Magnetic resonance spectroscopy of the brain in children with sickle cell disease

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ABSTRACT

الأهداف: دراسة موجودات ونتائج الرنين المغناطيسي الطيفي MRS لمخ مرضى الأنيميا المنجليه (SCD).

الطريقة: شملت هذه الدراسة 22 مريض بالأنيميا المنجليه SCD تتراوح أعمارهم ما بين 6 إلى 17 عام. قمنا باستبعاد من لديه تغير حديث في وظائف الدماغ. تم تقييم 22 مريض في مجموعة التحكم تتراوح أعمارهم ما بين 7 إلى 19 عام (13 ذكر و 9 أنثى). تم إجراء MRI وMRS لجميع المرضى.

النتائج: ارتفاع نسبة إن أستيل أسبرتات(NAA) في الكتل العصبية القاعدية في مرضى الأنيميا المنجليه مقارنه بالأفراد الطبيعيين. و ارتفاع نسبة إن أستيل أسبرتات(NAA) إلى الكولين أيضا (p=0.012) عند استخدام الصدى القصير و كذلك الطويل p=0.016. نسبة الكولين إلى الكرياتين كانت متقاربة في المرضى و الأشخاص الطبيعيين.

خاتمة: يرتفع أستيل أسبرتات (NAA) في الأطفال المرضى بالأنيميا المنجليه SCD بدون إصابة جديدة للمخ مما يشير إن أستيل أسبرتات (NAA) كمؤشر لحيوية العصب محل شك و بحث.

Objective: To study the findings of magnetic resonance spectroscopy (MRS) of the brain in patients with sickle cell disease (SCD).

Methods: This study was carried out from January 2007 to February 2009, in the Radiology Department of King Fahd Military Medical Complex, DhahranKingdom of Saudi Arabia. The study consists of 22 patients with SCD ranging from 6-17 years, excluding those with a recent change in brain function. Twenty-two control subjects ranging from 7-19 years (13 boys, 9 girls) were also evaluated. An MRI and MRS were carried out for all patients.

Results: Patients had a proportion of N-acetyl aspartate (NAA) in the basal ganglia that was higher than that of healthy control subjects. A higher ratio of NAA to choline (Cho) in patients compared to control subjects (p=0.012) was shown on short-echo and long-echo spectra (p=0.016). The ratio of Cho to creatine (Cr) was similar in patients and control subjects.

Conclusion: The NAA is strikingly increased in the brain spectra of children with SCD, with no recent brain insult, questioning what is known of it as an indicator of neuronal viability.

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Sickle cell disease (SCD) is a chronic hemolytic anemia that includes the different hemoglobin (Hb) variants. It is one of the most common genetic diseases worldwide. It is seen commonly in sub-Saharan Africa, but also occurs in the Mediterranean, India, and the Arabian Peninsula.[1] A high prevalence of SCD is present in the Kingdom of Saudi Arabia (KSA), being common in the eastern and southern regions of the country.[2] A higher prevalence of imaging abnormalities in patients with SCD was detected by Steen et al,[3] compared with earlier studies.[4,5] Magnetic resonance spectroscopy (MRS) uses the signal from hydrogen protons to detect the concentration of brain metabolites, a unique character that states it as a complement to MRI for non-invasive characterization of disease, especially the CNS disorders.[6] The aim of this work is to study the MRS findings of the brain in SCD patients, and to detect the difference of brain metabolites between normal children and children suffering from SCD.

Methods. Our study was conducted from January 2007 to February 2009, in King Fahd Military Medical

Complex, Dhahran,, Kingdom of Saudi Arabia. It consists of 22 patients ranging from 6-17 years (15 boys, 7 girls). Pediatric patients with SCD, yet, without recent brain insult (within the past 6 months), according to the patients' clinical profiles, were included in the study, and underwent MRI. If the MRI was positive for brain lesions in any of these patients, they were excluded from the study. Only patients with normal MRI proceeded to MRS. Nineteen patients did not receive any treatment, and 3 were receiving transfusion at the time of evaluation. Twenty-two control subjects ranging from 7-19 years (13 boys, 9 girls) were also evaluated. The ages of the selected patients and control subjects were not specified. They were chosen from the normal workflow in the pediatric clinics, if their parents or guardians agreed to the performance of the MR study. Fortunately, no patients required sedation for the examination. Parents or guardians of all subjects provided written informed consent after they received a brief description of the protocol. A letter of consent from the hospital's ethical committee for performance of the study was obtained prior to study commencement.

