^{1}H-NMR Metabonomic View on Schizophrenia

10th IAPC Meeting

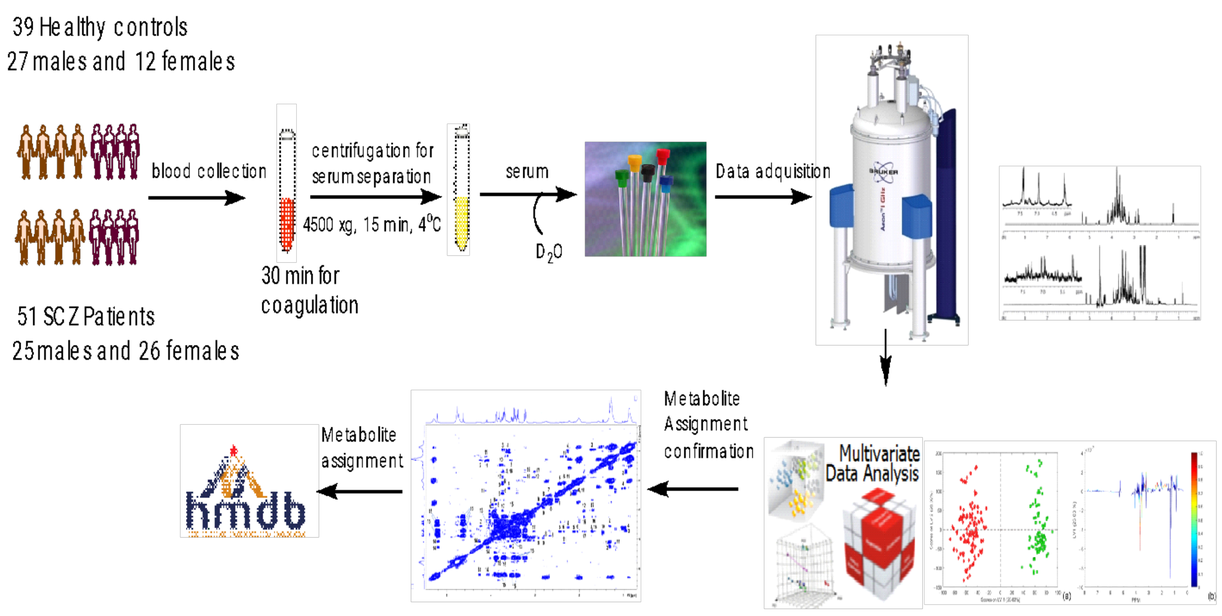
Nataša Avramović[1], Katarina Simić[2], Zoran Miladinović[3], Nina Todorović[2], Snežana Trifunović[4], Aleksandra Gavrilović[5], Silvana Jovanović[5], Dejan Gođevac[2], Ljubodrag Vujisić[4], Vele Tešević[4], Ljubica Tasic[6] and Boris Mandić[4]

[1]University of Belgrade-Faculty of Medicine, Institute of Medical Chemistry, Višegradska 26, 11000 Belgrade, Serbia;
[2]Institute of Chemistry, Technology and Metallurgy, National Institute, University of Belgrade, Studentski trg 12-16, 11000 Belgrade, Serbia;
[3]Institute of General and Physical Chemistry, Studentski trg 12-16, 11158 Belgrade, Serbia;
[4]University of Belgrade - Faculty of Chemistry, Studentski trg 12-16, 11000 Belgrade, Serbia;
[5]Special Hospital for Psychiatric Diseases "Kovin", Cara Lazara 253, 26220 Kovin, Serbia;
[6]Institute of Chemistry, Organic Chemistry Department, State University of Campinas, Campinas 13083-970, SP, Brazil.

INTRODUCTION

Schizophrenia (SCZ) is a brain disease leading to significant functional impairments and premature death, and it affects 20 million people worldwide. Due to the complexity of this disease including different genetic and environmental factors, there is a lack in understanding pathophysiology and diagnosis of schizophrenia. In order to overcome existing gaps, the establishment of a universal set of SCZ biomarkers has a crucial role.

METHODOLOGY



RESULTS

The set of 26 potential serum SCZ biomarkers were identified by performing analyses of spectral 1D and 2D NMR data accomplished in TOCSY, JRES and HSQC experiments and associated with chemometrics.

