ELEVATED PLASMA HOMOCYSTEINE LEVELS AND ITS RELATION WITH OXIDATIVE STRESS IN PATIENTS WITH STROKE

Luana Aparecida Cossentini,[1] Sayonara Rangel Oliveira,[2] Andréa Name Colado Simão,[3] Isaias Dichi,[4]

Abstract

Background: Elevated plasma homocysteine levels have been indicated as an independent risk factor for ischemic stroke. Objective: The objective of this study was to evaluate oxidative and nitrosative stress in patients with ischemic stroke with and without hyperhomocysteinemia (HHcy) and to verify whether HHcy is associated with oxidative stress in these patients. Methods: This study included 170 stroke patients, who were divided according to their levels of homocysteine (Hyperhomocysteinemia ≥13.59 μmol/L) and 220 healthy individuals (control group). Results: Stroke patients showed high levels of homocysteinemia when compared to the control group (p <0.001) adjusted for the variables: sex, ethnicity and age. Patients with stroke and hyperhomocysteinemia showed higher frequency of male subjects (p=0.001), lower Rankin scale (p=0.034), and lower glucose levels (p=0.020) when compared with stroke patients with lower levels of homocysteine. Patients with ischemic stroke and hyperhomocysteinemia had a trend to reduced NOx (p= 0.071) and increased TRAP (p= 0.003) levels when compared to the patients without hyperhomocysteinemia. The binomial logistic regression analysis showed that sex (p=0.032), glucose (p=0.017) and TRAP (p=0.019) were independently associated with hyperhomocysteinemia in patients with stroke. Conclusion: This is the first study in which homocysteinemia and oxidative and nitrosative stress markers were investigated in stroke patients. HHcy were associated with male sex, decreased glucose and increased TRAP. More studies are necessary to elucidate the complex relationship between homocysteine and redox status in these patients.

Keywords: Ischemic Stroke; Homocysteine; Oxidative Stress.

NÍVEIS DE ELEVADOS DE HOMOCISTEÍNA PLASMÁTICA E SUA RELAÇÃO COM O ESTRESSE OXIDATIVO EM PACIENTES COM ACIDENTE VASCULAR ENCEFÁLICO

Resumo

Introdução: Níveis elevados de homocisteína no plasma têm sido indicado como um fator de risco independente para acidente vascular cerebral isquêmico. Objetivo: O objetivo deste estudo foi avaliar o estresse oxidativo e nitrosativo em pacientes com AVC isquêmico com e sem hiper-homocisteinemia (HHcy) e verificar se o HHcy está associado ao estresse oxidativo nesses pacientes. Métodos: Este estudo incluiu 170 pacientes com AVC, que foram divididos de acordo com os níveis de homocisteína (Hiperhomocisteinemia ≥13,59 μmol/L) e 220 indivíduos saudáveis (grupo controle). Resultados: Os pacientes com AVC apresentaram níveis elevados de homocisteinemia quando comparados ao grupo controle (p <0,001) ajustado para as variáveis: sexo, etnia e idade. Pacientes com acidente vascular cerebral e hiper-homocisteinemia apresentaram maior frequência de indivíduos do sexo masculino (p=0,001), menor escala de Rankin (p=0,034) e menores níveis de glicose (p=0,020) quando comparados com pacientes com acidente vascular cerebral e homocisteína. Pacientes com acidente vascular cerebral isquêmico e hiper-homocisteinemia apresentaram tendência a redução do NOx (p=0,071) e aumento dos níveis de TRAP (p=0,003) quando comparados aos pacientes sem hiper-homocisteinemia. A análise de regressão logística binomial mostrou que sexo (p=0,032), glicose (p=0,017) e TRAP (p=0,019) foram independentemente associados à hiper-homocisteinemia em pacientes com acidente vascular cerebral. Conclusão: Este é o primeiro estudo em que a homocisteinemia e os marcadores de estresse oxidativo e nitrosativo foram investigados em pacientes com AVC. HHcy foram associados ao sexo masculino, diminuição da glicose e aumento da TRAP. Mais estudos são necessários para elucidar a complexa relação entre homocisteína e status redox nesses pacientes.

---

[1] PhD student at the Postgraduate Program in Experimental Pathology of the University of Londrina

[2] Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, University of Londrina, Paraná, Brazil

[3] Department of Pathology, Clinical Analysis and Toxicology, Health Sciences Center, University of Londrina, Paraná, Brazil

[4] Department of Internal Medicine - University of Londrina, Londrina, Paraná, Brazil. E-mail: dichi@sercomtel.com.br

INTRODUCTION

Acute ischemic stroke is the most common cerebrovascular disease and is one of the leading causes of death and long-term disability through the world. With the increase in the aging population, stroke has become the disease with higher indices of global health charges, especially in low- and middle-income countries (GLOBAL BURDEN OF DISEASE, 2015). Among all Latin American countries, Brazil has the highest mortality due to stroke (Lotufo, 2005).

