A.M. Peeters, A. Pijpers, D.A.A. Pevernagie, Management of insomnia in sleep disordered breathing, Eur Respir Rev 28 (2019).

[8] A. Sweetman, L. Lack, C. Bastien, Co-Morbid Insomnia and Sleep Apnea (COMISA): Prevalence, Consequences, Methodological Considerations, and Recent Randomized Controlled Trials, Brain Sci 9 (2019).

[9] R.H. Breteler, R. Rombouts, C.P. van der Staak, Smoking cessation studies: a methodological comparison, Int J Addict 23 (1988) 1297-1309.

[10] M. Goyal, J. Johnson, Obstructive Sleep Apnea Diagnosis and Management, Mo Med 114 (2017) 120-124.

[11] B. Gerstenslager, J.M. Slowik, Sleep Study, StatPearls, StatPearls Publishing



[12] V.K. Kapur, D.H. Auckley, S. Chowdhuri, D.C. Kuhlmann, R. Mehra, K. Ramar, C.G. Harrod, Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea: An American Academy of Sleep Medicine Clinical Practice Guideline, J Clin Sleep Med 13 (2017) 479-504.

[13] L.F. Drager, P.R. Genta, R.P. Pedrosa, F.B. Nerbass, C.C. Gonzaga, E.M. Krieger, G. Lorenzi-Filho, Characteristics and predictors of obstructive sleep apnea in patients with systemic hypertension, Am J Cardiol 105 (2010) 1135-1139.

[14] H. Xu, X. Zheng, Y. Qian, J. Guan, H. Yi, J. Zou, Y. Wang, L. Meng, A. Zhao, S. Yin, W. Jia, Metabolomics Profiling for Obstructive Sleep Apnea and Simple Snorers, Sci Rep 6 (2016) 30958.

[15] Mohit, M.S. Tomar, D. Sharma, S. Nandan, A. Pateriya, A. Shrivastava, P. Chand, Emerging role of metabolomics for biomarker discovery in obstructive sleep apnea, Sleep Breath (2022).

[16] B.B. Misra, V. Das, M. Landi, M.R. Abenavoli, F. Araniti, Short-term effects of the allelochemical umbelliferone on Triticum durum L. metabolism through GC-MS based untargeted metabolomics, Plant Sci 298 (2020) 110548.

[17] S.A. Sansone, T. Fan, R. Goodacre, J.L. Griffin, N.W. Hardy, R. Kaddurah-Daouk, B.S. Kristal, J. Lindon, P. Mendes, N. Morrison, B. Nikolau, D. Robertson, L.W. Sumner, C.

Taylor, M. van der Werf, B. van Ommen, O. Fiehn, The metabolomics standards initiative, Nat Biotechnol 25 (2007) 846-848.
[18] Z. Pang, J. Chong, G. Zhou, D.A. de Lima Morais, L. Chang, M. Barrette, C. Gauthier, P. Jacques, S. Li, J. Xia, MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights, Nucleic Acids Res 49 (2021) W388-W396.
[19] C.H. Johnson, J. Ivanisevic, G. Siuzdak, Metabolomics: beyond biomarkers and towards mechanisms, Nat Rev Mol Cell Biol 17 (2016) 451-459.
[20] A. Koulman, G.A. Lane, S.J. Harrison, D.A. Volmer, From differentiating metabolites to biomarkers, Anal Bioanal Chem 394 (2009) 663-670.
[21] A. Zhang, H. Sun, G. Yan, P. Wang, X. Wang, Metabolomics for Biomarker Discovery: Moving to the Clinic, Biomed Res Int 2015 (2015) 354671.
[22] M.R. Bonsignore, M.C. Suarez Giron, O. Marrone, A. Castrogiovanni, J.M. Montserrat, Personalised medicine in sleep respiratory disorders: focus on obstructive sleep apnoea diagnosis and treatment, Eur Respir Rev 26 (2017).
[23] Mohit, M.S. Tomar, F. Araniti, A. Pateriya, R.A. Singh Kushwaha, B.P. Singh, S.K. Jurel, R.D. Singh, A. Shrivastava, P. Chand, Identification of metabolic fingerprints in severe obstructive sleep apnea using gas chromatography-Mass spectrometry, Front Mol Biosci 9 (2022) 1026848.
[24] S. He, X. Zhang, S. Qu, Glutamate, Glutamate Transporters, and Circadian Rhythm Sleep Disorders in Neurodegenerative Diseases, ACS Chem Neurosci 10 (2019) 175-181.
[25] O.A. Petroff, GABA and glutamate in the human brain, Neuroscientist 8 (2002) 562-573.
[26] P.M. Macey, M.K. Sarma, R. Nagarajan, R. Aysola, J.M. Siegel, R.M. Harper, M.A. Thomas, Obstructive sleep apnea is associated with low GABA and high glutamate in the insular cortex, J Sleep Res 25 (2016) 390-394.
[27] X. Zhang, S. Wang, H. Xu, H. Yi, J. Guan, S. Yin, Metabolomics and microbiome profiling as biomarkers in obstructive sleep apnoea: a comprehensive review, Eur Respir Rev 30 (2021).
[28] A. Romero-Corral, S.M. Caples, F. Lopez-Jimenez, V.K. Somers, Interactions between obesity and obstructive sleep apnea: implications for treatment, Chest 137 (2010) 711-719.
[29] L. Simpson, N. McArdle, P.R. Eastwood, K.L. Ward, M.N. Cooper, A.C. Wilson, D.R. Hillman, L.J. Palmer, S. Mukherjee, Physical Inactivity Is Associated with Moderate-Severe Obstructive Sleep Apnea, J Clin Sleep Med 11 (2015) 1091-1099.
[30] S.N. Framnes, D.M. Arble, The Bidirectional Relationship Between Obstructive Sleep Apnea and Metabolic Disease, Front Endocrinol (Lausanne) 9 (2018) 440.
[31] Mohit, A. Pateriya, M.S. Tomar, A. Shrivastava, P. Chand, Immunometabolism: An evolutionary perspective in obstructive sleep apnea, Sleep Med Rev 65 (2022) 101668.
[32] L. Vacek, A. Dvorak, K. Bechynska, V. Kosek, M. Elkalaf, M.D. Trinh, I. Fiserova, K. Pospisilova, L. Slovakova, L. Vitek, J. Hajslova, J. Polak, Hypoxia Induces Saturated Fatty Acids Accumulation and Reduces Unsaturated Fatty Acids Independently of Reverse Tricarboxylic Acid Cycle in L6 Myotubes, Front Endocrinol (Lausanne) 13 (2022) 663625.
[33] D. Nolfi-Donegan, A. Braganza, S. Shiva, Mitochondrial electron transport chain: Oxidative phosphorylation, oxidant production, and methods of measurement, Redox Biol 37 (2020) 101674.

