

Body mass index and some biochemical parameters among valproate treated male epileptic patients

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(Ann. Coll. Med. Mosul 2011; 37 (1 & 2): 114-121).
Received: 22nd Nov 2010; Accepted: 10th Oct 2011.

ABSTRACT

Objective: To assess the effect of continuous valproate monotherapy (VPA) on body mass index (BMI), serum leptin, malondialdehyde (MDA) and lipid profile in male epileptic children and adult patients in comparison to healthy male controls.

Subjects, materials and methods: A case-control study design was adopted. Samples from 44 male patients (22 less than 18 years old, and 22 over than 18 years old), with primary generalized epilepsy, on continuous VPA monotherapy, for at least six months before participation in the study, were collected over the period from October 2009 to March 2010. Forty-four apparently healthy male volunteers (22 less than 18 years old, and 22 over than 18 years old) without previous history of epilepsy were recruited as controls. Serum levels of leptin, and lipid profile indices were estimated using commercially available kits and a manually prepared reagent for MDA assay. (BMI) was calculated as weight in kilograms divided by the squared height in meters.

Results: The results revealed that epileptic children and epileptic adults receiving continuous VPA monotherapy had a significantly higher BMI ($p < 0.001$), serum leptin, serum MDA and atherogenic index (AI) and a significantly lower ($p < 0.001$) serum high density lipoprotein cholesterol (HDL-c) as compared to their matched control subjects. The results also revealed insignificant difference in serum total cholesterol (TC), triglycerides (TGs) and low density lipoprotein cholesterol (LDL-c) between epileptic children and epileptic adults on VPA and their matched control subjects. Serum leptin was positively correlated with body mass index standard deviation score (BMI SDS) of epileptic children ($r = 0.542$; $P < 0.001$) and duration of using VPA ($r = 0.215$; $P < 0.001$) in epileptic children. The results showed insignificant difference in serum TC, TGs and LDL-c between epileptic adults on VPA and control subjects. This study also revealed that the increase in serum leptin was significantly higher ($p < 0.001$) in epileptic adults receiving continuous VPA monotherapy than in epileptic children.

Conclusion: Continuous VPA monotherapy was associated with higher BMI and serum leptin and an increase in the oxidative stress marker MDA in both male epileptic children and adults. There was no effect of VPA use on lipid profile indices in both epileptic children and adults except significant decrease in serum HDL-c and significant increase in AI.

Keywords: Valproate, male epileptic children and adult, BMI, leptin, malondialdehyde, lipid profile

الخلاصة

أهداف الدراسة: لتقييم تأثير الفالبروات كعلاج أحادي مستمر على دليل كتلة الجسم، اللبتين والمالونديالدهيد بصورة الدهون في مصل الدم عند مرضى الصرع من الأطفال والبالغين الذكور بالمقارنة مع مجموعة الضبط من الأصحاء.

المرضى وطرق العمل: تم اعتماد تصميم دراسة العينة والشاهد. عينات من ٤٤ مريضاً من الذكور (٢٢ أعمارهم دون ١٨ سنة و ٢٢ أعمارهم فوق ١٨ سنة) مصابين بالصرع الأولي العام مستمرين بالعلاج الأحادي لعقار الفالبروات على الأقل ٦ أشهر قبل البدء بالدراسة تم تجميعها للفترة من تشرين الأول ٢٠٠٩ الى آذار ٢٠١٠. أيضاً تم تجميع ٤٤ شخصاً سليماً (٢٢ أعمارهم دون ١٨ سنة و ٢٢ أعمارهم فوق ١٨ سنة) كمجموعة سيطرة. تم سحب نماذج الدم في حالة الصيام وتم فصل مصل الدم واستعماله لقياس لإجراء الفحوص الكيميائية باستعمال عدد الشغل التجارية. أما مستوى المالوندايديهايد فقد تم قياسه بطريقة الكشف المحضر يدويًا ومستوى الكولستيرول الواطئ الكثافة بمعادلة فريدوالد وتم حساب دليل كتلة الجسم من خلال قسمة الوزن (كغم) على مربع الطول (متر^٢).