Elevated plasma homocysteine levels have been indicated as an independent risk factor for ischemic stroke (Lu et al., 2018; Wu et al., 2016). Homocysteine is a sulfur amino acid, produced inside cells by demethylation of methionine (Oikonomidi, et al. 2016) and evidences have been reported that hiperhomocysteinemia (HHcy) is associated with many neurologic disorders including stroke, independent of long-recognized factors such as hyperlipidemia, hypertension and diabetes mellitus (Moretti & Caruso, 2019; Lu et al., 2018; Wu et al., 2016). HHcy is also related with functional disability in the acute phase of stroke and patients with acute stroke with elevated serum homocysteine levels are at an increase risk for early neurological deterioration (Kwon et al., 2014).

HHcy may cause toxicity by a variety of mechanisms, which includes vascular endothelial injury, excitotoxicity via stimulation of N-methyl-D-aspartate receptors (NMDA), and increased production of reactive oxygen species (Faraci and Lentz 2004.

The hypothesis of a possible association between elevated plasma levels of homocysteine and oxidative stress is corroborated by some studies indicating that cultivated endothelial cells with elevated levels of homocysteine may promote the formation of ROS, especially superoxide anion (Weiss, 2005). There are also reports that homocysteine causes significant disorders in the antioxidant defense system, for example in the enzymes glutathione peroxidase and superoxide dismutase (Weiss, 2005). A study performed *in vitro* with endothelial cells from human umbilical vein showed that homocysteine induces malondialdehyde (MDA) levels and inhibits eNOS (Feng et al., 2018), suggesting that HHcy can modify these oxidative stress biomarkers.

The literature on the level of homocysteine in patients with stroke is still scarce as well as the understanding of its role in stroke pathophysiology. In addition, to our knowledge, the relation between hyperhomocysteinemia and oxidative stress has not been investigated in

stroke to date. Thus, the objective of this study was to evaluate oxidative and nitrosative stress in patients with ischemic stroke with and without hyperhomocysteinemia. A second objective was to verify whether hyperhomocysteinemia is associated with oxidative stress in patients with ischemic stroke.

SUBJECTS AND METHODS

Study participants and clinical data

One hundred and seventy stroke patients with ischemic stroke and two hundred and twenty healthy controls were recruited at the University Hospital of Londrina. Demographic data and inflammatory biomarkers were analyzed in two groups of 85 patients: the first group without and the other group with hyperhomocysteinemia.

The group without hyperhomocysteinemia had patients with levels of homocysteine <13.59, whereas the group with hyperhomocysteinemia was composed by patients with serum homocysteine levels ≥ 13.59 all from the same geographical area. All groups were classified according to age, sex, ethnicity and body mass index (BMI). None of the study participants had clinical or laboratory findings of cardiac, thyroid, renal, hepatic, gastrointestinal or oncological diseases. Patients with various conditions that could interfere with homocysteine values such as renal insufficiency, B12 insufficiency, hypothyroidism, and hemolysis or drug use, such as phenytoin, isoniazid, methrotexate and L-dopa (Panunzio et al., 2003) were excluded from the study. None of the subjects were placed on a specific diet and were receiving antioxidant supplements. Patients were instructed by a nutritionist to maintain their usual diets, alcohol consumption, physical activity level, or other lifestyle factors during the intervention period. All subjects signed a free and informed consent form, and the study protocol was fully approved by the Ethical Committee of the University of Londrina, Londrina, Paraná, Brazil (CAAE 0250.0.268.000-11).

Anthropometric measurements, blood pressure and clinical data

Body weight was measured to the nearest 0.1 kg by using an electronic scale, with individuals wearing light clothing, but no shoes, in the morning; height was measured to the nearest 0.1 cm by using a stadiometer. BMI was calculated as weight (kg) divided by height (m) squared. The waist circumference (WC) was measured with a tape in the region between the last rib and the iliac crest, always in the standing position. Three blood pressure

measurements using a calibrated sphygmomanometer were taken with a 1-min interval after the participant had been seated and were recorded on the left arm. The mean of these measurements was used in the analysis. Neurological deficit score was evaluated using the modified Rankin Scale (mRS) (Bonita and Beaglehole 1988) applied within eight hours of the admission (baseline) and after three-month follow-up at the Neurology Outpatient Department of the University of Londrina. The Rankin original scale is divided in six grades, where zero corresponds to subjects with residual symptoms or light disability and grade five are subjects with severe disability, confined to bed or in a wheel chair. Thereafter, Rankin scale was modified with addition of death as the sixth grade (ANDRÉ, 2006).

