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Serum matrix metalloproteinase-2: A potential biomarker for diagnosis of epilepsy

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ABSTRACT

Objectives: In this study, we evaluate the utility of serum metalloproteinase-2 (MMP-2) as a biomarker for the diagnosis of epilepsy.
Methods: We assessed serum MMP-2 levels in 233 epileptic and 97 healthy control subjects. Control subjects had no complaints or signs of neurological disorders for at least 12 months prior to serum collection. Serum MMP-2 levels were determined using the Luminex technology.
Results: Compared with controls, subjects with epilepsy had significantly lower serum MMP-2 concentrations ($P < 0.05$). There was no significant difference between males and females in either group ($P > 0.05$). Serum MMP-2 concentrations were highly correlated with age in both groups, and this correlation was strongest for males. When an MMP-2 cut-off value of 175.40 ng/ml was used, the sensitivity for distinguishing subjects with epilepsy from controls was 71.13% and the specificity was 62.66%.
Conclusions: Our results reveal that serum MMP-2 may be a potential biomarker for the diagnosis of epilepsy.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by recurrent seizures. The diagnosis of epilepsy is largely based on a patient's detailed, reliable medical history, and electroencephalogram (EEG), just assists in the diagnosis if positive. However, medical histories are not always sufficient, making diagnosis difficult in some cases. Therefore, exploring novel methods for the accurate diagnosis of epilepsy is of great importance.

Matrix metalloproteinases (MMPs) comprise a family of zinc-dependent proteases with a wide range of substrates. They are major executors of extracellular matrix (ECM) remodeling throughout the body and have complex functions under normal and pathological conditions (Yong, 2005; Yong et al., 2001). The most abundantly expressed MMPs in the brain are MMP-2 and MMP-9 (Ikonomidou, 2014). MMPs have been implicated in seizure-induced cell death, breakdown of the blood–brain barrier, neuroinflammation, and aberrant synaptic plasticity, all of which occur during epileptogenesis (Wilczynski et al., 2008; Yang et al., 2011; Yong, 2005). Furthermore, MMP-2 is activated in the brain in the kainic acid rat seizure model (Zhang et al., 2000). Thus, MMP-2 may be a potential epileptic biomarker for epilepsy in human serum. Recent studies indicate that MMP-9 is generally sensitive to seizures (Li et al., 2012; Suenaga et al., 2008; Wilczynski et al., 2008), however, serum MMP-2 levels in epilepsy patients have not been previously reported.

The objective of the current study is to evaluate the utility of serum MMP-2 measurement as a biomarker for the occurrence and severity of epilepsy. We measured MMP-2 concentrations in serum from epileptic and control subjects using the Luminex technology. We then examined the effects of several factors (gender, age, and clinical variables) on MMP-2 levels. Finally, we evaluated the diagnostic value of serum MMP-2 measurement.

2. Methods

2.1. Participants

All procedures described in this study were approved by the Research Ethics Committee of the Medical School of Sichuan

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University. Each participant provided written informed consent prior to study enrollment. Outpatients and inpatients were recruited from a tertiary epilepsy center between November 2010 and October 2014. All were pre-screened for eligibility by the attending physicians. If diagnostic and inclusion criteria were met (see below), a research assistant introduced the study and obtained informed consent.

All patients included in the study underwent a comprehensive clinical examination including a detailed medical history, a neurological examination, neuropsychological testing, an EEG, and neuroradiological studies. Epilepsy was diagnosed and classified according to criteria proposed by the International League against Epilepsy (1981). Patients were excluded from the study if they had: (i) had epilepsy surgery; (ii) a history of psychogenic seizures or uncertain diagnosis; (iii) an active neurological disorder; (iv) a history of alcohol or substance abuse in the previous 12 months; (v) other major neurological disabilities, including a learning disability; (vi) taken part in a study of a new anti-epileptic drug (AED); (vii) active comorbid psychiatric illness; (viii) taken psychotropic medication in the previous 6 months; (ix) seizures due to electrolyte disturbances, metabolic causes, drug intoxication, infections, encephalitis, trauma or abnormal MRI suggestive of acute brain diseases; or (x) withdrawn consent.

All participants were asked about their socio-demographic details, age at onset of seizures, seizure frequency, duration of the disease, type of seizures, and current and past AED use. Each patient was placed in one of the four prognostic categories according to seizure frequency, treatment response, and history. These categories were: (1) newly diagnosed epilepsy (NDE) (duration of less than 1 year and drug-naive), (2) epilepsy in remission (SR) (no seizures for more than 2 years), (3) non-drug-resistant (NDR) epilepsy (active or occasional seizures which, in the opinion of the attending physician, could be improved by treatment), and (4) drug-resistant (DR) epilepsy (active seizures which, in the opinion of the attending physician, were unlikely to improve with further changes in drug treatment).

