Epilepsy Research (2013) **105**, 292—298

journal homepage: www.elsevier.com/locate/epilepsyres

The concentration of cell-free DNA in focal epilepsy

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Received 12 November 2012; received in revised form 21 January 2013; accepted 17 March 2013
Available online 11 April 2013

KEYWORDS
Cell-free DNA;
Neurodegeneration;
Inflammation;
Focal Epilepsy;
Etiology

Summary
Background: Increased production of cell-free DNA (cf-DNA), a marker of inflammation, cell death and degeneration, has been observed in stroke and severe traumatic brain injury among other medical conditions. The purpose of the present study was to evaluate the significance of cf-DNA in patients with focal epilepsy.
Methods: cf-DNA was measured in 167 consecutive well-evaluated patients with focal epilepsy (147 with refractory epilepsy). Epilepsy was characterized based on the patient history, electroclinical findings, neuroimaging results and etiology. 250 healthy individuals served as control subjects.
Results: The majority of the patients (125/167; 74.8%) had increased concentrations of cf-DNA. The median concentration of cf-DNA was significantly higher in the patients (0.867 μg/ml) compared to the control group (0.759 μg/ml) ($p < 0.001$). Symptomatic etiology was associated with increased concentrations of cf-DNA compared to probably symptomatic etiology ($p = 0.036$).
Conclusions: The study confirms that the release of cf-DNA is more active in symptomatic refractory focal epilepsy, whereas this process is less pronounced in patients with unknown cause of epilepsy.

Abbreviations: AED, antiepileptic drug; CD, celiac disease; cf-DNA, cell-free DNA; CNS, central nervous system; FLE, frontal lobe epilepsy; HS, hippocampal sclerosis; IL, interleukin; IL1-RA, interleukin 1 receptor antagonist; iNOS, inducible nitric oxide synthase; MRI, magnetic resonance imaging; NSE, neuron-specific enolase; OLE, occipital lobe epilepsy; PLE, parietal lobe epilepsy; S-100b, S-100b protein; SD, standard deviation; TBI, traumatic brain injury; TLE, temporal lobe epilepsy; TNF-α, tumor necrosis factor-α; UGT1A1, UDP-glucuronosyltransferase 1; XTLE, extra-temporal lobe epilepsy.

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http://dx.doi.org/10.1016/j.eplepsyres.2013.03.005

Introduction

Neurodegenerative and inflammatory reactions are stimulated in experimental and clinical studies of epilepsy. Refractory epilepsy with recurrent seizures may lead to progressive neurodegeneration and cell loss in the epileptic brain. The severity of seizures correlates with the degenerative processes, and the clinical evidence of neurodegenerative cascade in epilepsy is demonstrated with brain magnetic resonance imaging showing progressive atrophy of mesial temporal lobe of epilepsy patients.

Activation of inflammatory pathways is evident in experimental and human epilepsy; both acute and chronic proinflammatory reactions have been observed. Acute seizure disorders are associated with a rapid increase with proinflammatory cytokines, such as interleukin-6 (IL-6) (Lehtimäki et al., 2003; Alapirtti et al., 2009); this specific cytokine is also active in patients with chronic temporal lobe epilepsy (TLE) compared to extra-TLE (XTLE) (Liimatainen et al., 2009). IL-1β has pro-convulsant properties in limbic seizures, and inhibition of IL-1β activity has anti-convulsant effects (Vezzani et al., 2000; Ravizza et al., 2006).

Cell-free-DNA (cf-DNA) seems to reflect inflammation, cell death and tissue damage in several medical conditions (Tong and Lo, 2006; Tsang and Lo, 2007; Peters and Pretorius, 2011; Mittra et al., 2012). Cf-DNA was originally detected in fetal medicine and oncology but since then increased concentrations have been found in many other pathological states such as trauma, sepsis, myocardial infarction, transplantation and diabetes mellitus among others (Mittra et al., 2012). Two main sources of cf-DNA have been observed: apoptosis or necrosis and metabolic secretion from cells (Gahan et al., 2008), and concentrations of cf-DNA and proinflammatory cytokines has been shown to be correlated with each other (Mittra et al., 2012). Plasma DNA concentration was significantly increased in critically ill patients who died when compared to those who survived; this finding suggests that cf-DNA could be used as a prognostic biomarker (Wijeratne et al., 2004; Rhodes et al., 2006).

