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ORIGINAL ARTICLE

EFFECT OF VALPROATE AND OXCARBAZEPINE THERAPY ON ASYMMETRIC DIMETHYL ARGININE (ADMA) AND HOMOCYSTEINE LEVELS IN NEWLY DIAGNOSED EPILEPTIC CHILDREN

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ABSTRACT:

Aim: The aim of our study was to evaluate the effect of valproate and oxcarbazepine therapy in children newly diagnosed with epilepsy. Changes in serum asymmetric dimethyl arginine (ADMA), homocysteine, folate and B12 levels were assessed as possible markers of cardiovascular risk and correlation between homocysteine and ADMA levels was ascertained. **Materials and Methods**: Drug naïve patients newly diagnosed with epilepsy of age 7-17 years (n= 62), with no known cause of hyperhomocysteinemia, were enrolled for the study. A pre treatment analysis of homocysteine, folate and B12 was done using chemiluminescent competitive immunoassay. Serum ADMA levels were measured by ELISA. Thereafter, treatment of 34 children was started on valproate and 28 were prescribed oxcarbazepine. After six months of continuous treatment, the analysis was repeated. **Results**: Sixty two newly diagnosed, drug naïve patients of epilepsy were recruited for the study. After initial assessment of biochemical parameters, treatment was initiated. Valproate was started on 34 patients and 28 were given oxcarbazepine. No significant variations were observed in age, gender and seizure type. There was a significant increase in ADMA and Hcy levels in both the groups after six months of therapy ($p < 0.05$). A decrease in folate levels was registered in both the groups, but the change was significant only in the group on OXC treatment ($p = 0.003$). A significant increase in B12 concentration was observed in the group on VPA ($p = 0.002$) and a decrease was registered in children on OXC treatment ($p = 0.026$). . Differences between the groups were significant in ADMA, Hcy and B12 levels ($p = 0.000$, 0.000 and 0.003 respectively). 41.1% (14/34) children on valproate therapy and 21.4% (6/28) patients on oxcarbazepine had hyperhomocysteinemia after six months of therapy. No correlation was observed between ADMA and Hcy changes in both the groups. In subjects on valproate, at follow up, ADMA levels exhibited a positive correlation with B12 ($r= 0.389$; $p= 0.023$) and Hcy levels correlated negatively with folate ($r= -0.444$; $p= 0.008$). In patients on OXC therapy, ADMA correlated negatively with B12 at recruitment($r= -0.503$; $p= 0.006$). After six months of therapy, Hcy and folate levels had a significant negative correlation ($r= -0.427$; $p= 0.024$). **Conclusion**: Anti-epileptic therapy can affect ADMA, Hcy and related vitamins but increase in ADMA may be independent of Hhcy. Further studies are needed to understand the etiology of ADMA increase.

Key words: Valproate, Oxcarbazepine Therapy, Homocysteine, Epileptic Children

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INTRODUCTION

Epilepsy is a chronic neurological disorder requiring long-term or even lifetime treatment. Patients on anti epileptic drugs exhibit several vascular risk factors like abnormal lipid profile, increased oxidative stress and elevation in homocysteine levels. Homocysteine is an amino acid derived from another amino acid methionine and enhances risk of atherosclerosis, myocardial infarction and cerebrovascular disorders.[1-6] Endothelial dysfunction precedes atherosclerotic disturbances[7] and was shown to be caused by homocysteine in several pathological conditions.[8,9] Therapy by antiepileptic drugs (AEDs) has been associated with many occlusive vascular diseases.[10-13] AED therapy was also responsible for increased levels of plasma total homocysteine, serum vitamin B12 and low serum folate, vitamin B6.[1,14-17] An increased risk of fatal cardiovascular disease has been reported in epilepsy but its relation with AED therapy has not been confirmed.[11,18)]

Vascular disease may begin early in childhood or adolescence with a probability of being reversed, hence understanding the impact of cardiovascular risk factors is essential with emphasis on endothelial disturbances in children with epilepsy.