**Figure Legends:**

**Figure 1: (A)** Heat Map of top 75 differential features identified through metabolomics analysis on comparision of OSA and Healthy volunteers. Red colour is representative of higher accumulation of metabolites in respective group whereas blue colour shows the trend of lower accumulation. **(B)** Volcano plot reveals substantial differences between the metabolomic profiles of OSA patients and non-OSA patients. Coloured area of volcano plot emphasizes metabolites with p-value of less than 0.05 and Fold Change of greater than 2.0. Red colour indicates up accumulation while blue colour indicates lower accumulation.

**Figure 2: Figure 5.7: Multivariate exploratory analysis of urine samples**. (A) Score plot of PCA model for OSA samples. (B) Two-dimensional OPLS-DA score plots further increase discrimination between the OSA (green) and Healthy individual (Red) groups. (C) Model overview of the OPLS-DA model for the provided dataset. It shows the R2X, R2Y, and Q2 coefficients for the groups; (D) Permutation test representing the observed and cross-validated R2Y=0.991 and Q2=0.981 coefficients. (E) Variable importance in projection (VIP) scores indicating the top 30 metabolites contributing to the separation of OSA patients metabolic profile with Non-OSA.

**Figure 3: Biomarker Identification. (A)** Model performance evaluation for each of the six SVM classifiers with a growing number of metabolites. ROC curve of each SVM using the average cross-validation efficiency with the confidence interval of 95%. **(B)** Predictive accuracy of study model with number of different metabolite features. **(C)** Identified top 25 variables for classification of OSA patients to healthy volunteers.