The outcome was assessed by clinical examination or using telephone interviews with the patients or their relatives (Oh et al., 2011; Babu et al. 2013). The mRS is commonly used for measuring the degree of disability or dependence in the daily.

Biochemical biomarkers

After fasting for 12 hours, the subjects underwent the following laboratory blood analysis evaluated through a biochemical auto-analyzer (Dimension Dade AR Dade Behring, Deerfield, IL, USA) using Dade Behring® kits: total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol (TG), and glucose. Plasma insulin level was determined by chemiluminescence microparticule immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA).

Oxidative and Nitrosative analysis

Tert-butyl hydroperoxide-initiated chemiluminescence

Plasma levels of lipid hydroperoxide were evaluated by tertbutyl hydroperoxide-initiated chemiluminescence (CL-LOOH) as described previously (GONZALEZ, LLESUY & BOVERIS, 1991), and the results were expressed in relative light units.

Determination of advanced oxidation protein products (AOPPs)

AOPPs were determined in the plasma using the semi-automated method described by Witko-Sarsat and cols. (Witko-Sarsat, et al., 1996). AOPP concentrations were expressed as micromoles per liter (µmol/L) of chloramines-T equivalents.

Total radical-trapping antioxidant parameter

Total radical trapping antioxidant parameter (TRAP) was determined as reported by Repetto et al. This method detects hydrosoluble and/or liposoluble plasma antioxidants by measuring the chemiluminescence inhibition time induced by 2,2-azobis (2-amidinopropane). The system was calibrated with the vitamin E analogue Trolox. Serum uric acid levels were determined using a biochemical auto-analyser (Dimension Dade AR; Dade Behring) and were used to correct the TRAP values. TRAP measurements in conditions associated with hyperuricemia, such as the MetS, may be inaccurate because uric acid concentration accounts for 60 % of total plasma antioxidant capacity. Thus, a correction of TRAP based on uric acid concentration was performed (Venturini, et al., 2012).

Determination of nitric oxide metabolites

Serum levels of nitric oxide metabolites (NOx) were assessed by nitrite (NO2−) concentration according to the Griess reaction, supplemented by the reduction of nitrate to nitrite with cádmium (Guevara, et al., 1998 e Navarro-Gonzálvez, García-Benayas & Arenas, 1998).

Determination of homocysteine

Plasma levels of homocysteine were determined by chemiluminescence microparticule immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA).

Statistical analysis

Categorical variables were evaluated using the chi-square test and the data were expressed in absolute number (%). To verify the distribution of the data, a Shapiro-Wilk normality test was used. To evaluate the homogeneity of the variances, a Levene test was used. Parametric data were evaluated by Test t of Student and were expressed as mean ± Standard deviation (±SD). Non-parametric data were assessed by Mann-Whitney test and were expressed as median and interquartile range (25%-75%). Statistical difference was considered when p <0.05. Binominal logistic regression was performed to determine associations. The variables that presented in the univariate analysis p<0.10 were included in the binomial logistic regression analysis. Odds ratio and confidence interval were calculated.

All statistical analyzes were performed using statistical software SPSS version 22.0 (IBM, USA).

RESULTS

Serum homocysteine levels in patients with ischemic stroke and controls

Figure 1 shows the differences in homocysteine levels between healthy subjects (control group) and the group of patients with ischemic stroke. Patients with stroke have higher levels of homocysteinemia than controls (p <0.001) when the results were adjusted for gender, ethnicity and age. The median of the group of patients was 13.59 (25-75% interquartis: 9.73-17.47), whereas that of the control was 11.46 (25-75% interquartis: 9.71-14.04).