النتائج: أظهرت نتائج الدراسة ان مرضى الصرع من الأطفال المستمرين على الفالبروات لديهم ارتفاع معنوي في دليل كتلة الجسم، اللبتين والمالونديهايد في مصل الدم وكذا مؤشر تصلب الشرايين. وكان لديهم انخفاض معنوي في الكولستيرول عالي الكثافة ولا توجد فروق معنوية في مستوى الكولستيرول الكلي، الدهون الثلاثية، الكولستيرول واطئ الكثافة بالمقارنة مع مجموعة السيطرة.

أظهرت الدراسة أيضاً ان مرضى الصرع البالغين المستمرين على الفالبروات لديهم ارتفاعاً معنوياً في دليل كتلة الجسم، مستوى اللبتين والمالونديهايد وكذا مؤشر تصلب الشرايين ولديهم انخفاض معنوي في مستوى الكولستيرول عالي الكثافة في مصل الدم. بينما لم يكن هنالك فروقاً معنوية في مستويات الكولستيرول الكلي، الدهون الثلاثية، والكولستيرول واطئ الكثافة.

الاستنتاج: استنتجت هذه الدراسة ان الاستمرار بالعلاج الأحادي لعقار الفالبروات ترافق مع زيادة في وزن الجسم واللبتين في مصل الدم مع زيادة في مؤشر جهد الأوكسدة (المالوندايديهايد) في مرضى الصرع من الأطفال والبالغين. كان هنالك نقصاناً معنوياً في الكولستيرول عالي الكثافة وزيادة في مؤشر تصلب الشرايين مما يتوجب إجراء دراسة أوسع من حيث عدد المرضى للتحقق من الأمر.

مفتاح الكلمات: الفالبروات، مرضى الصرع من الأطفال والبالغين الذكور، دلالة كتلة الجسم، اللبتين، المالوندايديهايد، دهون الدم.

Epilepsy and its medications are associated with weight changes in which weight gain is the most common and distressing particularly with Valproate (VPA) therapy^(1,2). Weight gain is a difficult problem at any age, particularly in adolescence, a period of increased awareness to body weight and image⁽³⁾. Epilepsy and its medications may alter weight homeostasis regulating process including the two main homeostatic hormones, leptin and insulin. Increased blood levels of leptin and insulin due to leptin and insulin resistances are observed in patients with epilepsy^(4,5).

The most important effect of free radicals is lipid peroxidation which results in disruption of cell membranes, and this may explain the role of oxidative stress in the etiology of seizure-induced neuronal death⁽⁶⁾. It has been reported that increased generation of free radicals or decreased activity of antioxidant defense systems can cause some forms of seizures and in addition can increase the risk

of seizure recurrence^(7,8). Many antiepileptic drugs (AEDs) are metabolized to generate reactive metabolites with the capability of covalent binding to macromolecules as proteins or other vital biomolecules and hence eliciting systemic toxicity^(8,9). Lipid peroxidation caused by increased generation of free radicals or decreased activity of antioxidant defense systems have been suggested to be critically involved in seizure control⁽⁷⁾.

Many studies, mainly comprising adult patients, have provided the evidence that there is a significant influence of long-term AED therapy on total cholesterol (TC), triglycerides (TGs), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and very low-density lipoprotein cholesterol (VLDL-c)^(10,11). VPA is not a microsomal enzyme inducer, and lipid profile during VPA treatment is controversial. Decreased, no effect, and increased levels of serum lipids have been reported in epileptic patients^(12,13).

The aim of this study was to assess the effect of continuous VPA monotherapy on BMI, serum leptin, MDA and lipid profile in epileptic children and adults in comparison with those of healthy controls.

Subjects, materials and methods

Epileptic patients

This study included 44 male patients (22 less than 18 years old with mean age \pm SD of 11.26 ± 4.41 , and 22 more than 18 years old with mean age \pm SD of 30.55 ± 6.54), with primary generalized epilepsy, on continuous valproate [VPA, Depakine, Sanofi-Aventis, France] monotherapy in a mean \pm (SD) dose of $386.36\pm(99.02)$ mg/ d, for at least six months before participation in the study. These patients were referred from a private clinic over the period from October 2009 to March 2010. Patients with the following criteria were excluded from this study:

1. Patients with secondary epilepsy.
2. Patients with other neurological, medical, or psychiatric disorders.
3. Patients with rapidly progressive disorders that could alter their weight.
4. Patients with family history of body weight disorders.