**Figure 4:** The bubble plot representing significantly altered metabolic pathways in OSA patients.

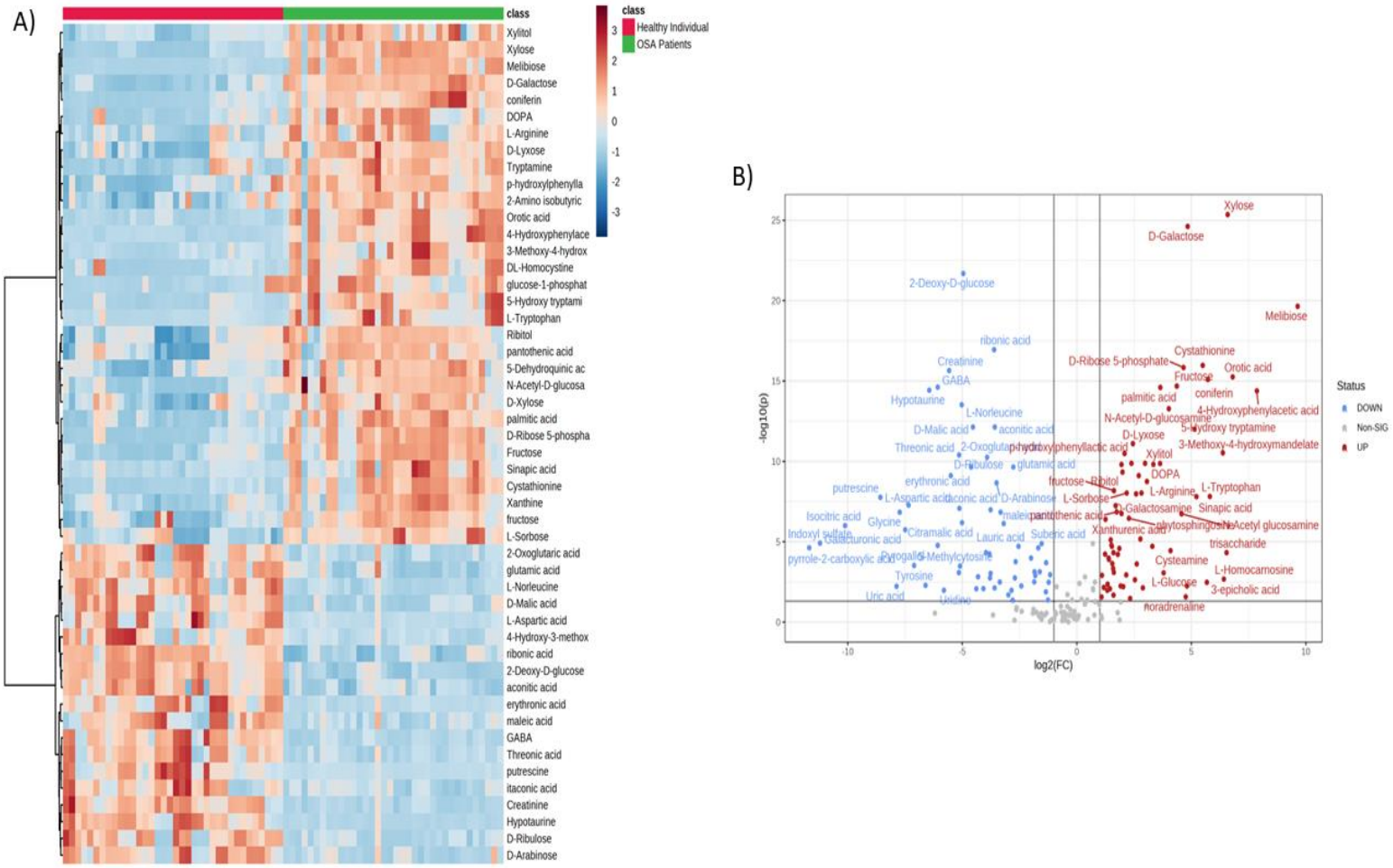

A)
class
Healthy Individual
OSA Patients
Xylitol
Xylose
Melibiose
D-Galactose
coniferin
DOPA
L-Arginine
D-Lyxose
Tryptamine
p-hydroxylphenylla
2-Amino isobutyric
Orotic acid
4-Hydroxyphenylace
3-Methoxy-4-hydrox
DL-Homocystine
glucose-1-phosphat
5-Hydroxy tryptami
L-Tryptophan
Ribitol
pantothenic acid
5-Dehydroquinic ac
N-Acetyl-D-glucosa
D-Xylose
palmitic acid
D-Ribose 5-phospha
Fructose
Sinapic acid
Cystathionine
Xanthine
fructose
L-Sorbose
2-Oxoglutaric acid
glutamic acid
L-Norleucine
D-Malic acid
L-Aspartic acid
4-Hydroxy-3-methox
ribonic acid
2-Deoxy-D-glucose
aconitic acid
erythronic acid
maleic acid
GABA
Threonic acid
putrescine
itaconic acid
Creatinine
Hypotaurine
D-Ribulose
D-Arabinose
B)
-log10(p)
log2(FC)
Status
DOWN
Non-SIG
UP
Xylose
D-Galactose
2-Deoxy-D-glucose
Melibiose
ribonic acid
Cystathionine
Creatinine
D-Ribose 5-phosphate
Orotic acid
GABA
Fructose
Hypotaurine
palmitic acid
coniferin
L-Norleucine
4-Hydroxyphenylacetic acid
N-Acetyl-D-glucosamine
D-Malic acid
aconitic acid
5-Hydroxy tryptamine
D-Lyxose
Threonic acid
3-Methoxy-4-hydroxymandelate
Xylitol
D-Ribulose
glutamic acid
DOPA
erythronic acid
fructose-Ribitol
putrescine
L-Arginine
L-Tryptophan
L-Aspartic acid
Sinapic acid
Glycine
Citramalic acid
Lauric acid
Suberic acid
Xanthurenic acid
trisaccharide
Cysteamine
Tyrosine
L-Homocarnosine
L-Glucose
3-epicholic acid
Uric acid
noradrenaline