Methylation of arginine results in formation of asymmetric dimethyl arginine (ADMA), which is an endogenous competitive inhibitor of nitric oxide synthase.[19] Hence, ADMA impairs synthesis of nitric oxide, a potent vasodilator. Increased Hcy levels inhibit the activity of enzyme dimethylarginine dimethylamino hydrolase (DDAH), which is required for ADMA metabolism, resulting in elevated levels of ADMA. Many researches have revealed that increased ADMA levels are associated with incidence of cardiovascular morbidity, which leads to enhanced mortality.[20-24] Thus, ADMA has emerged as a novel and reliable cardiovascular risk factor[25] and is a better indicator of endothelial function than Hcy, due to minimal interference by fasting status and physical activity.[26]

Elevated Hcy, an independent risk factor for atherosclerosis, ADMA and impaired lipid profiles, have been observed in many investigations with children on AEDs.[27-29] In this study, we aimed to explore the changes and correlations in plasma levels of ADMA, homocysteine and serum levels of folate and vitamin B12 under AED treatment. Valproic acid (VPA) and oxcarbazepine (OXC) are prescribed extensively in children with epilepsy. The aim of our study was to assess variation in serum ADMA, hcy, folate and B12 as possible cardiovascular risk factors, in children on these common antiepileptics and to assess correlation between homocysteine and ADMA levels, if any.

Materials and methods

The study included 62 drug naïve patients (7-17 years of age), recently diagnosed with epilepsy, visiting the out-patient clinic at department of neurology, DrRML Institute of Medical Sciences, Lucknow, India. The study was approved by institutional ethics committee. Patients were recruited in the study after signing written informed consent. Patients of ischemic stroke, with history of hypertension, cardiac and peripheral vascular disease, diabetes mellitus, renal or thyroid disease were excluded from the study. Subjects on regular consumption of vitamins or any other drug were also excluded.

Biochemical Analyses:

Following an overnight fasting period, blood was drawn, between 8-10 AM, from the antecubital vein in sitting position. Serum was immediately separated by centrifugation and stored at -80^{0}C until assayed. The procedure was repeated in both the groups after six months of continued therapy.

Analysis of ADMA: Serum ADMA levels were measured by ELISA (kits obtained from Qayee-Bio For Life Science). Absorbances were measured using a wavelength of 450 nm. All samples and standards were analyzed in duplicate.

Hcy, Folate and B12 assessment: Serum Hcy, folate and B12 concentrations levels were measured through chemiluminescent immunoassay by using commercially available kits (Siemens) by using kits (Siemens) available for Advia centaur auto analyzer. Normal reference ranges in fasting conditions are 5-15µM/L for tHcy, 3-17 ng/ml for serum folate and 178-800 pg/ml for vitamin B12. After a pre treatment assessment of the above biochemical parameters, treatment with valproate or oxcarbazepine was initiated and all assessments were repeated after six months of continuous therapy.

Statistical Analysis: Data were analyzed using the SPSS Version 20.0. There were two groups of patients in accordance with the type of drug used for therapy (VPA or OXC). Discrete variables (gender, seizure type) were compared by the Pearson chi square test. Shapiro-Wilk test confirmed that the data did not follow Gaussian distribution, hence non-parametric tests were employed for analysis. Baseline and follow-up values of serum ADMA, tHcy, B12 and folate in each group were compared using the Wilcoxon signed ranks test. Variation between the groups was assessed by Mann Whitney U test. Spearman correlation analysis was employed to evaluate correlation between the variables. Statistical significance was set at $p<0.05$.

RESULTS

TABLE 1: Demographic distribution of children with epilepsy

	VPA (n=34)	OXC (n=28)	p-value between groups
Age (years)	12.18(0.57)	13.36(0.50)	0.134
Gender(Male: Female)	24:10	20:8	0.942
Type of seizure (Partial: Generalized)	10:24	6:22	0.475
AED dose (mg/day)	682.4(29.39) (range,400-1000)	600(8.909) (range,450-650)	

Valproate(VPA), oxcarbazepine(OXC), anti epileptic drug (AED)
Values are expressed as mean and standard error(SE)
***Level of significance at P<0.05**

TABLE 2: ADMA, total homocysteine, folate and B12 levels before and after six months of anti-epileptic therapy:

