Identification of Alcohol-Dependence Biomarkers in plasma by using Metabolomics Analysis

Hamza Mohamed Amin Mostafa[1], Arwa Mohamed Amin Mostafa[1], Nor Hayati Arif[2], Chin-Hoe Teh[3], Vikneswaran a/l Murugaiyah[1], Baharudin Ibrahim[1]*

[1]School of Pharmaceutical Sciences, Universiti Sains Malaysia, Malaysia
[2]Psychiatry Department, Hospital Pulau Pinang, Malaysia
[3]Bruker (Malaysia) Sdn Bhd

Background: The main clinical methods to diagnose Alcohol-dependence (AD) in clinical practice currently depend on AD assessment questionnaires and some biomarkers such as Carbohydrate-Deficient Transferrin (CDT), Gamma Glutamyl Transferase (GGT) and Phosphatidylethanol (PEth). These two methods have been shown to have lack of specificity and sensitivity. Metabolomics technique by using nuclear magnetic resonance spectroscopy (NMR) of plasma can help us to identify novel biomarkers which could help in the more accurate diagnosis of AD.

Objectives: The main objective of this study is to identify a metabolic fingerprint (metabotype) in plasma that is able to discriminate between the alcohol dependent, the social drinkers non-dependent and the controls using metabolomics approach.

Methods: Blood samples were collected from 30 alcohol-dependent (mean age: 45.7), 54 social drinkers (mean age: 39.5) and 60 controls (mean age: 37.1). Plasma was separated by centrifugation and mixed with phosphate buffer which was prepared by dissolving di-sodium hydrogen Phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 (TSP), sodium azide (NaN_3) and deuterium oxide (D_2O) and then analyzed using NMR spectroscopy. Data analysis was done using multivariate analysis including principal component analysis (PCA) and orthogonal partial least square discriminate analysis (OPLS-DA) to develop a model to identify AD biomarkers.

Results: In PCA score plot, outliers were excluded from the three groups. PCA-X plot showed a similarity between the social drinkers and the controls groups, however, Alcohol-dependent group was clearly discriminated from them. After the combination of social drinkers and controls groups in one group, the OPLS-DA was done by comparing the combined group to the alcohol-dependent group. The OPLS-DA model showed a clear separation between the two groups (R^2= 0.567 and Q^2= 0.549) with 98.18% specificity, 62.07% sensitivity, and 90.65% accuracy.

Conclusion: The applied plasma metabolomics technique could differentiate with good accuracy between alcohol-dependent and the social drinkers and controls. The identification of the discriminating metabolites is ongoing.

Keywords: Alcohol-Dependence, Metabolomics, Phenotyping, Nuclear Magnetic Resonance, Diagnosis, TSP, D_2O.

*Corresponding author **:** baharudin.ibrahim@usm.my, Phone +604-6535839, Fax +604-6534594