**Figure 1**

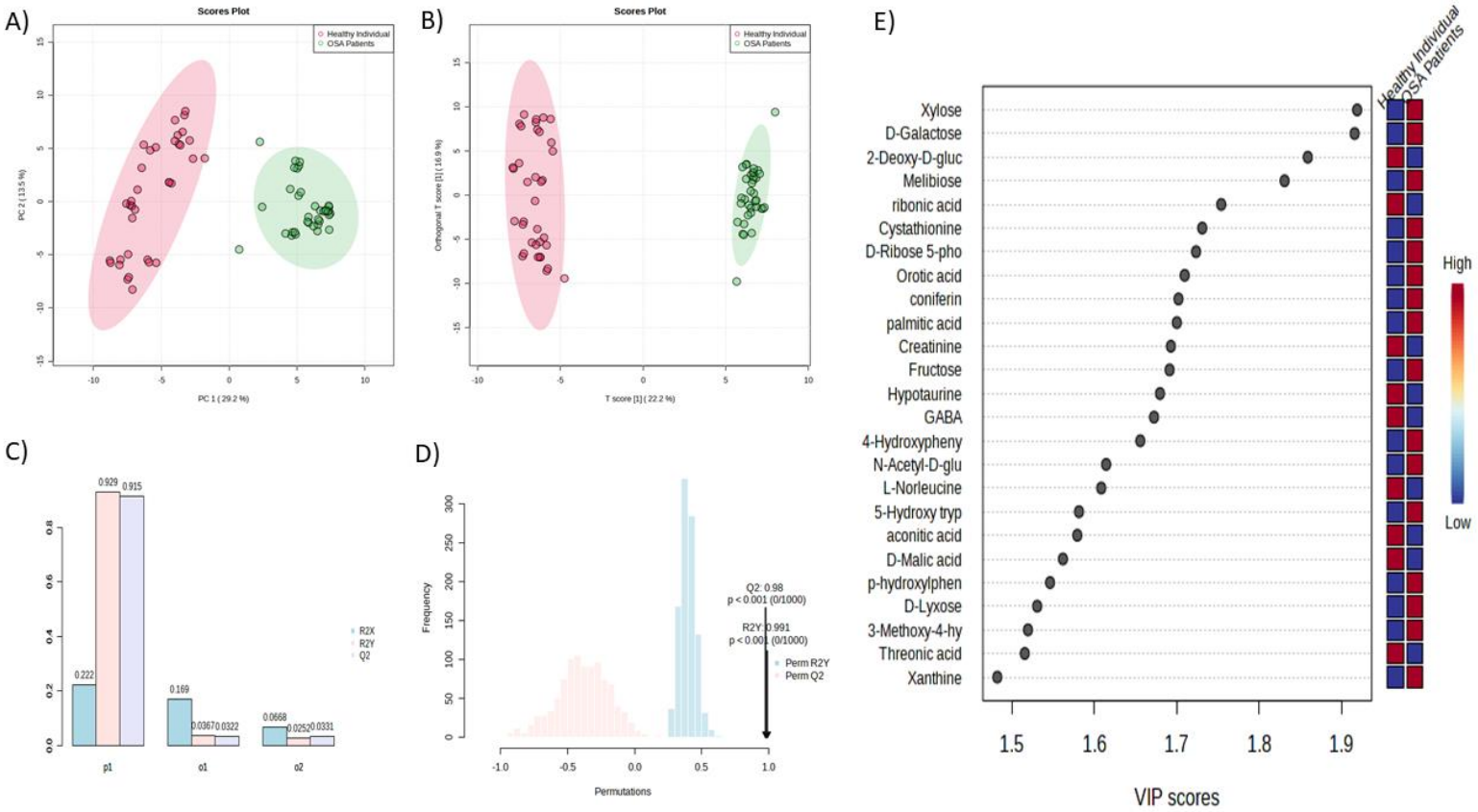

A)
Scores Plot
Healthy Individual
OSA Patients
PC 1 ( 29.2 %)
PC 2 ( 13.5 %)
B)
Scores Plot
Healthy Individual
OSA Patients
T score [1] ( 22.2 %)
Orthogonal T score [1] ( 16.9 %)
C)
0.929
0.915
0.222
0.169
0.0367
0.0322
0.0668
0.0252
0.0331
R2X
R2Y
Q2
p1
o1
o2
D)
Frequency
Permutations
Q2: 0.98
p < 0.001 (0/1000)
R2Y: 0.991
p < 0.001 (0/1000)
Perm R2Y
Perm Q2
E)
Healthy Individual
OSA Patients
Xylose
D-Galactose
2-Deoxy-D-gluc
Melibiose
ribonic acid
Cystathionine
D-Ribose 5-pho
Orotic acid
coniferin
palmitic acid
Creatinine
Fructose
Hypotaurine
GABA
4-Hydroxypheny
N-Acetyl-D-glu
L-Norleucine
5-Hydroxy tryp
aconitic acid
D-Malic acid
p-hydroxylphen
D-Lyxose
3-Methoxy-4-hy
Threonic acid
Xanthine
High
Low
VIP scores