MR imaging. The MR images were obtained using a 1.5-T unit (Signa; GE Medical Systems, Milwaukee, Wisconsin, USA). The standard imaging protocol for our department included T1-weighted, T2-weighted, fluid attenuation inversion recovery (FLAIR) imaging in transverse orientation. The MR imaging was used to screen for brain injury. The T1-weighted gradient-echo image set was acquired with the following parameters: TR/TE = 175/ 3.77, section thickness = 5 mm, field of vision (FOV) = 21 cm, matrix = 256 x 256, sections = 19, distance factor = 0.2 and time for one acquisition = 1 minute, 42 seconds. The T2-weighted turbo spin-echo image set was acquired using the following parameters: TR/TE1/TE2 = 3500/16/109, section thickness = 5 mm, FOV = 21 cm, matrix = 256 x 256, sections = 19, distance factor = 0.2, and time for one acquisition = 4 minutes, 7 seconds. Inversion-recovery sequence parameters were as follows: TR/TE/TI = 2500/20/100, 500, 900, and 2389; section thickness = 5 mm; FOV = 21 cm; and time for acquisitions = approximately 4 minutes.

MR spectroscopy. The MRS was carried out using a double spin-echo pulse sequence with water suppression. We used single-voxel spectroscopy using point-resolved spectroscopy (PRESS) technique. The region of interest was centered in the basal ganglia. Appropriate automatic shimming and water suppression were achieved using a 4-8-Hz line width, 1-kHz spectral width, and the automated software supplied by the manufacturer. The time domain signal intensity was processed to avoid the residual water. Several MRS sequences provided by the manufacturer and modified by us, to show different metabolites, were included: 1) Short-echo sequence with a TR/TE/NEX of 1500/30/128 to show lipids and other metabolites; 2) Long-echo sequence with a TR/TE/NEX of 1500/144/128 to show major metabolites against a flat baseline; and 3) In some subjects (12 patients and 12 control subjects), a sequence with a TR/TE/NEX of 5000/144/40 to show most metabolites in the fully-relaxed state with minimal metabolite saturation. This sequence was repeated again without water suppression (NEX = 1) using the water signal intensity as an internal concentration standard for metabolites.[7]

Data analysis. The resonance areas were integrated using standard software provided with the machine (Signa; GE Medical Systems, Milwaukee, WI, USA). Automatic processing developed by the manufacturer includes frequency shift, and phase, and linear baseline corrections after Fourier transformation. In a few cases, spectra were distorted and manual processing was used, particularly for phase and baseline corrections. Resonance areas proportional to metabolite concentration were determined by fitting Gaussian peaks to the spectral data. Use of ratios for metabolite quantitation can compensate for differences in coil loading or magnetic field homogeneity.[8]

Statistical analysis. The Mann-Whitney test, a nonparametric test, was used to test for significant differences in metabolite measurements when comparing the MRS findings of control subjects and selected SCD children. The level of statistical significance was set at $p<0.05$.

Results.

Metabolite levels. The selected patients had a proportion of NAA in the basal ganglia higher than that of the healthy control subjects. In addition, the ratio of NAA/Cho differed between long and short-echo sequences (**Figures 1**). Short-echo spectrum (**Figure 2**) showed a higher ratio of NAA to Cho in patients compared to control subjects ($p=0.012$). Long-echo spectra confirmed this finding ($p=0.016$). The ratio of Cho to Cr was similar in patients and control subjects. The Cho concentration was not reduced in the patients. Comparable brain Cho values between patients and control subjects would confirm that the brain tissue of patients with SCD has more NAA.[9] Lipid resonances did not differ between the 2 groups, but the central gray matter of all subjects had lipid levels higher than those reported in spectra from adults.

Discussion.

The high prevalence of SCD in KSA, especially in the eastern and southern regions of the country, was detected in a national wide community-based survey.[2] Proton magnetic resonance spectroscopy (1H-MRS), has been used as a complement to MRI, to study the metabolic status of the brain.[10] However,

MRS has its limitations, and is not always specific but, in combination with clinical information and conventional MRI, can be very helpful in diagnosing different diseases.[6] N-acetylaspartate is the nervous system-specific metabolite that is synthesized from aspartate and acetyl-coenzyme A in neurons. It is a key link between different biochemical processes in CNS metabolism.[11] In our study, the concentration of metabolites in patients with SCD were within the range of reported values for the human brain,[7,12,13] except the significant increase in the ratio of NAA/Cho at short (**Figure 1**) and long TEs. Not only this, but also the NAA/Cr ratio was also significantly increased at a short TE, compared to control subjects. There was nearly no notable difference at long TE. These findings were consistent with that of Steen and Ogg[14] who found abnormally high level of NAA in SCD patients.