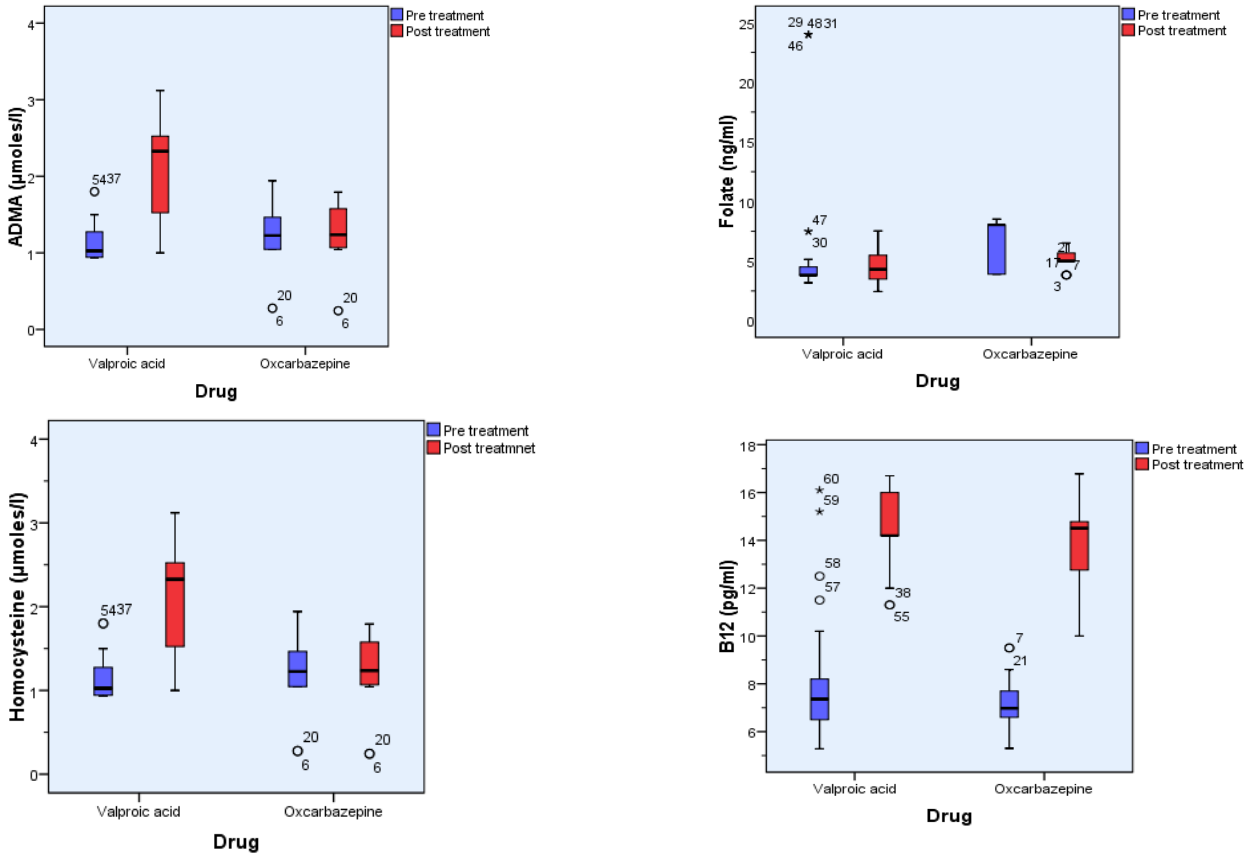
	ADMA		Homocysteine		Folate		B12	
Treatment groups	**Pre treatment**	**Post treatment**	**Pre treatment**	**Post treatment**	**Pre treatment**	**Post treatment**	**Pre treatment**	**Post treatment**
VPA	1.14 (0.04)	2.10 (0.11)	7.07 (0.17)	14.61 (0.27)	6.50 (1.12)	4.67 (0.27)	296.09 (14.42)	334.76 (12.83)
OXC	1.23 (0.07)	1.27 (0.08)	7.19 (0.19)	13.89 (0.33)	6.48 (0.38)	5.19 (0.13)	330.14 (11.77)	304.64 (13.38)
p-value for pre-treatment values between groups	0.412		0.644		0.666		0.111	

Valproate(VPA), oxcarbazepine(OXC), asymmetric dimethyl arginine(ADMA)
Values are expressed as mean and standard error(SE)
***Level of significance at P<0.05**

TABLE 3: Variation in ADMA, total homocysteine, folate and B12 levels after six months of monotherapy