**Figure 2**

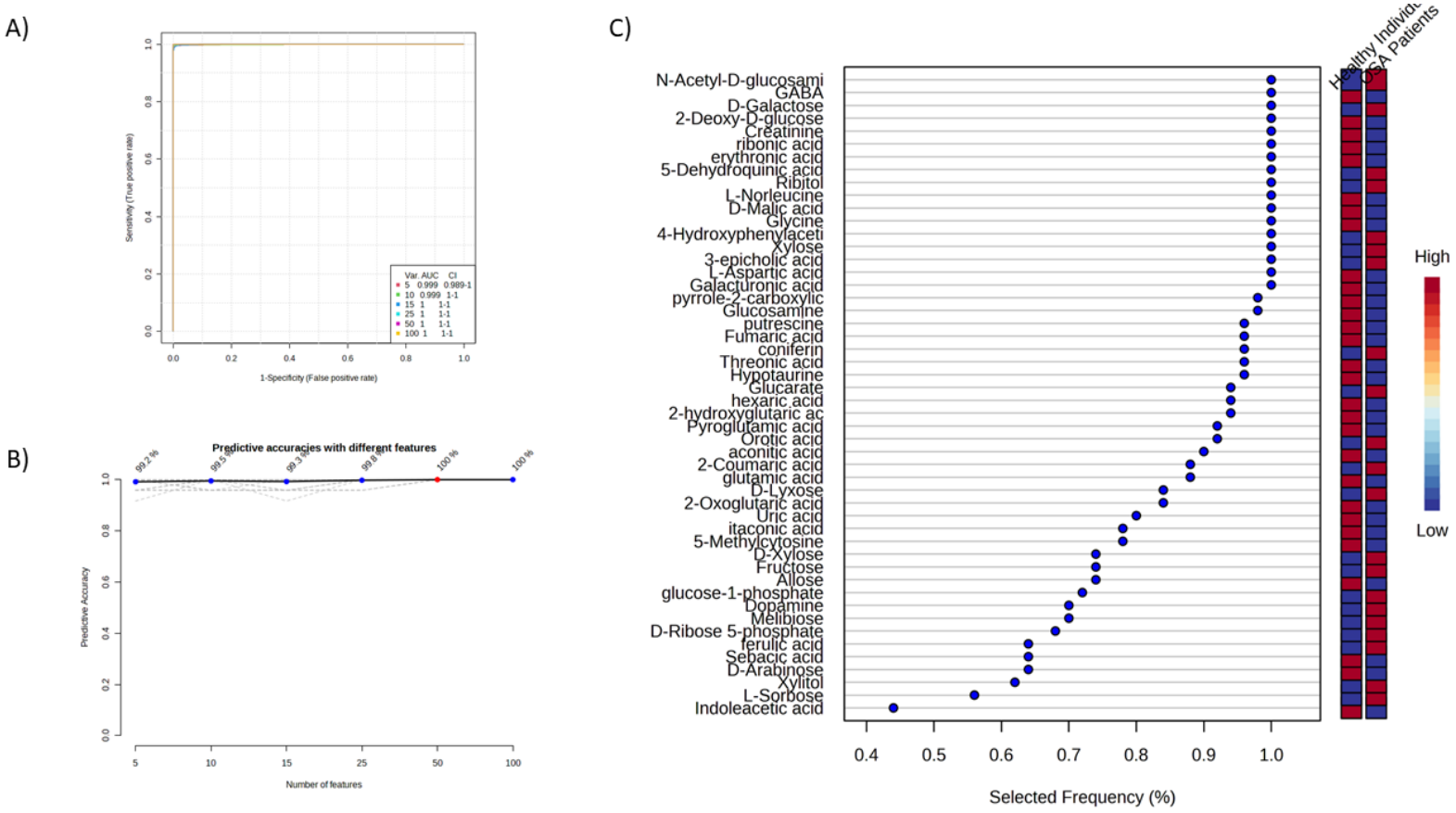
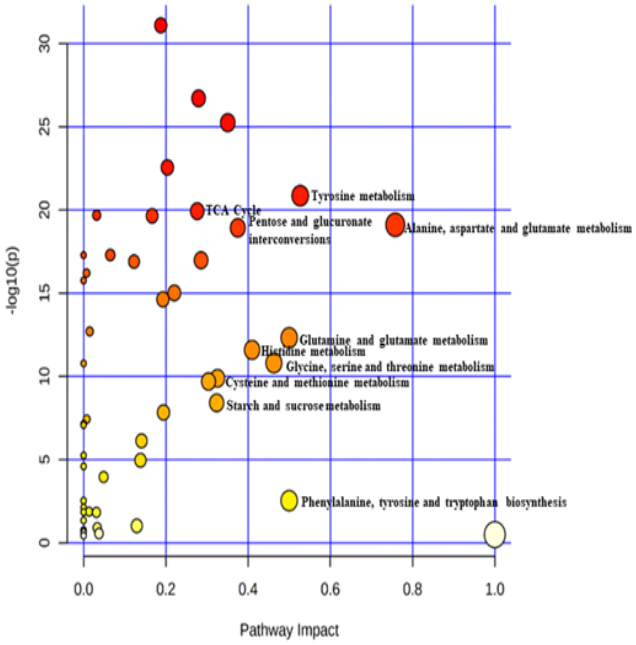

A)
Sensitivity (True positive rate)
1-Specificity (False positive rate)
Var. AUC CI
5 0.999 0.989-1
10 0.999 1-1
15 1 1-1
25 1 1-1
50 1 1-1
100 1 1-1
B)
Predictive accuracies with different features
99.2 %
99.5 %
99.3 %
99.8 %
100 %
100 %
Predictive Accuracy
Number of features
C)
N-Acetyl-D-glucosami
GABA
D-Galactose
2-Deoxy-D-glucose
Creatinine
ribonic acid
erythronic acid
5-Dehydroquinic acid
Ribitol
L-Norleucine
D-Malic acid
Glycine
4-Hydroxyphenylaceti
Xylose
3-epicholic acid
L-Aspartic acid
Galacturonic acid
pyrrole-2-carboxylic
Glucosamine
putrescine
Fumaric acid
coniferin
Threonic acid
Hypotaurine
Glucarate
hexaric acid
2-hydroxyglutaric ac
Pyroglutamic acid
Orotic acid
aconitic acid
2-Coumaric acid
glutamic acid
D-Lyxose
2-Oxoglutaric acid
Uric acid
itaconic acid
5-Methylcytosine
D-Xylose
Fructose
Allose
glucose-1-phosphate
Dopamine
Melibiose
D-Ribose 5-phosphate
ferulic acid
Sebacic acid
D-Arabinose
Xylitol
L-Sorbose
Indoleacetic acid
Healthy Individu
OSA Patients
High
Low
Selected Frequency (%)