Statistical Analysis

The PCA model using Pareto scaling with mean centering data accounted for a total variance of 90.95%, and the first two components provided a very good separation between the two main classes (Figure 1). The most positive contribution to the PC 2 loading graph (Figure 1b), corresponding to the class 'Schizophrenia', could be identified around 1.33 ppm (doublet: 1.32 ppm; 1.34 ppm) and around 4.11 ppm (quartet: 4.09 ppm; 4.10 ppm; 4.12 ppm; 4.13 ppm), which could be assigned to the signals of lactate, and in the area between 3.71 to 3.61 ppm typical for sugar molecules.

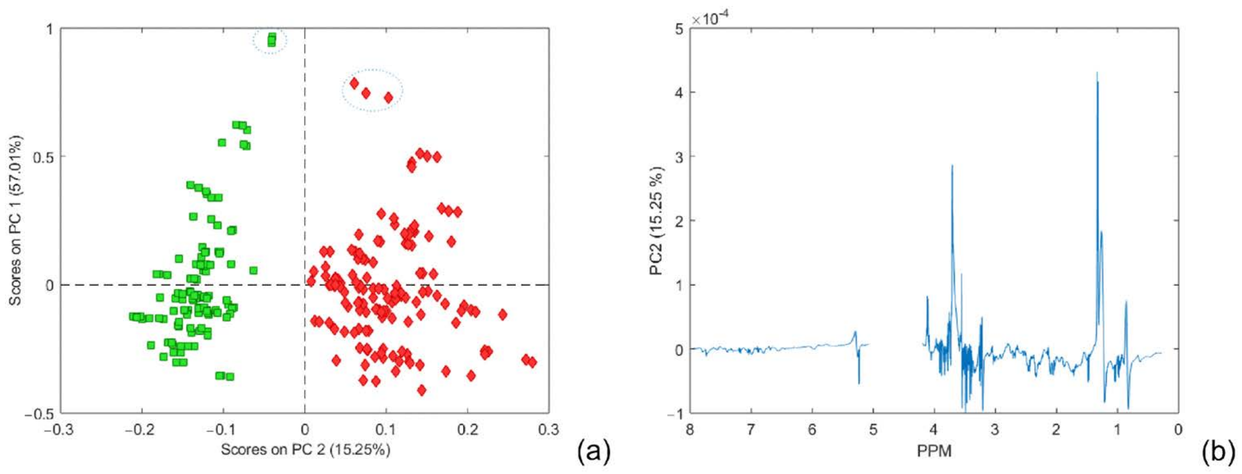


Figure 1. (a) PCA score plots of the first two components. The schizophrenia cohort is shown in red, and the control group in green. (b) PC 2 back-scaled projection of loading coefficients. The empty part of the loading plot belongs to the water resonances region.

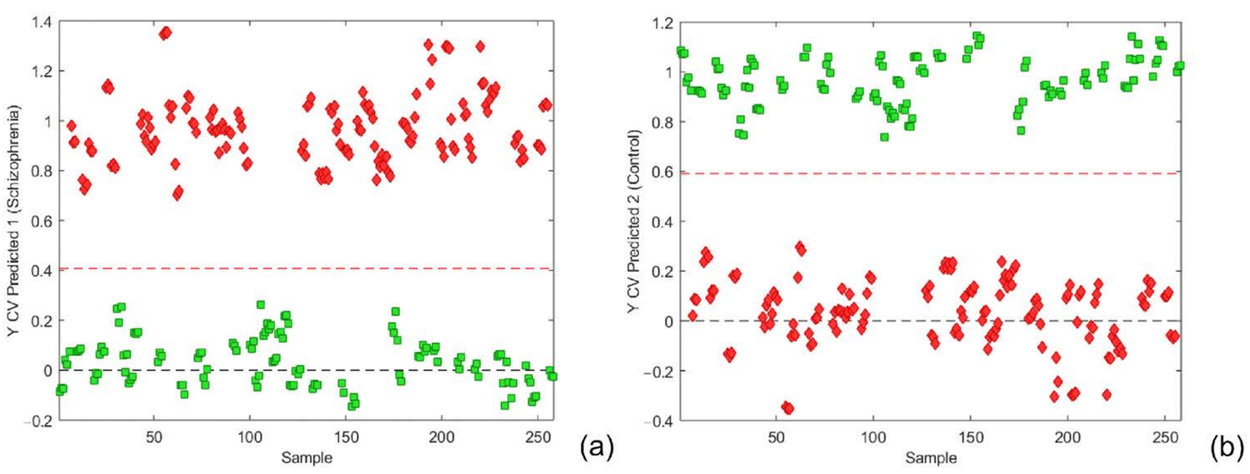


Figure 2. (a) Y CV Predicted for the class 'Schizophrenia' and threshold value of 0.4086; (b) Y CV Predicted for the class 'Control' and threshold value of 0.5914 using autoscaling. The schizophrenia cohort is shown in red and the control group in green.