In the context of neurological disorders, increased concentrations of cf-DNA were associated with severity of stroke and severe traumatic brain injury (TBI) (Lam et al., 2006; Tsai et al., 2011; Campello Yurgel et al., 2007). In these studies, the measurement of cf-DNA was performed in the acute phase of the disease. However, increased concentrations of this marker of inflammation and denegeration have also been detected in chronic brain disorders, such as Friedrich ataxia or spinocerebellar ataxia (Swarup et al., 2011). It has to be emphasized that the finding is not specific for brain diseases and in some neurological conditions such as in Parkinson's disease no increased levels have been detected (Scalzo et al., 2009). Nevertheless, studies on the significance of cf-DNA in neurological disorders are few. No previous studies exist on the effect of seizures and epilepsy in the concentrations of cf-DNA. The purpose of the present study was to evaluate the significance of cf-DNA in focal epilepsy. Furthermore, we searched for the association between specific clinical features in patients with epilepsy and concentration of cf-DNA to find which clinical parameters would be associated with changed concentration of this marker.

Materials and methods

This prospective study included 167 consecutive patients with focal epilepsy attending the Outpatient Department of Neurology and Rehabilitation of Tampere University Hospital, Finland. Patient population represents the overall cohort of refractory epilepsy in our hospital district (population of 440,000) since practically all the patients with refractory epilepsy are treated in our center. 147 patients had refractory epilepsy according to the following definition: persistency of seizures after two different antiepileptic drugs (AEDs) (Kwan et al., 2010). The remaining 20 patients had gained seizure freedom before recruiting to the study or had new-onset epilepsy. Epilepsy was classified based on patient history, electroclinical findings, neuroimaging results and etiology. Focal epilepsy types were categorized to TLE, frontal (FLE), parietal (PLE), occipital (OLE), and multifocal epilepsy according to the ILAE guidelines (Commission on Classification, 1989). No primary generalized epilepsies existed in the study. The etiology was defined according to the brain magnetic resonance imaging (MRI), histological analysis of resected lesions and medical history into the following categories: hippocampal sclerosis (HS9, HS + dual pathology (HS associated with another brain lesion), cortical dysplasia (CD) (cortical dysgenesis, heterotopia, tuberosis sclerosis), other [tumor, vascular malformation, vascular lesion, trauma, other hippocampal abnormality, central nervous system (CNS) infection, local or diffuse atrophy, nonspecific signal change, demyelination or nonspecific gliosis], and probably symptomatic (cryptogenic, unknown) etiology. Data regarding autoimmune diseases, surgery for epilepsy or other lesional surgery were collected, as were data regarding epilepsy duration and seizure frequency for the previous one, three and 12 months. Informed consent was obtained from every patient, and blood samples were drawn. The study was approved by the Ethics

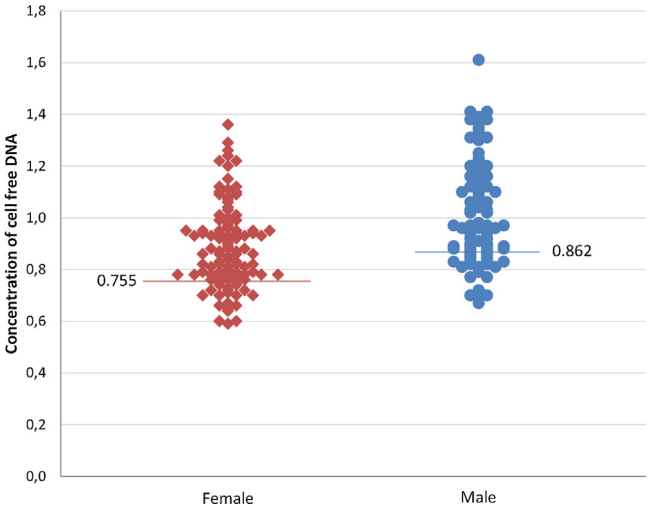

Figure 1 Concentration of cell-free DNA (μg/ml) in female and male patients with epilepsy. The majority of the patients (125/167; 74.8%) had increased concentrations of cf-DNA (μg/ml), median values. The figure presents the results of female and male patients. 80.2% of female and 68.4% of male patients had increased concentration of cf-DNA.