The control group was comprised of ninety-seven clinically healthy adults with no history suggesting medical, neurological or psychiatric disorders.

2.2. Collection of serum

Five milliliters of blood was collected from the antecubital vein into vacutainer tube(s) without anticoagulants. Blood was incubated in an upright position at room temperature for 30 min to clot, then centrifuged (10 min at 3000 rpm). Serum was removed by aspiration, then flash-frozen in liquid nitrogen until assays were performed.

2.3. Measurement of serum MMP-2

MMP-2 levels were quantified using the magnetic Luminex screening assay and human premixed multianalyte kit (R & D Systems, USA and Canada, Europe, China) according to the manufacturer's instructions. This assay system has been independently validated as measuring serum MMP-2 levels accurately and with high reproducibility.

Prior to the assay, serum samples were diluted (15 μL serum in 75 μL of calibrator diluent RD6-52), which means serum samples were diluted 1 in 6 with calibrator diluent RD6-52. To prevent precipitation with storage, the microparticle cocktail was resuspended and 50 μL was placed in each well of a 96-well microplate. 50 μL of diluted standard or sample was then added to each well. Plates were covered with a foil plate sealer and incubated for 2 hours at room temperature on a horizontal orbital microplate shaker set (800 ± 50 rpm). A magnet was placed under the microplate to retain the beads in the wells, liquid was removed, and the beads were washed (100 μL Wash Buffer, 3 times). Next, diluted biotin antibody cocktail and diluted streptavidin-PE was added to each well step-by-step according to instructions. Microparticles were resuspended in 100 μL wash buffer and incubated for 2 min with shaking. Plates were read within 90 min using the Luminex FLEXMAP 3D (Merck Millipore, USA).

2.4. Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 21.0, SPSS Inc., Chicago, IL, USA). Continuous variables were compared using the Student's *t*-test and are reported as mean ± standard deviation. Categorical variables were compared using the Chi-squared test or Fisher's exact test, as indicated, and are displayed as percentages. MMP-2 levels were compared between the epileptic and control groups using the Student's *t*-test. The correlation between MMP-2 levels and age was assessed using the non-parametric Spearman's test. One-way analysis of variance (ANOVA) was used to assess differences between prognostic groups and seizure types.

As levels of MMP-2 were normally distributed (Kolmogorov–Smirnov test), multiple linear regression analysis was used to estimate the effects of different baseline risk factors on MMP levels. In this analysis, epilepsy duration (years), seizure frequency (seizures/month), age at onset (years), time since last seizure (days), and history of AED use (years) were used as continuous variables. All tests were 2-tailed, with clinical significance defined as values of $P < 0.05$.

The area under the receiver operator characteristic (ROC) curve (AUC) analysis was used to calculate the relationship between sensitivity and specificity for distinguishing the disease group from healthy controls, and hence evaluate the diagnostic performance of MMP-2 measurements. The 'optimum' cut-off value from the ROC curve is the point at which the sum of sensitivity and specificity is maximal.

3. Results

3.1. Demographics of study participants

Demographic details of patients with epilepsy and controls are presented in Table 1. Two hundred and thirty-three epileptic subjects (133 females and 100 males) and 97 controls (53 females and 44 males) provided data for the study. The age of subjects ranged from 5 to 79 years (27 ± 12 years) in the epilepsy group and 2 to 78 years (28 ± 17 years) in the control group. Age was not significantly different between the control and epilepsy groups ($P > 0.05$). Gender was not matched between epilepsy and control groups ($P < 0.05$).

Epileptic subjects had mean disease duration of 8.2 years (range 0.04–52 years). Seizures occurred at a rate of 0–30 per week. Patients' most recent seizures occurred between 1 day and 4.4 years prior to sample collection. Epilepsy was classified as generalized in 85 (36.5%) and localization-related in 148 (63.5%).

Of the epileptic subjects, only 42 were not taking AEDs and two were taking traditional Chinese medicines. Subjects who used AEDs had been taking them for 0–31 years (4.2 ± 5.7 years).

3.2. Effects of age and gender on serum MMP-2 levels

The age dependence of serum MMP-2 levels was also assessed (Fig. 1a). Linear regression and association analyses demonstrated that serum MMP-2 levels significantly decreased as a function of age in epileptic subjects ($R = -0.179$, Pearson correlation, $P = 0.006$)