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Vascular risk factors and oxidative stress as independent predictors of asymptomatic atherosclerosis in adult patients with epilepsy

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KEYWORDS
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Summary
Purpose: This study aimed to evaluate the carotid artery intima-media thickness (CA-IMT) in adult patients with epilepsy and its contribution to oxidative stress and vascular risk biomarkers.
Methods: This study included 225 adult epileptic and 60 control subjects. For all, CA-IMT, fasting lipid profile (TC, TG, HDL-c and LDL-c), total homocysteine (tHcy), von Willbrand factor (vWF), fibrinogen, oxidized LDL (Ox-LDL), malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARs), uric acid, total antioxidant capacity (TAC) and glutathione peroxidase (GSH.Px), were assessed.
Results: Compared to control group, the IMT of patients' common carotid artery, bifurcation area and internal carotid arteries was significantly thickened in 51.1%, 73.3% and 43.6% in various groups of patients (treated and untreated). In the studied patients, the levels of tHcy, vWF, fibrinogen, MDA, TBARs, Ox-LDL levels were increased while HDL-c and TAC were decreased. Patients on CBZ showed the most significant changes in the levels of tHcy, vWF and HDL-c while patients on VPA showed significant alteration in uric acid, TBARs and GSH.Px.
Conclusion: This study supports that in patients with epilepsy, various vascular risk factors and CA-IMT, get worse which could be attributed to epilepsy itself and/or its antiepileptic medications.

Abbreviations: BMI, body mass index; AEDs, antiepileptic drugs; EI-AEDs, enzyme inducer AEDs; CBZ, carbamazepine; VPA, valproate; TC, total cholesterol; TG, triglycerides; LDL-c, low-density lipoprotein-cholesterol; HDL-c, high density lipoprotein-cholesterol; Ox-LDL, oxidized low density lipoprotein; MDA, malondialdehyde; TBARs, thiobarbituric acid reactive substance; GSH.Px, glutathione peroxidase; TAC, total antioxidant capacity; tHcy, total homocysteine; vWF, von Willbrand factor; ROS, reactive oxygen species; IMT, intima-media thickness; CCA, common carotid artery; ICA, internal carotid artery; CA-IMT, carotid artery IMT; MW, Mann—Whitney test

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Introduction

Epilepsy is a frequent chronic medical problem (Hauser et al., 1993). Neurologists have little concern about the prevalence of vascular risk factors and their contribution in the development of atherosclerosis among patients with epilepsy (reviewed in Hamed and Nabeshima, 2005). In the last decade, several data are available about number of vascular markers that are critically implicated in structural and functional changes of the vessel wall predisposing to atherosclerosis in patients with chronic epilepsy. Chronic epilepsy and prolonged antiepileptic drug (AED) treatment, particularly enzyme inducer AEDs (EI-AEDs; carbamazepine, CBZ, phenytoin, PHT and phenobarbitone, PB) have been found to induce hypercholesterolemia/dyslipidemia (Eris et al., 2000; Mahmoudian et al., 2005; Hamed et al., 2005), hyperhomocysteinemia (Schwaninger et al., 1999; Verrotti et al., 2000; Karabiber et al., 2003; Sener et al., 2006) and hyperuricemia (Hamed et al., 2004). The harmful effect of recurrent epilepsy and the vascular risk factors has been contributed to over production of reactive oxygen species (ROS), oxidation of low density lipoproteins (LDLs) into an atherogenic particles, a pivotal role in the pathogenesis of atherosclerosis (Heinecke, 1988).

In this study, we proposed to test the hypothesis that epileptic patients are at high risk to develop atherosclerosis. The central questions are:

(1) What is the prevalence of subclinical atherosclerosis and the possible vascular risk factors in patients with epilepsy with no previous history of vascular disease?
(2) What is the effect of epilepsy and long-term conventional AED therapy on vascular risk factors, lipid peroxidation and antioxidant biomarkers?

