METABOLOMICS ANALYSIS OF PLASMA SAMPLES FROM PATIENTS WITH SJÖGREN'S SYNDROME WITH ^{1}H NMR

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Sjögren's syndrome (SS) is a systemic inflammatory autoimmune disease, more prevalent in women, which presents with excessive oral and eye mucosal dryness. Although often under-diagnosed, SS is a relatively common disease, which develops concurrently with other autoimmune diseases, commonly lupus and rheumatoid arthritis. Despite extensive research, the pathogenesis is not yet well understood. Biopsy of the labial minor salivary gland (LMSG) remains the single most important test in the diagnosis of SS, with the focus score (the cluster of ≥50 lymphocytes in a 4 mm^2 glandular section, FS) considered as the gold standard. High throughput technologies in combination with clinical data can provide insight into disease mechanisms. Plasma metabolomics offers a non-invasive, holistic approach that could aid in diagnosis, differentiation and disease monitoring.

Plasma samples were collected from 76 patients with sicca symptoms from the special clinic of General Hospital of Athens "Laiko". The patients were classified based on LMSG biopsies into the following groups: Control group with FS<1 (n=40), Low Focus Score group (LFS, n=18) with FS 1-3, and High Focus Score group (HFS, n=18) with FS > 3. Plasma metabolic profiling was achieved by means of ^{1}H 1D NMR spectra, applying special filtering pulse sequences for small molecule (LED) and macromolecules (CPMG). Spectra features were transformed to numerical data, subsequently normalized, scaled, and subjected to both univariate (t-test, one-way ANOVA, ROC) and multivariate (unsupervised, supervised) analytical approaches.

Spectral data analysis resulted in the identification of 40 metabolites including amino acids and derivatives, ketone bodies, hydrocarbons, polyols, and organic acids. Applying SMolESY software, 22 metabolites were also quantified. In LED spectra, signals of characteristic chemical groups and molecular moieties of lipoproteins were observed, providing additional information. Both statistical analyses were informative of the similarity of the investigated groups: no clustering of the entire metabolic profiles were observed, nor statistically significant changes in metabolite levels. However, specific trends were detected: (1) aromatic amino acids, alanine and creatinine were relatively increased in LFS, (2) isoleucine, valine and glycine showed reduced levels in HFS, while (3) choline and acetic acid were elevated in HFS. Interestingly, FS was found highly correlated with ^{1}H NMR fingerprint.

Metabolomics in plasma of SS patients showed trends of altered metabolite levels in groups defined according to FS. These results are in accordance with the existing literature in blood, which is based on more sensitive techniques (GC-MS) and investigate changes in comparison with a healthy population. Currently, research in SS disease is mainly focused on saliva, being the biofluid closely related to the affected glands. However, the limited salivary availability of these patients, calls for the discovery of sensitive markers in plasma.