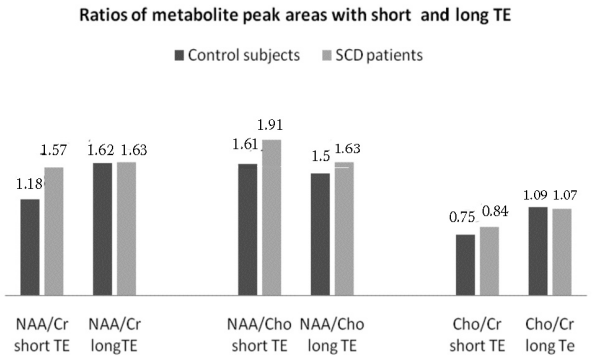

Figure 1 - Ratios of metabolite peak areas with short and long TE. Values are expressed in parts/million

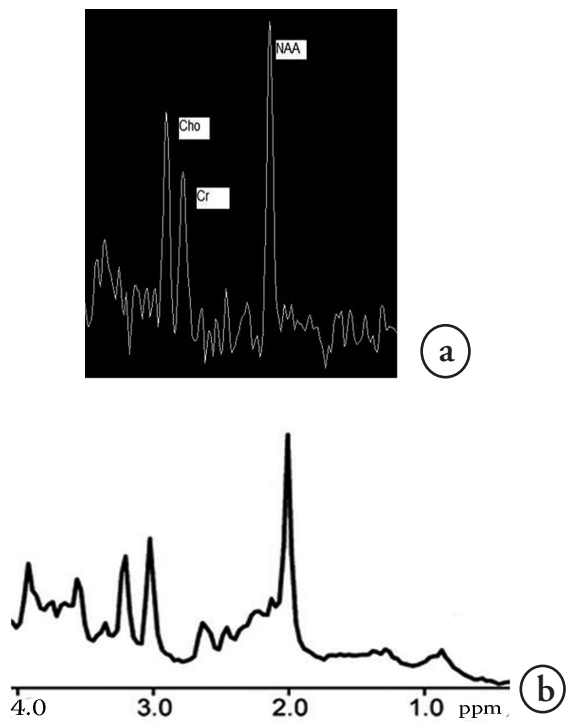

Figure 2 - Magnetic resonance spectroscopic findings obtained in **a**) control subject, and **b**) patient with SCD using short-echo spectrum (TR/TE of 1500/30). Cho, Cr, NAA peaks are labeled in (a). Increased ratio of NAA/Cho in SCD patients compared to control subject is evident.

The NAA is a neuronal and axonal marker. Decreased NAA values represent either neuronal loss or neuronal dysfunction.[15] If NAA is a neuronal marker, patients would have been expected to have stable or reduced NAA levels, as children with SCD often have both brain injury and cognitive impairment.[3] Yet, this is not in agreement with our findings, or with that of Steen and Ogg,[14] who reported that their findings call into question the role of NAA as a marker of the number of viable neurons in brain tissue. Further more, others call into question the role of NAA as a neuronal marker. De Stefano et al[15] found that the NAA peak transiently decreased after acute brain injury, Grodd et al,[16] Takanashi et al,[17] and Deicken et al,[18] reported that NAA levels can be chronically elevated in disease states not associated with neuronal proliferation, including Canavan disease, Pelizaeus-Merzbacher disease, and familial bipolar I disorder. So, they suggest that NAA is not a reliable marker of viable neurons.

The possible explanations of our results of increased NAA/Cho ratios in SCD patients are; one explanation is the redistribution of NAA from the intracellular space to the extracellular space to maintain osmotic balance in tissue.[13,19,20] Yet, this is weakened by the findings of Verbalis,[21] which debates the involvement of NAA in brain osmoregulation. Another explanation postulated by Burlina et al[22] and Demougeot et al,[23] who concluded that mast cells and other immune cells may synthesize or store NAA, and thus leukocyte infiltration or microglial activation in SCD patients may play a role in the raised levels of NAA in the brains of sickle cell patients

A potential limitation of our study was the relative low number of SCD patients (22 cases). Using single-voxel MR spectroscopy with a relatively large voxel size, resulting in partial volume effect, furthermore, the presence of CSF within the voxel affects the level of metabolites, is another limitation. The last minor limitation was difficulty in achievement of magnetic field homogeneity, which was solved to a major extent by using ratios for metabolite quantitation.

In conclusion, NAA is strikingly increased in the brain spectra of children with SCD, with no recent brain insult, questioning what is known of it as an indicator of neuronal viability. Future studies must include a

larger patient population, using both short and long echo sequences. The use of multivoxel technique for MR spectroscopy must also be considered, along with the addition of perfusion and diffusion MR studies. A comprehensive prognostic study with comparison of the results of MR spectroscopy in patients with poor and good prognoses must also be included.

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