Metabolic Characterization of Asymptomatic Sickle Cell Disease Patients Using ^{1}H Magnetic Resonance Spectroscopy

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INTRODUCTION

Sickle Cell Disease (SCD) is a genetic disorder in which, a single aminoacid substitution in the β-chain of hemoglobin leads to intracellular polymerization and subsequent vaso-occlusion[1]. Pathological changes in a number of metabolites has been described in SCD using different biochemical assays and despite our increasing understanding of pathophysiology, SCD continues to affect millions of people worldwide. Understanding intrinsic variability is important for diagnostic purposes but the quantity of variables involved in the analysis adds extra complexity to this subject.

Magnetic Resonance Spectroscopy (MRS) of biofluids and tissues has been shown to be a powerful tool in the diagnosis of a number of diseases where a metabolic fingerprint provides a single and useful source of information[2]. Magnetic Resonance techniques in SCD have so far focused on the use of imaging modalities to assess central nervous system damage and ^{1}H MRS has been used to study hemoglobin S polymers and 2,3 DPG metabolism[3, 4]. To our knowledge there has been no study to evaluate multivariate metabolic differences between SCD patients and control subjects nor correlating these changes to pathophysiology of the disease. The aim of our study was to assess differences in the metabolic profile of whole blood extracts from asymptomatic SCD patients and control subjects and to correlate these changes in pathophysiology. We also tested Principal Components Analysis (PCA) to extract relevant features of our data. Our results shown that significant differences can be found in the pattern of asymptomatic SCD patients ^{1}H MRS profile compared to control subjects and PCA can be used to further explore the source of those differences.

METHODS

Blood was drawn from 11 asymptomatic SCD patients and 16 volunteers. Blood proteins were removed from whole blood using 100% acetonitrile. After removal of the acetonitrile the samples were made up in D_2O which contained a small amount of sodium trimethylsilyl proprionate as a frequency reference and quantification standard. Samples were run on a Varian Unity Plus 500MHz NMR spectrometer (Varian Inc, Palo Alto, Ca) using a 45° pulse, 6kHz sweep width, 32k data points and 256 scans at 25°C with a water presaturation pulse. A line broadening of 1 Hz was applied prior to peak integration using SpecNMR software (JEOL UK, Welwyn Garden City, UK). Statistical processing and principal component analysis (PCA) was carried out using available commercial programs and an in-house written Matlab code (for PCA). All clinical procedures were ethically approved by the Universidad de Oriente.

RESULTS

^{1}H MRS profiles of asymptomatic SCD patients and control subjects were remarkable similar under visual inspection and resonances from valine, 3-hydroxybutyrate (3HB), lactate, alanine, acetate, creatine, creatinine, N-methyl-containing compounds (N-Met-C) and glucose were assigned. No signal arising from glutathione was found probably because it was removed in the extraction. Significant differences were found for valine, alanine, acetate, 3HB and N-Met-C compounds ($p<0.05$), see Table 1. Lactate, creatine, creatinine and glucose did not reach significance, however a PCA which including all these metabolites shown good correlation between lactate, glucose and valine as the major sources of variability included in principal component 1, Figure 1.

DISCUSSION

Our results showed that ^{1}H MRS profile of extracted blood is different between asymptomatic SCD patients and control subjects. These differences probably reflect different composition of membranes and lipid turnover and changes in amino-acid metabolism associated to the primary defect in hemoglobin. However no single signal can be used as a biomarker of the disease. Multivariate analysis reveals that despite the fact that lactate and glucose did not reach significance, these two metabolites with valine are the major sources of variability in our database. This fact anticipates its role in differentiating classes between asymptomatic SCD patients and control subject enhancing the discriminatory power of the ^{1}H MRS profile.

CONCLUSION

We had shown that ^{1}H MRS profile of asymptomatic SCD patients is significantly altered and that exploratory analysis like PCA in combination with ^{1}H MRS may help to elucidate the origin of changes in the disease. Also, reduction of dimensionality can be possible in the dataset. Further analysis should be done including more patients to identify other important metabolites like glutathione and ergothionine and to discriminate classes between asymtomatic SCD patients and control subjects, as well as those patients in crisis.

REFERENCES

1. Bookchin R.M., Lew V.L., *Hematol. Oncol. Clin. North Am.*, (1996), **10**, 1241-53.
2. Bell J.D. In de Certaines JD, Bovée WMMJ and Podo F (eds). Pergamon Press Ltd: Oxford, (1992); 529-557
3. Schrader MC, Simplaceanu V, Ho C., *Biochim. Biophys. Acta*, (1993), **1182**, 178-88.
4. Lam Y.F., Lin A.K., Ho C., *Blood*, (1979), **54**, 196-209.

	Control	SCD Patients
Valine	0.22±0.09	0.07±0.05
3HB	0.05±0.03	0.03±0.03
Lactate	1.19±0.45	2.32±2.30
Alanine	0.44±0.13	0.24±0.19
Acetate	0.09±0.05	0.20±0.05
Creatine	0.34±0.09	0.35±0.24
N-Met-C	0.03±0.01	0.05±0.04
α-Glucose	2.83±0.82	2.42±1.32

Table 1. Concentrations of a number of blood metabolites quantified from the ^{1}H MRS spectroscopy data. All concentrations are in mM ±SD.

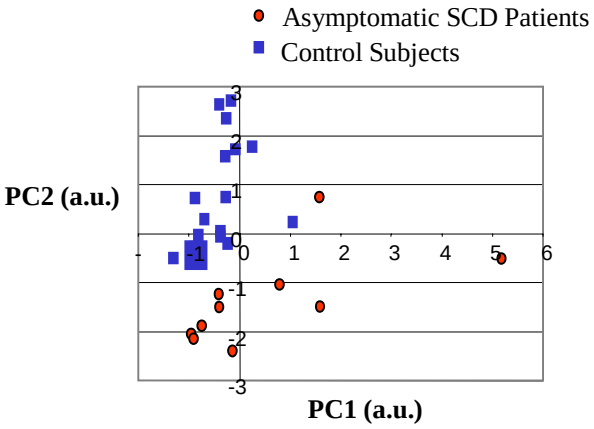

Figure 1: PCA analysis of selected metabolites