Figure1 - Patients with ischemic stroke showed higher plasma levels of homocysteine (median: 13.59 ,25-75% interquartis: 9.73-17.47) than control group (median: 11.46, 25-75% interquartis: 9.71-14.04). Mann-Whitney test. *p<0,001 adjusted by sex, ethnicity and age.
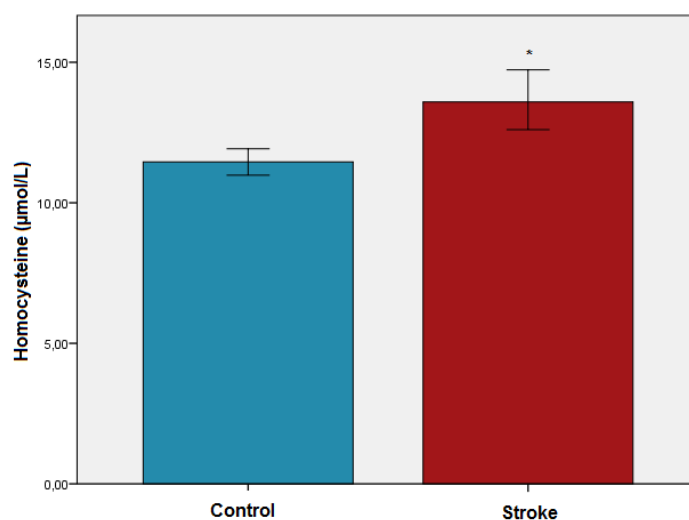
Characteristics of patients according to the homocysteine levels

Data from Table 1 show the demographic characteristics, lipid profile, drug use and blood pressure of the patients with ischemic stroke. The patients were divided according to the median obtained in homocysteine levels: homocysteine <13.59, and with hyperhomocysteinemia ≥ 13.59. There were significant differences regarding sex (p= 0.001), modified Rankin scale at the baseline (p= 0.034) and glucose (p= 0.020).

Table 1 - Demografic characteristics and metabolic biomarkers according homocysteine levels in patients with ischemic stroke.

Parameters	Reference group (n=85)	Hyperhomocysteinemia (n=85)	p
Age (years)	68 (60-77)	71 (56-77)	0,899
Female/Male (%)	46 (54,1)/39 (45,9)	25 (29,4)/60 (70,6)	0,001
Caucasian/Not Caucasian (%)	68 (80,0)/17 (20,0)	64 (75,2)/21 (24,8)	0,461
Antihypertensive (N/Y) (%)	68 (80,8)/16 (19,1)	55 (73,3)/20 (26,7)	0,252
Hypoglycemic agents (N/Y) (%)	27 (32,9)/55 (67,1)	18 (24,3)/56 (75,7)	0,236
Hypolipidemic agents (N/Y) (%)	28 (34,1)/54 (65,9)	20 (27,0)/54 (73,0)	0,336
Smoker (N/Y) (%)	15 (17,6)/79 (82,4)	22 (27,5)/58 (72,5)	0,129
MetS (N/Y) (%)	36 (42,3)/49 (57,7)	25 (30,4)/57 (69,6)	0,111
Hypertension (N/Y) (%)	73 (85,8)/12 (14,2)	64 (78,0)/18 (22,0)	0,187
Stroke PRÉVIO(N/Y) (%)	30 (36,1)/53 (63,9)	35 (42,6)/47 (57,4)	0,390
Rankin (Baseline)	4 (3-5)	4 (2-4)	0,034
Rankin (After 3 Months)	4 (2-6)	4 (2-6)	0,413
BODY MASS INDEX (Kg/M²)	26,00 (5,72)	26,57 (5,15)	0,467
PAS	147 (120-160)	150(129-170)	0,300
PAD	85 (80-100)	90 (80-100)	0,703
CHOLESTEROL TOTAL (mg/dL)	179 (53)	170 (49)	0,323
LDL-C (mg/dL)	102 (80-129)	129 (98-74)	0,450
HDL-C (mg/dL)	43 (17)	42 (14)	0,806
Triglycerides (mg/dl)	137 (98)	129 (85)	0,732
Glucose (mg/dl)	129 (105-207)	117 (98-148)	0,020
Insulin (µU/ml)	27,22 (40,30)	25,49 (33,93)	0,660

Qui-square test. Data are expressed as absolute number (%). Teste t de Student. Data are expressed as media (±SD). Teste de Mann-Whitney. Data are expressed as median (25%-75%). F: Female; M: male; C: Caucasian; NC: not Caucasian; N: No; Y:Yes; MetS: Metabolic Syndrome; PAS: Sistolic arterial pressure; PAD: diastolic blood pressure; LDL-C: low density lipoprotein; HDL-C: High density lipoprotein. Reference Group: Homocysteine <13,59. hyperhomocysteinemia ≥ 13,59.

Oxidative stress biomarkers according to the homocysteineinemia levels

Patients with ischemic stroke and hyperhomocysteinemia had a trend to reduced NOx (p= 0.071) and increased TRAP (p= 0.003) levels when compared to the patients without hyperhomocysteinemia (table 2).