	ADMA		Homocysteine		Folate		B12	
	Change in variable at six months	p-value within group	Change in variable at six months	p-value within group	Change in variable at six months	p-value within group	Change in variable at six months	p-value within group
VPA	0.96(0.13)	0.000*	6.64(0.47)	0.000*	-1.82(1.05)	0.939	38.67(12.79)	0.002*
OXC	0.04(0.02)	0.022*	6.70(0.36)	0.000*	-1.29(0.46)	0.009*	-25.50(11.66)	0.038*
p-value between groups	0.000*		0.0011*		0.237		0.018*	

Valproate (VPA), oxcarbazepine (OXC), asymmetric dimethyl arginine (ADMA)
Values are expressed as mean and standard error (SE)
***Level of significance at P < 0.05**



RESULTS

TABLE 4: Correlation between biochemical parameters in children on VPA therapy

	$ADMA_1$	HCY_1	$Folate_1$	$B12_{(1)}$	$ADMA_2$	HCY_2	$Folate_2$	$B12_{(2)}$
$ADMA_1$	1	-0.008	-0.197	0.070	-0.423*	0.008	-0.092	0.092
HCY_1		1	0.249	0.093	0.110	0.265	0.015	0.18
$Folate_1$			1	-0.077	0.004	-0.293	0.725*	-0.048
$B12_{(1)}$				1	-0.031	-0.252	0.090	0.622*
$ADMA_2$					1	0.251	-0.046	0.389*
HCY_2						1	-0.444*	-0.233
$Folate_2$							1	0.015
$B12_{(2)}$								1
Subscript $_1$ represents pre treatment values and subscript $_2$ represents values after six months of treatment								

TABLE 5: Correlation between biochemical parameters in children on OXC therapy

	$ADMA_1$	HCY_1	$Folate_1$	$B12_{(1)}$	$ADMA_2$	HCY_2	$Folate_2$	$B12_{(2)}$
$ADMA_1$	1	0.274	-0.360	-0.503*	0.926*	-0.056	0.333	-0.140
HCY_1		1	-0.200	-0.282	0.332	0.116	0.065	-0.240
$Folate_1$			1	0.321	-0.326	0.039	-0.396*	0.735
$B12_{(1)}$				1	-0.421	0.060	-0.116	0.237
$ADMA_2$					1	0.041	0.292	-0.087
HCY_2						1	-0.427*	0.060
$Folate_2$							1	-0.720*
$B12_{(2)}$								1
Subscript $_1$ represents pre treatment values and subscript $_2$ represents values after six months of treatment								

Sixty two newly diagnosed, drug naïve patients of epilepsy were recruited for the study. After initial assessment of biochemical parameters, treatment was initiated. Valproate was started on 34 patients and 28 were given oxcarbazepine. Mean daily dose was 682.4 mg (range, 400-1000) for VPA group and 600 mg (range,450-650)for OXC group. Table 1 summarizes the demographic details, no significant variations were observed in age (p=0.134), gender ($\chi2$=0.005, df= 1, p=0.942) and seizure type ($\chi2$=0.511, df=1, p=0.475). Variation in pre-treatment levels between the VPA and OXC groups was statistically insignificant (ADMA, p=0.412; Hcy, p=0.644; folate, p=0.666; B12, p=0.111). There was a significant increase in ADMA (VPA: z=-4.595, p= 0.000; OXC: z= -2.325, p= 0.020) and Hcy (VPA: z= --5.036, p= 0.000; OXC: z= -4.626, p= 0.000) levels in both the groups after six months of therapy. A decrease in folate levels was registered in both the groups, but the change was significant only in the group on OXC treatment (z=-2.948; p= 0.003). A significant increase in B12 concentration was observed in the group on VPA (z= -3.147; p= 0.002) and a decrease was registered in children on OXC treatment (z= -2.234; p= 0.026).

Changes in ADMA, folate and B12 levels were higher in the VPA group, while changes in Hcy concentrations were marginally higher in the group on OXC. Differences between the groups were significant in ADMA, Hcy and B12 levels (p= 0.000, 0.000 and 0.003 respectively).

Cut off value for hyperhomocysteinemia (Hhcy) was set at 15.1μM/L. In the group on VPA therapy, 41.1% (14/34) children had Hcy levels above the cut off value of 15.1μM/L, while 21.4% (6/28) patients on OXC had Hhcy after six months of therapy. At recruitment, in subjects on VPA therapy no significant correlations were observed between ADMA and Hcy, folate or B12 levels. At follow up, ADMA levels exhibited a positive correlation with B12 (r= 0.389; p= 0.023) and Hcy levels correlated negatively with folate (r= -0.444; p= 0.008).

