Proton MR and a New Software as Predictors for the Differentiation of Meningitis in Children

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INTRODUCTION

Meningitis is one of the most devastating infectious diseases of childhood in developing as well as in underdeveloped countries. The cornerstone of management of meningitis in children depends on the rapid diagnosis and prompt treatment. The differential diagnosis of meningitis in children has been reported to be difficult due to various non-specific clinical features. Several techniques are currently in use, but none of them is foolproof. The analysis of CSF is often crucial for the diagnosis of neurological disorders as the chemical components of CSF reflect the state of the CNS in healthy and diseased state[1]. The potential advantages of NMR and the persistent problems regarding the timely diagnosis of childhood meningitis have prompted us to explore ^{1}H NMR spectroscopy as a diagnostic utility based on the analysis of CSF from 191 cases to bring out an additional diagnostic tool involving the quantitative NMR information and the routine clinical symptoms features of patients in the form of an expert system MENEXSYS.

MATERIALS AND METHODS

The CSF samples of children (6-12 years of age) comprising bacterial meningitis (BM, n=85), tuberculous meningitis (TBM, n=47), viral meningitis (VM, n=35) and control cases (n=24) were obtained by lumbar puncture over a period of five years (1998-2003). The study was approved by the Institute's as well as the Hospitals' Scientific and Ethical Committees following established guidelines. The snap-frozen CSF sample (450 µl) was transferred to a 5 mm NMR tube containing 50 µl of D_2O for the purpose of internal locking and immediately subjected to NMR analysis. The NMR experiments were performed using Bruker's *Avance* DRX 300 MHz FT-NMR spectrometer equipped with a 5 mm multinuclear inverse probehead with a Z-shielded gradient, operating at a proton frequency of 300.13 MHz. ^{1}H NMR spectra were acquired with WATERGATE water suppression at 298K with 32768 data points, spectral width (SW) of 3591 Hz, 128 scans, with a total relaxation time of 7.68 s per scan, and the binomial 90° pulse was set to 32 ms, with a line-broadening of 0.30 Hz prior to Fourier transformation. The quantification of the metabolites in all 191 CSF samples was carried out using Bruker's NMRQUANT with respect to a known concentration of TSP (49 mg/dl). The percentage occurrence for eleven clinical symptoms, the seven unquantified metabolites as seen in the NMR spectra and the respective clinical data were further subjected to Kruskal-Wallis test (non-parametric ANOVA) for statistical significance followed by a post hoc Dunn's multiple comparisons test. The statistical significance for the twelve NMR-based quantified metabolites was determined by one-way ANOVA followed by a post hoc Student-Newman-Keuls multiple comparisons test, where a *P*-value of less than 0.05 indicated statistical significance in all. The data were subsequently subjected to DFA with a stepwise-forward variable selection procedure so as to define important descriptors for the differentiation of meningitis from the control group, followed by the discrimination of the three types of meningitis based on the discriminant function based Z-cut-off values. For the expert system development that would be responsible for any future prediction of the meningitis type, the classification logic has been divided into three phases as: (i). Differentiation of control from disease groups; (ii). Differentiation of tuberculous meningitis from bacterial and viral meningitis; (iii). Differentiation of bacterial from viral meningitis.

RESULTS

Resonance signals arising due to the presence of valine, leucine, isoleucine, 1,2-propanediol, β-hydroxy butyrate, lactate, alanine, acetate, acetone, acetoacetate, pyruvate, glutamine, citrate, creatine/creatinine, α,β-glucose, glycine, *myo*-inositol, tyrosine and formate were readily assigned at 300 MHz. The exchangeable proton at 5.76 ppm that could be detected by the use of WATERGATE ^{1}H NMR pulse sequence was assigned to the amide NH_2 of urea in all CSF samples. Cyclopropane was observed overall in 85.1% cases of TBM, and has therefore been concluded as a 'finger-print' marker for this disease class. Further, the presence of glutamine (not as a mixture with glutamate) in the meningitis CSF samples of children has also been established. The unique observation of glutamine in our study could be attributed to the acuteness of the disease. Further, it was observed that with an increase in the concentrations of urea, there was an associated increase in the concentrations of glutamine. Subjecting the data further to DFA involving the disease and control groups, a combination of the NMR-derived metabolite variables and the clinical symptom variables could classify overall 96.4% cases into the respective groups (F-Ratio: 10.85, P-value: < 0.001). When the non-tuberculous meningitis (BM+VM) and tuberculous meningitis cases were subjected to DFA, overall 77.2% cases could be classified into the respective groups (F-Ratio: 1.79, P-value: 0.03). Subjecting the bacterial meningitis and viral meningitis cases for DFA with clinical and NMR-derived metabolite variables, overall 84.2% cases could be classified into the respective groups (F-Ratio: 1.83, P-value: 0.04). The prediction possibility of the classification model was further checked with a 75/25 data split, by using 75% of the patients' data as training sets and the remaining 25% data as test sets. The test sets groups could be successfully classified as 85.7%, 66.7%, and 78.9% comprising control vs. BM+TBM+VM, TBM vs. BM+VM, and BM vs. VM, respectively, followed by 94.4%, 76.2%, and 87.7% for the training sets groups, respectively, thereby revealing an overall improvement in the differential diagnosis of meningitis when important clinical and NMR descriptors where combined together and taken for the analysis.

DISCUSSION

It has recently been observed that the analysis of CSF based on CSF cytology, sugar and protein content is not the primary information to serve for the purpose of differential diagnosis[2]. Our study has therefore involved a combination of clinical symptoms, various metabolite concentrations as observed through NMR and identification of fingerprint marker(s) as a 'method of choice' for a quick differential diagnosis of meningitis in children. Among the clinical symptom variables, DFA analysis defined that fever was necessary to separate out the disease cases from the control group, but it was ineffective for the differentiation of the TBM from non-TBM cases as well as for the differentiation of BM from VM cases. Similarly among the NMR-derived metabolite variables, the contribution of acetoacetate was noteworthy; moreover, presence of cyclopropane has further refined the results for the TBM group. For the purpose of prediction of the type of any new 'suspected to be meningitis' case, the respective mid-point cut-off values and discriminant function weights obtained for the clinical symptoms and NMR-derived metabolite variables were incorporated into the expert system MENEXSYS. It is hoped that MENEXSYS would simplify the analysis for the differential diagnosis of meningitis in children, besides being very user-friendly in the form of an easy-to-use Graphical User Interface[3].

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