Control subjects

Forty four apparently healthy male volunteers (22 less than 18 years old with mean age \pm SD of 10.95 ± 4.10 , and 22 more than 18 years old with mean age \pm SD of 30.05 ± 5.95) without previous history of epilepsy were recruited as controls (with age matching to the patients group) from employees at the College of Pharmacy and Mosul College of Medicine, and from relatives who fulfilled the criteria adopted for the study. The control group was judged free of any illness by history and clinical examination. They were included in the study to compare the normal values for serum leptin, MDA and lipid profile indices.

Specimens collection and analysis

For epileptic patients, about 5 mL of venous blood was drawn, using a disposable syringe between 8.00 to 10.00 a.m., and after an overnight fasting. The blood was allowed to clot in a plain tube at room temperature, and

then the serum was separated by centrifugation at 3000 rpm for 10 min., and kept frozen at -20°C to be analyzed later on. Samples from the control subjects were collected and processed in the same way. Serum leptin was measured by enzyme linked immunosorbent assay (ELISA) technique, using the IBL leptin ELISA Kit (Germany), which is an immunoassay for the quantitative in vitro diagnostic measurement of leptin in serum and plasma. Serum MDA levels were estimated using TBA assay method⁽¹⁴⁾. Determination of serum TC and TGs concentrations was done by the enzymatic colorimetric method (Allain et al., 1974)⁽¹⁵⁾, using total cholesterol BIOLABO Kit (France). Determination of serum HDL-c concentration was done by the precipitation method (Lopez-Virella et al., 1977)⁽¹⁶⁾, using HDL-c BIOLABO Kit (France). Serum LDL-c concentration was calculated using Friedewald equation⁽¹⁷⁾:

$\text{LDL-c (mmol/L)} = \text{TC} - \text{HDL-c} - \text{TG}/2.2$.
Atherogenic index (A.I) was calculated by the following equation: $\text{AI} = \text{TC} / \text{HDL-c}$ ⁽¹⁸⁾.

BMI was calculated according to the equation: $\text{BMI} = \text{Weight (Kg)} / \text{Height (m}^2\text{)}$ ⁽¹⁹⁾

Age and sex specific standard deviation scores (SDS) for height, weight and BMI were calculated for children according to charts developed by the National Centre for Health Statistics in collaboration with the National Centre for Chronic Disease Prevention and Health Promotion⁽²⁰⁾.

Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) (version 11.5). Standard statistical methods were used to determine the mean and standard deviation. Unpaired t-test was used to compare the results of different biochemical parameters. Linear regression analysis [Pearson correlation coefficient (r)] was performed to identify the relationship between different biochemical parameters. $p\text{-value} \leq 0.05$ was considered to be statistically significant⁽²¹⁾.

Results

Tables 1 and 2 demonstrated the demographic characteristics of the epileptic patients and the control subjects respectively.

Table 3 demonstrated the comparison of BMI SDS, serum levels of leptin, MDA and lipid profile indices between epileptic children and epileptic adults receiving continuous VPA monotherapy and their matched control subjects.

The results of this study revealed that epileptic children receiving continuous VPA monotherapy had significantly higher values for BMI, serum leptin, MDA and AI and a significantly lower ($p < 0.001$) serum HDL-c as compared to their matched controls, while non significant differences were demonstrated for serum TC, TGs and LDL-c. Serum leptin was positively correlated with BMI SDS of epileptic children ($r = 0.542$; $P < 0.001$) and duration of usage VPA ($r = 0.215$; $P < 0.001$) in epileptic children. The results of this study also revealed that epileptic adults receiving continuous VPA monotherapy had a significantly higher ($p < 0.001$) BMI, serum leptin, MDA and AI and a significantly lower ($p < 0.001$) serum HDL-c as compared to their matched controls, with insignificant difference in serum, TC, TGs and LDL-c between epileptic adults on VPA and their matched controls. This study also revealed that the increase in serum leptin was significantly higher ($p < 0.001$) in epileptic adults receiving continuous VPA monotherapy than that in epileptic children. On the other hand, there

was insignificant difference in serum MDA, and lipid profile indices between epileptic children and epileptic adults receiving continuous VPA monotherapy.

Table (1): The characteristics of the epileptic patients.