In patients on OXC therapy, ADMA correlated negatively with B12 at recruitment(r= -0.503; p= 0.006). After six months of therapy, Hcy and folate levels had a significant negative correlation (r= -0.427; p= 0.024). Correlations between ADMA and Hcy variations form baseline to followup, in both the groups, are also insignificant.

DISCUSSION

Childhood epilepsy is a recurring chronic disorder, which often needs lifelong therapy. Epilepsy itself or long-term therapy by AEDs may be responsible for endothelial dysfunction, which promotes atherogenesis, resulting in several occlusive cardiovascular diseases.[11,12,13,30,31] Many studies have reported significant rise in fasting Hcy levels in patients on AED therapy.[32,33] ADMA, an endogenous inhibitor of nitric oxide synthase, has been associated with endothelial dysfunction and several investigations associate rising levels of ADMA with hyperhomocysteinemia.[34-36] Our study demonstrates significant elevations in ADMA and Hcy concentrations in children after six months of VPA and OXC therapy as compared to pre treatment levels. This is similar to a recent study by Oz *et al* (27). To our knowledge, this is the first study on Indian population that

monitors ADMA levels as an effect of VPA and OXC therapy in children. Folate is the most important determinant of tHcy levels but there are several other factors like vitamins B12, B6, glomerular filtration rate (GFR) and polymorphisms in genes involved in coding of enzymes involved in Hcy metabolism.[37] Carbamazepine (CBZ) shows increased Hcy levels, associated with a decrease in vitamin B12 and folate levels resulting from reduction in enzyme activity in the liver (38). A few studies have shown elevation in serum vitamin B12 levels in patients receiving VPA therapy.[39,40] Our study demonstrated increased levels of vitamin B12 in children on VPA therapy, while children on OXC, registered a decrease. Folate levels decreased in both the groups, though the decrease was significant in the group on OXC therapy. This decrease in folate may result from disruption of the enterohepatic circulation of folate. Elevated B12 levels in patients receiving VPA might be due to heightened conjugation between vitamin B12 and transcobalamin II.[29,41,42]). Contrary to these results, some other studies reveal no changes in folate and B12 levels in patients receiving VPA therapy.[29,39,40]

Information about effects induced by new-generation AEDs, such as OXC, lamotrigine, (LTG), levatiracetam (LEV), and topiramate (TPM), on ADMA and Hcy metabolism is limited.[43,44] These drugs may have fewer side-effects than the old AEDs, specially in children with epilepsy who are more prone to an early development of atherosclerosis. Oxcarbazepine has a chemical structure similar to parent compound carbamazepine (CBZ). CBZ utilizes the cytochrome P-450 system for oxidation, while OXC through reduction of its keto group forms a monohydroxy derivative (MHD), which after glucuronidation gets excreted in the urine. Whereas metabolism of OXC involves minimum use of the hepatic cytochrome P-450-dependent enzymes. Therefore, OXC is preferred over CBZ and other old generation drugs.[45]

In the present study, 20 children were detected with Hhcy, out of which 14 were on VPA and only 6 on OXC therapy. In a study by Kurul *et al* [44], no child using OXC developed HHcy. However, a major drawback in their study was a small sample size. Belcastro *et al* [43], in their study revealed that newer AEDs such as OXC and TPM may cause HHcy, while drugs like LTG and LEV did not affect Hcy concentrations.

Hyperhomocysteinemia enhances ADMA production, which lowers NO levels, increasing the risk of atherosclerosis.[26,35] Significantly increased ADMA levels were reported in adult patients taking VPA and CBZ.[27] Similar observations were reported in children on VPA therapy.[28] A third study[46] reported elevation in ADMA and Hcy in a group on OXC therapy. No significant correlation was observed between Hcy and ADMA in the above studies. Similarly in studies on vascular diseases, no significant relationship between ADMA and HHcy was reported.[47,48] Likewise, we observed no significant relationship between Hcy and ADMA levels and their variation after six months of therapy in children on VPA and OXC. This indicates that the negative vascular events induced by Hcy and ADMA may have a different etiology despite having a biochemical link.[49] Another study on patients with hyperhomocysteinemia and peripheral artery disease (PAD), revealed that oral folate supplementation for 6 weeks reduced Hcy levels substantially without influencing ADMA concentrations in plasma.[50]

CONCLUSION:

Therefore, we can conclude that anti-epileptic therapy can affect ADMA, Hcy and related vitamins but increase in ADMA may be independent of Hhcy. Further studies are needed to understand the etiology of ADMA increase.

