**1H NMR Based Serum Metabolic Profiling Reveals Differentiating Biomarkers in Patients with Diabetes and Diabetes Comorbidity**

Atul Rawat1,2, Gunjan Misra2,5$, Madhukar Saxena2$, Sukanya Tripathi3, Durgesh Dubey1,2, Sulekha Saxena4, Avinash Aggarwal4, Varsha Gupta3,M Y Khan2 and Anand Prakash2,6#

1Centre of Biomedical Research, Lucknow; 2Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow; 3Department of Zoology, Lucknow University, Lucknow; 4Department of Critical Care, King George Medical University, Lucknow; 5Department of Biotechnology, CSJMU, Kanpur; 6Department of Biotechnology, Mahatma Gandhi Central University, Bihar India

**Author’s Contributed Equally** -: **$**

**Corresponding Author** -:

 Dr.Anand Prakash,

Dean, School of Life Sciences,

Professor & Head, Department of Biotechnology,

Mahatma Gandhi Central University, Bihar, INDIA

E-mail: anandprakash@mgcub.ac.in

Tel (Off): +91-9628282357

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**Abbreviations:** NMR: Nuclear Magnetic Resonance; CPMG: Carr–Purcell–Meiboom–Gill; DB: Diabetes, DC: Diabetes-related Co-morbidity, ROC: Receiver operating characteristic; AUROC; area under the ROC curve; RF; Random Forest; ESM: Electronic Supplementary Material.

**Table S1:** Metabolites used in each group for pathway analysis in the metabolic pathway analysis (MetPA) module of MetaboAnalyst.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **#** | **Metabolite** | **DB vs. HC** | **DC vs. HC** | **DC vs. DB** |
| 1 | LDL | - | - | - |
| 2 | Iso/ | Isoleucine | Isoleucine | x |
| 3 | Leucine | Leucine | Leucine | x |
| 4 | Valine | x | x | Valine |
| 5 | LDL/VLDL | - | - | - |
| 6 | Arginine | x | Arginine | Arginine |
| 7 | NAG | - | - | - |
| 8 | Glutamate | Glutamate | Glutamate | Glutamate |
| 9 | Acetoacetate | x | Acetoacetate | x |
| 10 | Pyruvate | x | x | Pyruvate |
| 11 | Succinate | x | x | Succinate |
| 12 | Glutamine | Glutamine | Glutamine | x |
| 13 | Citrate | Citrate | Citrate | Citrate |
| 14 | Methylamine | Methylamine | Methylamine | x |
| 15 | Methionine | Methionine | x | Methionine |
| 16 | Trimethylamine | x | Trimethylamine | x |
| 17 | Creatine | Creatine | x | Creatine |
| 18 | Creatinine | Creatinine |  | Creatinine |
| 19 | Betaine | Betaine | Betaine | x |
| 20 | Glucose | Glucose | Glucose | Glucose |
| 21 | Lactate | x | Lactate | x |
| 22 | Proline | x | x | Proline |
| 23 | Threonine | Threonine | Threonine | Threonine |
| 24 | PUFA  | - | - | - |
| 25 | Phenylalanine | Phenylalanine | Phenylalanine | x |
| 26 | Histidine | Histidine | Histidine | X |

**Note:** LDL, VLDL, PUFA and NAG are not recognized by the programme thus were excluded from the analysis. Represents- x = Metabolite not found significant in RF analysis. - = Metabolite not recognized by the programme.

**Table S2:** Correlation Analysis (CA) was performed in statistical analysis module of MetaboAnalyst. CA was performed for the significant metabolites perturbed in DC compared to the DB group.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Proline** | **Methylamine** | **Citrate** | **Succinate** | **Glutamate** | **NAG** | **Arginine** | **Valine** | **V/LDL** |
| **PUFA** |  |  |  |  |  |  |  |  | 0.74 |
| **Threonine** | 0.76 |  |  |  |  |  |  |  |  |
| **Methionine** |  | 0.76 | 0.77 |  | 0.74 |  |  |  |  |
| **Methylamine** |  |  | 0.80 | 0.87 | 0.81 |  | 0.85 | 0.61 |  |
| **Citrate** |  |  |  | 0.75 | 0.76 |  | 0.75 | 0.64 |  |
| **Succinate** |  |  |  |  | 0.82 |  | 0.81 | 0.61 |  |
| **Glutamate** |  |  |  |  |  | 0.79 | 0.79 |  |  |
| **Arginine** |  |  |  |  |  |  |  | 0.82 |  |

PUFA showed positive (+ve) CA with LDL/VLDL;

Threonine showed +ve CA with proline;

Methionine showed +ve CA with Methylamine, Citrate, Glutamate;

Methylamine showed +ve CA with Citrate, Succinate, Glutamate, Arginine, Valine;

Citrate showed +ve CA with Succinate, Glutamate, Arginine, Valine;

Succinate showed +ve CA with Glutamate, Arginine, Valine;

Glutamate showed +ve CA with NAG, Arginine;

Arginine +ve CA with Valine.

**Figure S1:** Receiver operating characteristic (ROC) curve obtained from a combination of the significant metabolites identified via RF analysis in each group derived from the CPMG 1H NMR spectra.

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