OBJECTIVES

Metabonomic study of serum samples of Serbian patients with schizophrenia (51) and healthy controls (39) by ^{1}H-NMR analyses associated with chemometrics was explored in order to identify potential biomarkers for diagnosis of SCZ, reliable monitoring of treatment response and clinical outcomes.

RESULTS

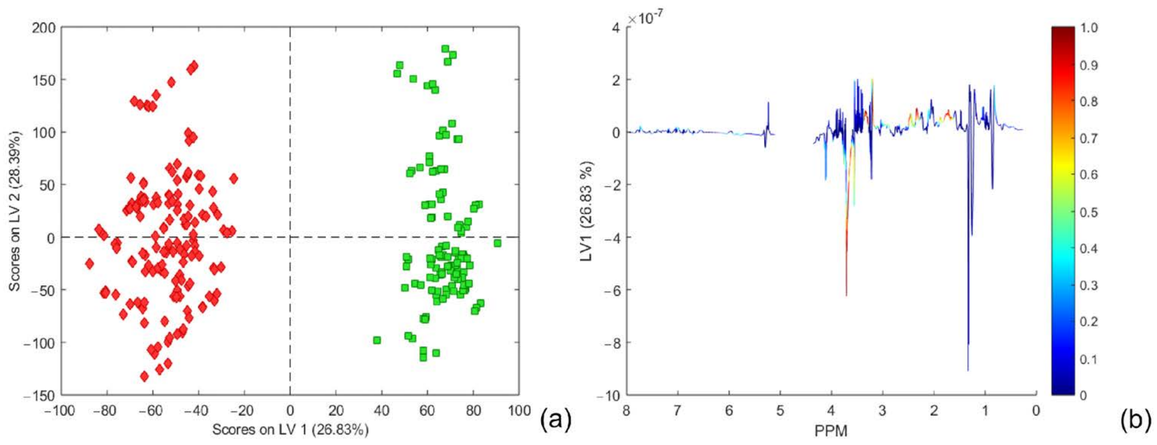


Figure 3. (a) Score plots of the first two LV components of the OPLS-DA model using mean-centering and unit variance scaling (for a 4-component model, RMSEC = 0.0934 and RMSECV = 0.1304). The schizophrenia cohort is shown in red, and the control group in green. (b) Back-scale projection of loading vector LV 1 to coloring coded according to the absolute value of the particular loading weighted by correlation of the spectral data set and score matrix from the OPLS-DA model.

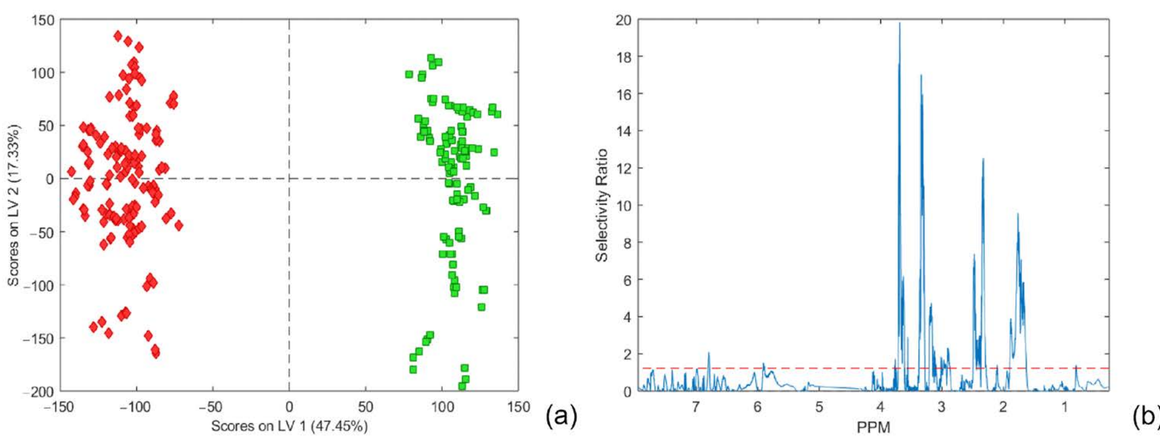


Figure 4. (a) Score plot of the first predictive LV 1 and the first orthogonal LV 2 components (for a 4-component model, RMSEC = 0.0845 and RMSECV = 0.1071). The schizophrenia cohort is shown in red, and the control group in green. (b) Selectivity ratio plot.