REFERENCES

1. Schwaninger M, Ringleb P, Winter R, Kohl B, Fiehn W, Rieser PA, Walter-Sack I. Elevated plasma concentrations of homocysteine in antiepileptic drug treatment. Epilepsia. 1999 Mar 1;40(3):345-50.
2. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullmann D, Tishler PV, Hennekens CH. A prospective study of plasma homocyst (e) ine and risk of myocardial infarction in US physicians. Jama. 1992 Aug 19;268(7):877-81.
3. Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PW, Belanger AJ, O'Leary DH, Wolf PA, Schaefer EJ, Rosenberg IH. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. New England Journal of Medicine. 1995 Feb 2;332(5):286-91.
4. Graham IM, Daly LE, Refsum HM, Robinson K, Brattström LE, Ueland PM, Palma-Reis RJ, Boers GH, Sheahan RG, Israelsson B, Uiterwaal CS. Plasma homocysteine as a risk factor for vascular disease: the European Concerted Action Project. Jama. 1997 Jun 11;277(22):1775-81.
5. Isojärvi JI, Airaksinen KE, Repo M, Pakarinen AJ, Salmela P, Myllylä VV. Carbamazepine, serum thyroid hormones and myocardial function in epileptic patients. Journal of Neurology, Neurosurgery & Psychiatry. 1993 Jun 1;56(6):710-2.
6. Eiris JM, Lojo S, Del Rio MC, Novo I, Bravo M, Pavon P, Castro-Gago M. Effects of long-term treatment with antiepileptic drugs on serum lipid levels in children with epilepsy. Neurology. 1995 Jun 1;45(6):1155-7.
7. Berenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. New England journal of medicine. 1998 Jun 4;338(23):1650-6.
8. Berg MV, Boers GH, Franken DG, KAMP G, Jakobs C, Rauwerda JA, Kluft C, Stehouwert C. Hyperhomocysteinaemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. European journal of clinical investigation. 1995 Mar 1;25(3):176-81.
9. Jin L, Caldwell RB, Li-Masters T, Caldwell RW. Homocysteine induces endothelial dysfunction via inhibition of arginine transport. Journal of Physiology and Pharmacology. 2007 Jun 1;58(2):191.