**Figure 3**




| | Total Cmpd | Hits | Raw p | FDR | Impact | Status in OSA |
|---|---|---|---|---|---|---|
| Alanine, aspartate and glutamate metabolism | 28 | 11 | 8.30E-20 | 4.70E-19 | 0.75802 | Down |
| Tyrosine metabolism | 42 | 11 | 1.37E-21 | 1.75E-20 | 0.52693 | Up |
| Glutamine and glutamate metabolism | 6 | 3 | 4.94E-13 | 1.26E-12 | 0.5 | Down |
| Phenylalanine, tyrosine and tryptophan biosynthesis | 4 | 1 | 0.00296 | 0.003972 | 0.5 | Down |
| Glycine, serine and threonine metabolism | 33 | 4 | 1.60E-11 | 3.56E-11 | 0.46284 | Down |
| Histidine metabolism | 16 | 5 | 2.59E-12 | 6.28E-12 | 0.40983 | Up |
| Pentose and glucuronate interconversions | 18 | 4 | 1.15E-19 | 5.85E-19 | 0.375 | Up |
| Citrate cycle (TCA cycle) | 20 | 7 | 1.39E-20 | 1.18E-19 | 0.35061 | Down |
| Cysteine and methionine metabolism | 33 | 5 | 1.26E-10 | 2.57E-10 | 0.32573 | Up |
| Starch and sucrose metabolism | 18 | 4 | 3.78E-09 | 7.14E-09 | 0.3236 | Up |

**Figure 4**

**Table-1: Intergroup Comparison of Study Characteristics between Case & Control group.** The significant differences were found between case and control groups for the characteristics age (older in case group, p<0.001), weight (heavier in case group, p<0.001), neck size (thicker in case group, p<0.001), BMI (all the cases of BMI>35 kg/m2 belong to the case group, p<0.001), hypertension (all the hypertensive cases belong to the case group, p<0.001), High risk of OSA (all the higher risk belong to case group, p<0.001), EPWORTH STOP BANG (case group contained all the high change of dosing, p<0.001) and BERLINE STOP BANG (All the cases at higher risk, p<0.001)

| **Variable** | | **Case group** | **Control group** | **p-value** |
|---|---|---|---|---|
| Age | years | 49.5 ± 12.7 | 38.6±3.6 | <0.001* |
| Height | cm | 170.2 ± 7.7 | 169.3 ± 6.5 | 0.605 |
| Weight | kg | 93.5 ± 15.3 | 73.6±11.2 | <0.001* |
| Neck Size | cm | 43.3 ± 1.6 | 33.5±1.7 | <0.001* |
| Gender | Male | 36 (100%) | 36 (100%) | NA |
| | Female | 0 (0.0%) | 0 (0.0%) | |
| BMI (>= 35 kg/$m^2$) | N (%) | 14 (38.9%) | 0 (0.0%) | <0.001* |
| Hypertension | N (%) | 7 (19.4%) | 0 (0.0%) | 0.006 |
| High Risk of OSA | N (%) | 36 (100%) | 0 (0.0%) | <0.001* |
| EPWORTH | would never dose | 0 (0.0%) | 29 (82.9%) | <0.001* |
| | slight change of dosing | 0 (0.0%) | 6 (17.1%) | |
| | moderate change of dosing | 0 (0.0%) | 0 (0.0%) | |
| | high change of dosing | 36 (100%) | 0 (0.0%) | |
| BERLINE (High Risk) | N (%) | 36 (100%) | 0 (0.0%) | <0.001* |

**Table 2.** List of metabolites have more then 2 fold up and down accumulation in OSA.

| **Metabolites Up accumulated in OSA** | | | | |
|---|---|---|---|---|
| Metabolite Name | FC | log2(FC) | p.ajusted | -log10(p) |
| Melibiose | 796.25 | 9.6371 | 1.80E-20 | 19.745 |
| 4-Hydroxyphenylacetic acid | 231.52 | 7.855 | 3.85E-15 | 14.414 |
| Orotic acid | 111.41 | 6.7997 | 5.05E-16 | 15.297 |
| Xylose | 95.688 | 6.5803 | 2.14E-26 | 25.67 |
| trisaccharide | 92.46 | 6.5308 | 4.79E-05 | 4.3201 |
| L-Homocarnosine | 85.337 | 6.4151 | 0.002106 | 2.6765 |
| 3-Methoxy-4-hydroxymandelate | 82.775 | 6.3711 | 2.83E-11 | 10.548 |
| L-Tryptophan | 55.808 | 5.8024 | 1.51E-08 | 7.8209 |
| coniferin | 52.618 | 5.7175 | 7.57E-16 | 15.121 |
| 3-epicholic acid | 51.05 | 5.6738 | 0.00336 | 2.4737 |
| Cystathionine | 44.99 | 5.4915 | 9.35E-17 | 16.029 |
| Sinapic acid | 37.151 | 5.2153 | 1.55E-08 | 7.8098 |
| 5-Hydroxy tryptamine | 35.138 | 5.135 | 9.53E-13 | 12.021 |
| D-Galactose | 28.557 | 4.8357 | 1.75E-25 | 24.756 |
| L-Glucose | 27.934 | 4.8039 | 0.005605 | 2.2514 |
| noradrenaline | 26.888 | 4.7489 | 0.026946 | 1.5695 |
| D-Ribose 5-phosphate | 25.17 | 4.6536 | 1.31E-16 | 15.882 |
| N-Acetyl glucosamine | 23.694 | 4.5665 | 1.82E-07 | 6.7411 |
| Fructose | 20.531 | 4.3597 | 1.95E-15 | 14.71 |
| Cysteamine | 16.984 | 4.0861 | 3.61E-05 | 4.4424 |
| N-Acetyl-D-glucosamine | 16.154 | 4.0138 | 5.38E-14 | 13.269 |
| Cytosine | 13.787 | 3.7852 | 0.000877 | 3.0571 |
| palmitic acid | 12.464 | 3.6397 | 2.44E-15 | 14.613 |
| Xylitol | 12.39 | 3.6311 | 1.38E-10 | 9.8604 |
| Xanthine | 10.108 | 3.3374 | 1.53E-10 | 9.815 |
| ferulic acid | 9.8097 | 3.2942 | 1.97E-05 | 4.7051 |
| DOPA | 8.3047 | 3.0539 | 1.77E-09 | 8.7514 |
| 5-Dehydroquinic acid | 7.8593 | 2.9744 | 1.34E-10 | 9.8736 |
| 4-hydroxyhippuric acid | 7.3625 | 2.8802 | 0.007349 | 2.1338 |
| L-Arginine | 7.0466 | 2.8169 | 9.48E-09 | 8.023 |
| Anthranilic acid | 6.8208 | 2.7699 | 6.94E-06 | 5.1584 |
| DL-Homocystine | 6.4895 | 2.6981 | 7.56E-10 | 9.1213 |
| heptadecanoic acid | 6.1081 | 2.6107 | 0.000243 | 3.6135 |
| glucose-1-phosphate | 5.9974 | 2.5843 | 1.06E-08 | 7.9758 |
| 2-Coumaric acid | 5.7832 | 2.5319 | 0.002351 | 2.6288 |
| D-Lyxose | 5.4425 | 2.4443 | 8.04E-12 | 11.095 |
| Tryptamine | 5.2003 | 2.3786 | 1.34E-10 | 9.8736 |
| Adenosine | 5.0082 | 2.3243 | 0.034102 | 1.4672 |
| phytosphingosine | 4.8165 | 2.268 | 3.55E-07 | 6.4502 |
| L-Sorbose | 4.5047 | 2.1714 | 9.48E-09 | 8.023 |
| mucic acid | 4.4482 | 2.1532 | 0.001171 | 2.9314 |
| p-hydroxylphenyllactic acid | 4.2222 | 2.078 | 3.31E-11 | 10.481 |
| Glucarate | 4.0666 | 2.0238 | 0.00628 | 2.202 |