In accordance with the results of chemometric analyses, the identification of metabolites as potential biomarkers in blood samples of patients with schizophrenia from a Serbian cohort was performed based on analyses of spectral 2D NMR data obtained in TOCSY, 2DJ, and HSQC experiments. TOCSY spectral data (Figure 5) led to the identification of 20 metabolites, while 25 metabolites were identified based on 2DJ experiments

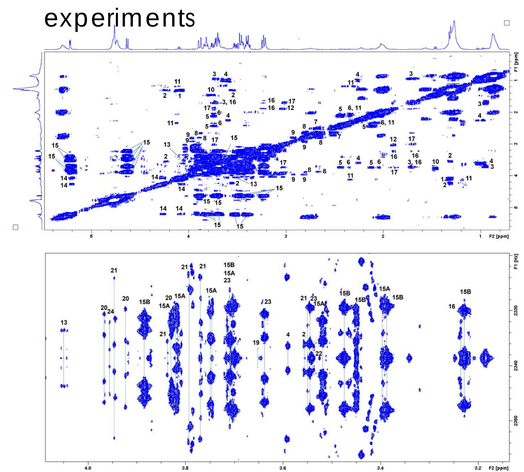


Figure 5. TOCSY (upper) panel) and 2DJ (lower) panel) spectra of one sample from the Serbian cohort of schizophrenia patients in 0.7–5.5 ppm and 3.1–4.1 ppm, respectively.

CONCLUSIONS

NMR-based serum metabolomics of a cohort of schizophrenia patients from Serbia established a set of 26 biomarkers for schizophrenia. The majority of identified metabolites are equal to the previous reports in Brazil and China on schizophrenia, which opens up a possibility for using these biomarkers as disease markers for diagnostics purposes. Furthermore, four metabolites, aspartate (aspartic acid), lysine, 2-hydroxybutyric acid, and acylglycerols, were identified for the first time in serum samples from this Serbian cohort of patients with schizophrenia based on NMR analyses associated with chemometrics

ACKNOWLEDGMENTS

We kindly acknowledge Organization for the Prohibition of Chemical Weapons (Project L/ICA/ICB-217652/18 and L/ICA/ICB-108/21) and the Ministry of Education and Science of the Republic of Serbia (Contract numbers: 451-03-68/2022-14/200168, 451-03-68/2022-14/200026, 451-03-68/2022-14/200051 and 451-03-9/2022-14/200110) for financial supports.

REFERENCES

Simić, K.; Todorović, N.; Trifunović, S.; Miladinović, Z.; Gavrilović, A.; Jovanović, S.; Avramović, N.; Gođevac, D.; Vujisić, L.; Tešević, V.; et al. NMR Metabolomics in Serum Fingerprinting of Schizophrenia Patients in a Serbian Cohort. *Metabolites* **2022**, *12*, 707.

Tasic, L. et al. Metabolomics and lipidomics analyses by ^{1}H nuclear magnetic resonance of schizophrenia patient serum reveal potential peripheral biomarkers for diagnosis. *Schizophr Res.* **2017**,*185*, 182.

Tasic, L.; Larcerda, A.T.L.; Pontes, J.G.M.; da Costa, T.B.B.C.; Nani, J.; Martins, L.G.; Santos, L.; Nunes, M.F.Q.; Adelino, M.P.M.; Pedrin, M.; et al. Peripheral biomarkers allow differential diagnosis between schizophrenia and bipolar disorder. *J. Psychiatr. Res.* **2019**, *119*, 67.

Pontes, J.G.M.; Brasil, A.J.M.; Cruz, G.C.F.; de Souza, R.N.; Tasic, L. NMR-based metabolomics strategies: Plants, animals and humans. *Anal. Methods* **2017**, *9*, 1078.