10. Luoma PV, Myllylä VV, Sotaniemi EA, Lehtinen IA, Hokkanen EJ. Plasma high-density lipoprotein cholesterol in epileptics treated with various anticonvulsants. European neurology. 1980;19(1):67-72.
11. Annegers JF, Hauser WA, Shirts SB. Heart disease mortality and morbidity in patients with epilepsy. Epilepsia. 1984 Dec 1;25(6):699-704.
12. Dasheiff RM. Sudden unexpected death in epilepsy: a series from an epilepsy surgery program and speculation on the relationship to sudden cardiac death. Journal of Clinical Neurophysiology. 1991 Apr 1;8(2):216-22.
13. Li X, Breteler M, Bruyne MC, Meinardi H, Hauser WA, Hofman A. Vascular determinants of epilepsy: the Rotterdam Study. Epilepsia. 1997 Nov 1;38(11):1216-20.
14. Tamura T, Aiso K, Johnston KE, Black L, Faught E. Homocysteine, folate, vitamin B-12 and vitamin B-6 in patients receiving antiepileptic drug monotherapy. Epilepsy research. 2000 Jun 1;40(1):7-15.
15. Verrotti A, Pascarella R, Trotta D, Giuva T, Morgese G, Chiarelli F. Hyperhomocysteinemia in children treated with sodium valproate and carbamazepine. Epilepsy research. 2000 Oct 2;41(3):253-7.
16. Apeland T, Mansoor MA, Strandjord RE. Antiepileptic drugs as independent predictors of plasma total homocysteine levels. Epilepsy research. 2001 Nov 30;47(1):27-35.
17. Apeland T, Mansoor MA, Strandjord RE, Vefring H, Kristensen O. Folate, homocysteine and methionine loading in patients on carbamazepine. Acta neurologica scandinavica. 2001 May 1;103(5):294-9.
18. Nilsson L, Tomson T, Farahmand BY, Diwan V, Persson PG. Cause-specific mortality in epilepsy: A cohort study of more than 9,000 patients once hospitalized for epilepsy. Epilepsia. 1997 Oct 1;38(10):1062-8.
19. Vallance P, Leone A, Calver A, Collier J, Moncada S. Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. Journal of cardiovascular pharmacology. 1992 Apr 1;20:S60-2.
20. Böger RH, Bode-Böger SM, Thiele W, Junker W, Alexander K, Frölich JC. Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. Circulation. 1997 Apr 15;95(8):2068-74.
21. Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, Imaizumi T. Endogenous nitric oxide synthase inhibitor. Circulation. 1999 Mar 9;99(9):1141-6.
22. KIELSTEIN JT, BÖGER RH, BODE-BÖGER SM, SCHÄFFER J, BARBEY M, KOCH KM, FRÖLICH JC. Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease relationship to treatment method and atherosclerotic disease. Journal of the American Society of Nephrology. 1999 Mar 1;10(3):594-600.
23. Yoo JH, Lee SC. Elevated levels of plasma homocyst (e) ine and asymmetric dimethylarginine in elderly patients with stroke. Atherosclerosis. 2001 Oct 31;158(2):425-30.
24. Valkonen VP, Päivä H, Salonen JT, Lakka TA, Lehtimäki T, Laakso J, Laaksonen R. Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. The Lancet. 2001 Dec 29;358(9299):2127-8.
25. Vallance P. Importance of asymmetrical dimethylarginine in cardiovascular risk. The Lancet. 2001 Dec 29;358(9299):2096-7.
26. Lentz SR, Rodionov RN, Dayal S. Hyperhomocysteinemia, endothelial dysfunction, and cardiovascular risk: the potential role of ADMA. Atherosclerosis supplements. 2003 Dec 31;4(4):61-5.
27. Öz O, Gökçil Z, Bek S, Çakır E, Odabaşı Z. Is asymmetric dimethylarginine responsible for the vascular events in patients under antiepileptic drug treatment?. Epilepsy research. 2009 Nov 30;87(1):54-8.
28. Ozdemir O, Yakut A, Dinleyici EC, Aydogdu SD, Yarar C, Colak O. Serum asymmetric dimethylarginine (ADMA), homocysteine, vitamin B12, folate levels, and lipid profiles in epileptic children treated with valproic acid. European journal of pediatrics. 2011 Jul 1;170(7):873-7.
29. Sener U, Zorlu Y, Karaguzel O, Ozdamar O, Coker I, Topbas M. Effects of common anti-epileptic drug monotherapy on serum levels of homocysteine, vitamin B12, folic acid and vitamin B6. Seizure. 2006 Mar 31;15(2):79-85.
30. Hamed SA, Hamed EA, Hamdy R, Nabeshima T. Vascular risk factors and oxidative stress as independent predictors of asymptomatic atherosclerosis in adult patients with epilepsy. Epilepsy research. 2007 May 31;74(2):183-92.
31. Hamed SA, Nabeshima T. The high atherosclerotic risk among epileptics: the atheroprotective role of multivitamins. Journal of pharmacological sciences. 2005;98(4):340-53.
32. Ono H, Sakamoto A, Eguchi T, Fujita N, Nomura S, Ueda H, Sakura N, Ueda K. Plasma total homocysteine concentrations in epileptic patients taking anticonvulsants. Metabolism. 1997 Aug 1;46(8):959-62.
33. James GK, Jones MW, Pudek MR. Homocyst (e) ine levels in patients on phenytoin therapy. Clinical biochemistry. 1997 Dec 31;30(8):647-9.
34. Krzyzanowska K, Mittermayer F, Krugluger W, Schnack C, Hofer M, Wolzt M, Schernthaner G. Asymmetric dimethylarginine is associated with macrovascular disease and total homocysteine in patients with type 2 diabetes. Atherosclerosis. 2006 Nov 30;189(1):236-40.
35. Dayal S, Rodionov RN, Arning E, Bottiglieri T, Kimoto M, Murry DJ, Cooke JP, Faraci FM, Lentz SR. Tissue-specific downregulation of dimethylarginine dimethylaminohydrolase in hyperhomocysteinemia. American Journal of Physiology-Heart and Circulatory Physiology. 2008 Aug 1;295(2):H816-25.
36. Zinellu A, Sotgia S, Scanu B, Deiana L, Carru C. Determination of protein-incorporated methylated arginine reference values in healthy subjects whole blood and evaluation of factors affecting protein methylation. Clinical biochemistry. 2008 Oct 31;41(14):1218-23.
37. Dinleyici EC, Kirel B, Alatas O, Muslumanoglu H, Kilic Z, Dogruel N. Plasma total homocysteine levels in children with type 1 diabetes: relationship with vitamin status, methylene tetrahydrofolate reductase genotype, disease parameters and coronary risk factors. Journal of tropical pediatrics. 2006 Jan 9;52(4):260-6.
38. Castro-Gago M, Novo-Rodríguez MI, Blanco-Barca MO, Urisarri-Ruíz de Cortázar A, Rodríguez-García J, Rodríguez-Segade S, Eirís-Puñal J. Evolution of serum lipids and lipoprotein (a) levels in epileptic children treated with carbamazepine, valproic acid, and phenobarbital. Journal of child neurology. 2006 Jan;21(1):48-53.
39. Gidal BE, Tamura T, Hammer A, Vuong A. Blood homocysteine, folate and vitamin B-12 concentrations in