Parameters Mean± SD	Children (≤ 18 years) (n=22)	Adults (> 18 years) (n=22)
Patients age (years)	6-18 (11.26±4.41)	19-44 (30.55±6.54)
Body mass index (BMI) (kg/m ²)	27.75±4.41	28.66±3.31
Duration of illness (years)	2.34±2.09	5.17±6.64
Age at onset (years)	9.38±4.42	25.39±9.38
Duration of using VPA (years)	2.07±2.18	2.86±2.98
Dose of VPA (mg/day)	386.36±99.02	595.45±247.80

Table (2): The characteristics of the controls.

Parameters Mean± SD	Children (≤ 18 years) (n=22)	Adults (> 18 years) (n=22)
age (years)	6-18 (10.95±4.10)	22-44 (30.05±5.95)
BMI (kg/m ²)	23.86±5.53	24.59±2.92

Table (3): Comparison of BMI, serum leptin, MDA and lipid profile indices levels between the epileptic patients and the controls.

Parameters Mean±SD	Children (≤ 18 yrs) (n=22)		Adults (> 18 yrs) (n=22)	
	Epileptic children	Controls	Epileptic adults	Controls
BMI (kg/m ²)	27.75±4.41**	23.86±3.53	28.66±3.31**	24.59±2.92
Leptin (ng/ml)	6.06±5.54 **	2.89±1.71	9.81±5.38 **	3.91±1.96
MDA (μ Mol/L)	2.03±0.44 **	1.26±0.48	2.06±0.51**	0.95±0.22
TC(mmol/L)	4.46±0.78	4.48±0.79	4.54±0.52	4.66±0.71
TGs (mmol/L)	1.44±0.45	1.36±0.48	1.34±0.44	1.29±0.39
LDL-c (mmol/L)	3.01±0.83	2.84±0.57	3.18±0.62	3.16±0.71
HDL-c(mmol/L)	0.79±0.13**	1.02±0.27	0.75±0.11**	0.93±0.15
AI	5.64±1.50 **	4.55±0.77	6.07±1.51**	5.11±1.11

** : significant differences $p < 0.001$.

Discussion

The current study demonstrated higher body weight and significant increase in BMI in both children and adult epileptics on VPA therapy. Clinically significant weight gain has been reported with several AEDs including the conventional agents VPA, carbamazepine (CBZ) and the newer medications gabapentin and vigabatrin and may result in lack of compliance with or discontinuation of therapy⁽²²⁾. Among all of the AEDs, VPA a broad-spectrum antiepileptic drug commonly used to treat children with focal and generalized epilepsy, has been the best studied with respect to effects on body weight⁽²³⁾. Its etiology is most likely multi-factorial and controversial. Pylvanen et al.⁽²⁴⁾ reported that 52% of men treated with VPA had BMI scores within the obesity category. Numerous pooled and specifically assigned data from clinical trials plus retrospective and cross-sectional analysis also reported that treatment with VPA is associated with a significant weight gain in 3—71% of patients which ranged from 5 to 49 kg^(25,26). In agreement with our results the study of Rauchenzauner et al.,⁽²⁷⁾ who reported that long-term therapy with VPA in childhood was associated with significant increase in body weight, as well as Hamed et al.,⁽²⁸⁾ who also concluded that BMI was significantly elevated in VPA treated epileptic children compared with controls, untreated and those treated with other AEDs.

Several mechanisms have been suggested to explain VPA-related weight gain including:

- 1- The effect on the hypothalamus: this is supported by the observation that epileptic patients treated with VPA and reported weight gain also developed increased appetite thirst, and quenching with calorie-rich beverages⁽²³⁾, all of which indicate hypothalamic stimulation⁽²⁹⁾.
- 2- VPA-induced hyperinsulinemia and insulin resistance: this is supported by the observation that weight gain during VPA treatment is related to increase in insulin concurrent with decrease in glucose level which can stimulate appetite and may cause weight gain⁽²³⁾.
- 3- VPA-induced hyperleptinemia and leptin resistance: this is supported by the observation that women who became obese after VPA therapy reported high leptin levels and insulin resistance while patients who remained lean did not show any changes⁽²⁾.
- 4- VPA-induced changes in circulating levels of leptin and its relation to ghrelin and adiponectin: this is supported by the observation that weight gain in VPA-treated patients was associated with increased concentrations of leptin and decreased concentrations of ghrelin and adiponectin. These changes were correlated with the extent of body weight, patients' BMI, the amount of adipose tissue and fasting plasma levels of insulin and leptin⁽³⁰⁾. The down-regulation of ghrelin is considered as a consequence of increased insulin or leptin.
- 5- Genetic susceptibility to develop hyperleptinemia: this is supported by the observation that VPA-treated patients may manifest high serum leptin levels without weight gain and leptin level is correlated to BMI⁽³¹⁾.