It has been known that increased arterial intima-media thickness (IMT) may pre-date the clinical manifestations of atherosclerosis by many years in subjects at risk of atherosclerosis (Raitakari, 1999). We therefore undertook an integral approach at investigating epileptic patients comparing them with a matched control group, by measuring the carotid artery IMT (CA-IMT) using duplex ultrasound. We also investigated the influence of epilepsy and conventional antiepileptic drug (AED) treatment on blood levels of some potential vascular risk factors including TC, TG, HDL-c, LDL-c, uric acid, total Hcy (tHcy), von Willbrand factor (vWF) and fibrinogen. Another important point we wanted to remark in this study, was to evaluate the extent of oxidative damage in epileptic patients through assessing the level of lipid peroxidation markers (oxidized low density lipoprotein or Ox-LDL, malondialdehyde or MDA and thiobarbituric acid reactive substance or TBARs) and also to evaluate the magnitude of cell protection against oxidative stress through assessing the levels of total antioxidant capacity (TAC) and glutathione peroxidase (GSH.Px). Correlation between CA-IMT with all measured parameters was also evaluated.

Patients and methods

This study included 225 adult patients (males = 122 and females = 103), (age range; 18—45 years), with primary epilepsy with no previous history of vascular risk factor or disease, recruited over a period of 2 years (2003—2005) from the out-patient epilepsy clinic of Assiut University Hospital, Egypt. Sixty normal healthy volunteer subjects matched for age and sex were chosen as controls. Control subjects were recruited from healthy physicians and technicians who had been undergone medical annual general health screening check up within the 6 months before the study took place. The protocol of the study was reviewed and approved by the local ethical committee and written informant consent was obtained from all subjects before participation in this study.

Excluded from this work (for all participants) were: (1) patients with diseases other than epilepsy or taking other regular medication besides antiepileptic drugs (AEDs), (2) subjects with any risk for atherosclerosis including: smoking, diabetes mellitus, hypertension, hypercholesterolemia or dyslipidemia, active gastrointestinal disease, severe medical or psychiatric illness, alcoholism, serum creatinine concentration >150 mmol/l, history of gout or acute renal failure and patients with concomitant manifest atherosclerotic vascular disease, i.e. history of vascular disease (i.e. myocardial infarction, cerebrovascular stroke or transient ischemic attacks), (3) family history of vascular disease or risk for vascular disease, (4) use of supplemental antioxidant or over-the-counter multivitamins and (5) subjects taking contraceptive pills.

Data collected per patient were the followings:

(1) Biological variables including: age, gender, weight, height, systolic and diastolic blood pressure, body mass index (BMI); calculated using the following formula [BMI = weight (kg)/height (m)]^2. Overweight was defined as a BMI between 25 and 30 kg m^{-2} and obesity as a BMI over 30 kg m^{-2}.
(2) Information on smoking habits was obtained by questionnaire and patients were divided into smokers (present or former) and non-smokers (when they never smoked regularly). Smokers were excluded from the study.
(3) All participants were subjected to full medical, cardiologic and neurological history and examination. We used a structured questionnaire to identify symptom-free controls and to exclude subjects who were suspected of having any form of arterial or venous disease.
(4) Seizures were analyzed determining seizure type, duration of illness, age at onset, type and duration of AEDs utilized and the degree of response to AEDs. Epilepsy type was classified according to the recommendations of the International League Against Epilepsy (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). AEDs were prescribed in recommended doses and according to the well-known guidelines. All treated patients gave history of compliance to AEDs. Regarding the degree of control on AEDs, patients were considered controlled, when seizure free for $\geq$1 year, partially controlled when seizure frequencies were occasional or rare and uncontrolled when seizures were frequent or very frequent. The frequency of seizures were divided as follows—(a) very frequent: seizures occurring several times a day or at intervals shorter than 7 days, (b) frequent: seizures at intervals longer than 7 days but shorter than 30 days, (c) occasional: seizures at intervals longer than 30 days but shorter than 1 year and (d) rare: seizures at intervals longer than 1 year (Hamed et al., 2005).

Specimen collection and analysis

Routine laboratory blood tests were done for all patients, including: complete blood count, fasting blood glucose level, kidney and liver function tests. After an overnight fast and patients were seizure free for at least 72 h, blood samples were drawn at (8.00—10.00 a.m). The serum sam-