Characterization of Glycoprotein and Lipoprotein Profiles of Rheumatoid Arthritis (RA) patients by 1H-Nuclear Magnetic Resonance Spectroscopy (1H-NMR).

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Background

Rheumatoid Arthritis (RA) is an autoimmune and chronic inflammatory disease associated with a high index of morbidity and mortality by cardiovascular diseases (CVDs). 1H-NMR is a technique capable of determining the lipoprotein and glycoprotein profiles to characterize dyslipidemias and to estimate the cardiovascular and to evaluate inflammatory state.

Recent studies have established the importance of glycoproteins in important biological processes: cell adhesion, transport, signal transduction and, especially, control of cellular inflammation. The proton nuclear magnetic resonance spectroscopy (1H-NMR) is emerging as a technique able to detect levels of circulating glycoproteins in a quick and accurate way [2].

Aim

This study aims to characterize by 1H-NMR the plasma glycoprotein profile of patients with RA versus healthy individuals and to identify patterns indicating the severity of the disease.

Methodology

Serum samples of 214 RA patients and 203 healthy individuals matched by sex, age and body mass index were analysed by 1H-NMR. The glycoprotein and lipoprotein [1] profile was characterized by the study of the 1H-NMR spectrum of both groups. A mathematical treatment of the signal was conducted as follows: the raw spectra was fitted with first analytic Lorentzian/Gaussian functions reproducing the peak associated with the glycoproteins (LMW, Glyc-B, Glyc-A, and Baseline) based on their chemical shift. From each of these functions the total area and height (proportional to the concentration), the position (characteristic of the magnetic environment) and the width (related to the flexibility and the aggregation state of the molecules generating the signal) were determined. For each function we calculated the derived parameters Height/Width/Height to capture the shape of the peaks. On the other hand, the traditional inflammatory markers such as C-reactive protein (CRP), (immunoglobulin k-mioeglobulin (IgM)) and the erythrocyte sedimentation rate (ESR) were determined. Lipid variables such as total cholesterol, LDL (low density lipoprotein) cholesterol, HDL (high density lipoprotein) cholesterol, very low density lipoproteins (VLDL), total triglycerides (TG), apolipoprotein AI (ApoA1) and apolipoprotein B (ApoB) were also determined by traditional biochemical methods.

• Univariate statistical analysis of the glycoprotein variables was conducted to identify differences between the RA patients group and the healthy individuals group.
• Associations between the lipid variables and inflammatory markers determined by biochemical methods and the 1H-NMR glycoprotein profile were also studied.
• A Partial Least-Squares-Discriminant Analysis (PLS-DA) was conducted to identify the characteristic glycoprotein profiles associated with the AR group and the healthy group.
• Different PLS-DA models were evaluated to build the best predictive model for the highest RA disease severity individuals according to the DAS28 index value (>75th Percentile) by using the traditional inflammatory markers and 1H-NMR parameters (glycoprotein and lipoprotein) as input variables.

Results

We found statistically significant differences in several 1H-NMR glycoprotein’s peak variables highlighting the values of total area, Height/Width Glyc-A and Height/Width Glyc-B variables between the AR patients and the control group (p values 0.127; 0.125 and 0.127 respectively) (Figure 1). The PLS-DA could perfectly discriminate the AR and healthy groups (Figure 2); Figure 3 depicts the contribution of each variable (loadings) on the maximized variance between the two groups.

The multivariate study showed that including glycoproteins to traditional inflammation parameters improved the activity classification and severity of RA being the area under the curve validated ROC curve 0.79 (Figure 4) while only with the traditional inflammatory markers was 0.74 (Figure 5).

Conclusions

The 1H-NMR is a useful technique to identify an atherogenic / pro-inflammatory profile in patients with RA. The glycoprotein and lipoprotein profiles extracted from 1H-NMR spectra, along with the classic inflammatory parameters, provide more accurate information about the cardiometabolic status of RA disease as well as severity and activity of the disease.

References