| | | | | |
|---|---|---|---|---|
| D-Galactosamine | 3.8541 | 1.9464 | 1.81E-07 | 6.7425 |
| 2-Amino isobutyric acid | 3.8514 | 1.9454 | 1.63E-10 | 9.7884 |
| L-Methionine | 3.7569 | 1.9095 | 0.005826 | 2.2346 |
| Sucrose | 3.6068 | 1.8507 | 2.73E-05 | 4.5641 |
| Cadaverine | 3.4421 | 1.7833 | 6.24E-05 | 4.2047 |
| Pantothenic acid | 3.3268 | 1.7341 | 1.42E-07 | 6.8483 |
| D-Xylose | 3.2225 | 1.6882 | 5.94E-08 | 7.2265 |
| Fructose | 3.0607 | 1.6138 | 6.85E-09 | 8.1645 |
| Galactitol | 3.0504 | 1.609 | 0.000818 | 3.0874 |
| L-Serine | 3.0219 | 1.5955 | 0.021295 | 1.6717 |
| $\alpha$-Lactose | 3.0185 | 1.5938 | 4.79E-05 | 4.3201 |
| Tagatose | 2.9871 | 1.5788 | 0.000475 | 3.3235 |
| Asparagine | 2.9179 | 1.5449 | 0.000236 | 3.627 |
| Dopamine | 2.8843 | 1.5282 | 2.01E-05 | 4.6975 |
| Lysine | 2.836 | 1.5039 | 1.68E-05 | 4.7758 |
| Histamine | 2.779 | 1.4746 | 7.90E-06 | 5.1026 |
| Maltose | 2.7151 | 1.441 | 0.008608 | 2.0651 |
| Myristic acid | 2.6333 | 1.3969 | 0.000131 | 3.8811 |
| 1-Methyl Histidine | 2.6071 | 1.3824 | 0.000106 | 3.9743 |
| Hippuric acid | 2.5255 | 1.3366 | 0.004532 | 2.3437 |
| Shikimic acid | 2.5056 | 1.3252 | 0.010761 | 1.9682 |
| Xanthurenic acid | 2.3754 | 1.2482 | 4.12E-07 | 6.3852 |
| Tyramine | 2.3435 | 1.2287 | 6.15E-05 | 4.2115 |
| Homovanillic acid | 2.2528 | 1.1717 | 0.007148 | 2.1458 |
| D-Mannitol | 2.1321 | 1.0923 | 0.001255 | 2.9014 |
| 5-Keto-D-Gluconic acid | 2.1059 | 1.0745 | 0.027984 | 1.5531 |