patients with epilepsy receiving lamotrigine or sodium valproate for initial monotherapy. Epilepsy research. 2005 May 31;64(3):161-6.
40. Vilaseca MA, Monrós E, Artuch R, Colomé C, Farré C, Valls C, Cardo E, Pineda M. Anti-epileptic drug treatment in children: hyperhomocysteinaemia, B-vitamins and the 677C→ T mutation of the methylenetetrahydrofolate reductase gene. European Journal of Paediatric Neurology. 2000 Nov 1;4(6):269-77.
41. Karabiber H, Sonmezgoz E, Ozerol E, Yakinci C, Otlu B, Yologlu S. Effects of valproate and carbamazepine on serum levels of homocysteine, vitamin B12, and folic acid. Brain and Development. 2003 Mar 31;25(2):113-5.
42. May RB, Sunder TR. Hematologic Manifestations of Long-Term Valproate Therapy. Epilepsia. 1993 Nov 1;34(6):1098-101.
43. Belcastro V, Striano P, Gorgone G, Costa C, Ciampa C, Caccamo D, Pisani LR, Oteri G, Marciani MG, Aguglia U, Striano S. Hyperhomocysteinemia in epileptic patients on new antiepileptic drugs. Epilepsia. 2010 Feb 1;51(2):274-9.
44. Kurul S, Ünalp A, Yiş U. Homocysteine levels in epileptic children receiving antiepileptic drugs. Journal of child neurology. 2007 Dec;22(12):1389-92.
45. Schmidt D, Elger CE. What is the evidence that oxcarbazepine and carbamazepine are distinctly different antiepileptic drugs?. Epilepsy & behavior. 2004 Oct 31;5(5):627-35.].21
46. Emeksiz HC, Serdaroglu A, Biberoglu G, Gulbahar O, Arhan E, Cansu A, Arga M, Hasanoglu A. Assessment of atherosclerosis risk due to the homocysteine–asymmetric dimethylarginine–nitric oxide cascade in children taking antiepileptic drugs. Seizure. 2013 Mar 31;22(2):124-7.
47. Jonasson TF, Hedner T, Hultberg B, Öhlin H. Hyperhomocysteinaemia is not associated with increased levels of asymmetric dimethylarginine in patients with ischaemic heart disease. European journal of clinical investigation. 2003 Jul 1;33(7):543-9.
48. Wanby P, Teerlink T, Brudin L, Brattstro¨m L, Nilsson I, Palmqvist P, et al. Asymmetric dimethylarginine (ADMA) as a risk marker for stroke and TIA in a Swedish population. Atherosclerosis 2006;185:271–7.
49. van Guldener C, Nanayakkara PW, Stehouwer CD. Homocysteine and asymmetric dimethylarginine (ADMA): biochemically linked but differently related to vascular disease in chronic kidney disease. Clinical Chemical Laboratory Medicine. 2007 Dec 1;45(12):1683-7.
50. Ziegler S, Mittermayer F, Plank C, Minar E, Wolzt M, Schernthaner GH. Homocyst (e) ine-lowering therapy does not affect plasma asymmetrical dimethylarginine concentrations in patients with peripheral artery disease. The Journal of Clinical Endocrinology & Metabolism. 2005 Apr 1;90(4):2175-8.

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