The results of the current study also revealed an elevated serum leptin in both epileptic adults and children on VPA monotherapy. This is consistent with the finding of Rauchenzauner et al.,⁽²⁷⁾ and Hamed et al.,⁽²⁸⁾, both reported an increase in serum leptin in epileptic patients on continuous VPA therapy and that hyperleptinemia was common among epileptic children who gained weight with VPA therapy suggesting a state of leptin resistance. The mechanism of hyperleptinemia and leptin resistance with VPA treatment is still controversial. The increase in serum leptin levels in VPA-induced weight gain may be a consequence of increase in adipose tissue⁽⁴⁾. It has been suggested that VPA causes direct secretion of leptin from adipocytes.

Lipid peroxidation is an indicator of free radical metabolism and oxidative stress in human beings and other organisms. Malondialdehyde (MDA), is an end product of lipid peroxidation that can react with thiobarbituric acid. The estimation of MDA is a

sensitive measure of lipid peroxidation⁽³²⁾. This study revealed a significantly higher serum MDA in both epileptic children and adults receiving VPA monotherapy. In agreement with our finding, is the study conducted by Yukset et al⁽³³⁾. They concluded that epileptic patients on VPA had an increased lipid peroxidation levels. Consistent with our results, also the study conducted by Solowiej and Solaniec⁽³⁴⁾ who reported that the concentration of MDA was elevated in all epileptic patients significantly in both VPA monotherapy and polytherapy, while insignificant elevation was noticed in newly diagnosed epileptics and in CBZ monotherapy group. In their more recent research, Sobaniec et al.,⁽³⁵⁾ and Yiş et al.,⁽⁶⁾ both reported insignificant elevation in MDA concentration in epileptics on VPA monotherapy. While in contrast to our results, Peker et al.,⁽³⁶⁾ who investigated the effects of VPA on lipid peroxidation among other parameters, reported no significant differences in serum MDA in comparison to healthy controls. In explanation to our results, there were several studies suggesting that excessive generation of free radical intermediates are associated with VPA administration possibly as a consequence of VPA biotransformation⁽³⁷⁾, or alterations in glutathione homeostasis⁽³⁸⁾ and/or depletion of cofactors required for antioxidant defense⁽⁹⁾.

The effect of VPA on lipid profile remains unclear. VPA has been reported to be associated with a decrease of serum LDL-c or TC^(39,40) and increased HDL-c⁽⁸⁾. No significant changes in serum lipid has been reported by others^(13,41). Our study revealed a significantly lower HDL-c and a highly significant increase in A.I in epileptic children on VPA monotherapy in comparison to healthy controls. In agreement with our results, is the study conducted by Zeitlhofer et al.,⁽⁴²⁾. They reported that VPA-treated epileptic patients showed decreased HDL-c levels. Eiris et al.,⁽⁴³⁾ also reported that epileptic children receiving continuous VPA monotherapy had significantly lower HDL-c than their matched control subjects. While in contrast to our results, Heldemberg et al.,⁽⁴⁰⁾ reported that

epileptic children receiving continuous VPA monotherapy had increased serum HDL-c.

This study also revealed insignificant differences in TC, TG and LDL-c in epileptic children on continuous VPA monotherapy which is in concordance with the results of Luef et al.,⁽⁴⁴⁾ who found insignificant differences in serum TC, TG and LDL-c between epileptic children receiving continuous VPA monotherapy and their matched controls. Furthermore, this study also demonstrated that epileptic adults receiving continuous VPA monotherapy had significantly lower HDL-c and significantly higher AI than their matched controls. This is consistent with the results of the study conducted by Hamed et al.,⁽²⁸⁾. They reported that epileptic adults on continuous VPA monotherapy had significantly lower HDL-c than their matched controls.

In conclusion., VPA monotherapy in epileptic children and adults can cause a rise in BMI, serum leptin and MDA (as a reflection of oxidative stress) and also causes a significant reduction in HDL-c and a significant rise in AI which needs further evaluation with larger number of patients.

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