| **Metabolites Down accumulated in OSA** | | | | |
|---|---|---|---|---|
| Metabolite Name | FC | log2(FC) | p.ajusted | -log10(p) |
| Sebacic acid | 0.44505 | -1.1679 | 0.001075 | 2.9686 |
| Uridine 5'-diphospho-N-acetylglucosamine | 0.42604 | -1.2309 | 0.003283 | 2.4837 |
| L-Ascorbic acid | 0.41624 | -1.2645 | 0.04152 | 1.3817 |
| Glutamine | 0.39373 | -1.3447 | 0.000191 | 3.7194 |
| Glucosaminic acid | 0.39082 | -1.3554 | 0.012816 | 1.8922 |
| Suberic acid | 0.34361 | -1.5412 | 1.23E-05 | 4.9097 |
| Threose | 0.32408 | -1.6256 | 0.000713 | 3.147 |
| $\beta$-Ketoadipic acid | 0.31015 | -1.6889 | 2.31E-05 | 4.6358 |
| Mannose | 0.28347 | -1.8188 | 0.001229 | 2.9105 |
| Rhamnose | 0.27699 | -1.8521 | 0.00072 | 3.1428 |
| 5-hydroxymethyl-2-furoic acid | 0.27471 | -1.864 | 0.002998 | 2.5232 |
| Allose | 0.24933 | -2.0038 | 0.000101 | 3.9946 |
| Diethanolamine | 0.18586 | -2.4277 | 0.005537 | 2.2567 |
| 2-Hydroxyglutaric acid | 0.17046 | -2.5525 | 1.88E-05 | 4.7265 |
| Glucosamine | 0.15637 | -2.677 | 0.000168 | 3.7737 |
| Threonic acid | 0.15271 | -2.7111 | 0.001739 | 2.7598 |
| Glutamic acid | 0.14587 | -2.7772 | 2.12E-10 | 9.6743 |

| | | | | |
|---|---|---|---|---|
| L-5-Oxoproline | 0.14287 | -2.8072 | 0.04278 | 1.3688 |
| Adipic acid | 0.13816 | -2.8556 | 0.010423 | 1.982 |
| 2-Aminoethanol | 0.12557 | -2.9935 | 0.019788 | 1.7036 |
| Lauric acid | 0.10888 | -3.1992 | 7.06E-07 | 6.1511 |
| Maleic acid | 0.099277 | -3.3324 | 1.42E-07 | 6.8483 |
| D-Ribose | 0.096738 | -3.3698 | 0.003136 | 2.5036 |
| D-Arabinose | 0.087692 | -3.5114 | 2.05E-09 | 8.6881 |
| Aconitic acid | 0.083117 | -3.5887 | 6.64E-13 | 12.178 |
| Erythritol | 0.082806 | -3.5941 | 0.007349 | 2.1338 |
| Ribonic acid | 0.081425 | -3.6184 | 8.75E-18 | 17.058 |
| Itaconic acid | 0.073281 | -3.7704 | 9.79E-08 | 7.0094 |
| 3,4-Dihydroxyphenylacetic acid | 0.073282 | -3.7704 | 0.000913 | 3.0397 |
| Fumaric acid | 0.07192 | -3.7975 | 0.001743 | 2.7588 |
| D-Xylulose | 0.070873 | -3.8186 | 5.59E-05 | 4.2529 |
| 2-Oxoglutaric acid | 0.065997 | -3.9215 | 5.21E-11 | 10.283 |
| 6-Aminohexanoic acid | 0.063712 | -3.9723 | 4.68E-05 | 4.3294 |
| hexaric acid | 0.058923 | -4.085 | 0.00809 | 2.0921 |
| 4-aminophenol | 0.050177 | -4.3168 | 0.001477 | 2.8306 |
| oxoproline | 0.047635 | -4.3918 | 0.008359 | 2.0778 |
| D-Malic acid | 0.042961 | -4.5408 | 6.64E-13 | 12.178 |
| D-Ribulose | 0.040557 | -4.6239 | 2.13E-10 | 9.6711 |
| 2-Deoxy-D-glucose | 0.032073 | -4.9625 | 1.49E-22 | 21.826 |
| Citramalic acid | 0.030889 | -5.0168 | 6.36E-07 | 6.1965 |
| L-Norleucine | 0.030626 | -5.0291 | 2.78E-14 | 13.555 |
| 5-Aminopentanoic acid | 0.029048 | -5.1054 | 0.000322 | 3.4916 |
| 4-Hydroxy-3-methoxymandelic acid | 0.028502 | -5.1328 | 8.07E-08 | 7.0933 |
| Threonic acid | 0.028264 | -5.1449 | 3.78E-11 | 10.422 |
| Pyroglutamic acid | 0.028085 | -5.1541 | 0.000818 | 3.0874 |
| erythronic acid | 0.021986 | -5.5073 | 7.56E-10 | 9.1213 |
| Creatinine | 0.020881 | -5.5817 | 1.93E-16 | 15.714 |
| Uridine | 0.017883 | -5.8053 | 0.010423 | 1.982 |
| GABA | 0.014812 | -6.0771 | 2.19E-15 | 14.659 |
| 5-Methylcytosine | 0.014783 | -6.0799 | 1.68E-05 | 4.7758 |
| Hypotaurine | 0.011465 | -6.4466 | 3.44E-15 | 14.464 |
| Tyrosine | 0.010223 | -6.612 | 0.005102 | 2.2922 |
| Pyrogallol | 0.007247 | -7.1085 | 0.000295 | 3.5298 |
| L-Aspartic acid | 0.006131 | -7.3497 | 5.15E-08 | 7.2883 |
| Galacturonic acid | 0.005524 | -7.5 | 1.76E-06 | 5.7553 |
| Glycine | 0.004688 | -7.7368 | 1.42E-07 | 6.8483 |
| Uric acid | 0.004257 | -7.8759 | 0.005863 | 2.2319 |
| putrescine | 0.002612 | -8.5805 | 1.68E-08 | 7.775 |
| Isocitric acid | 0.000895 | -10.125 | 9.78E-07 | 6.0099 |
| Indoxyl sulfate | 0.00042 | -11.218 | 1.21E-05 | 4.919 |
| pyrrole-2-carboxylic acid | 0.000304 | -11.686 | 2.31E-05 | 4.6358 |