Table 2 - Oxidative stress biomarkers according homocysteine levels in patients with ischemic stroke

Parameters	Reference group n=85	Hyperhomocysteinemia n=85	p
AOPP (µmol/Lof chloramine T equivalents)#	149 (116-220)	189 (120-259)	0,153
NOx (µmol/L)#	18,32 (11,90-22,80)	14,91 (9,78-19,54)	0,071
LOOH (cpm)#	20417,50 (14933,0-29389,0)	21105 (16266,0-30074,0)	0,482
TRAP (µM of Trolox)	759 (162)	839 (188)	0,003

Teste t de Student. Data are expressed as media (±SD). Teste de Mann-Whitney. Data are expressed as median (25%-75%).AOPP: advanced oxidation protein products; NOx: Nitric oxide; LOOH: Lipid hydroperoxide; TRAP: Total radical trapping antioxidant parameter. Reference group: Homocysteine <13,59. Hyperhomocysteine ≥ 13,59. # Data evaluated by Ln.

Association of homocysteine levels with biological and oxidative stress markers

After performing the binomial logistic regression analyses (Table 3), it was verified that sex (p= 0,032), glucose (p= 0.017) and TRAP (p= 0.019) were independently associated with hyperhomocysteinemia in patients with ischemic stroke.

Table 3 - Binomial logistic regression analysis in ischemic stroke patients according to the homocysteine levels (homocysteine <13,59 as reference group) and explanatory variables

Parameters	B	S.E.	Wald	Odds Ratio (IC 95%)	p
Sex (Female/Male)	0,902	0,421	4,584	2,464 (1,079-5,623)	0,032
Rankin	-0,013	0,156	0,007	0,987 (0,728-1,340)	0,935
Glucose	-0,009	0,004	5,698	0,991 (0,984-0,998)	0,017
TRAP	0,003	0,001	5,519	1,003 (1,000-1,005)	0,019
NOx	-0,016	0,022	0,523	0,984 (0,943-1,028)	0,469

. Data are expressed as absolute number. Teste t de Student. Data are expressed as media (±SD). Teste de Mann-Whitney. Data are expressed as median (25%-75%). NO: Nitric oxide; TRAP: Total radical trapping antioxidant parameter. Reference group: Homocysteine <13,59. Hyperhomocysteinemia ≥ 13,59.

DISCUSSION

The main findings of the present study were that increased TRAP, male sex and decreased glucose levels were independently associated with hyperhomocysteinemia in patients with ischemic stroke. In addition, this study confirms that patients with ischemic stroke have higher homocysteine levels than healthy subjects.

Recently, Anniwaer et al. (2018) showed the presence of hyperhomocysteinemia in patients with primary cerebral infarction. Some studies also revealed that patients with high homocysteine levels have approximately 2 times higher stroke risk (Iso et al., 2004; Han et al., 2015). Of note, besides being a risk factor, HHcy in stroke was associated with severity and worse prognosis (Kwonet al., 2014) as well as with recurrence (Ye et al., 2017; Gungor et al.,2018). The mechanisms by which HHcy is related to stroke include direct toxicity, vascular endothelial injury, inhibition of N-methyl-D-Aspartate receptors, and induction of oxidative stress (Moretti and Caruso, 2019). Furthermore, it has been reported that HHcy is related with both kinds of stroke, ischemic and hemorrhagic, inducing cerebrovascular atherosclerosis and atherothrombosis in the first case, and probably, in the second one by upregulating the matrix metalloproteinases-9 (MMP-9) expression, which takes responsibility for atherosclerotic plaque instability and rupture, and intracranial hemorrhage incidence (Moretti and Caruso, 2019).

In relation to oxidative and nitrosative stress and homocysteine, our results showed that patients with ischemic stroke and HHcy had a trend to reduced NOx and increased TRAP levels when compared to the patients without hyperhomocysteinemia. In addition, TRAP was independently associated with HHCy in these patients. Other study also demonstrated a decrease in NOx in stroke patients (Serrano - Ponz, et al., 2016). Evidences showed that in response to higher Hcy levels, there is a decrease in NO level (Sharma et al., 2015), which promotes endothelial damage (Hou et al., 2018). HHcy-induced ROS production decreases NO production and bioavailability triggering increased redox signaling, therefore HHcy may also be associated with reduced plasma levels of NO and impaired endothelium-dependent vasodilation (Moretti and Caruso, 2019; Hou et al., 2018).

The total plasma antioxidant capacity can also be related to HHcy. A previous study demonstrated that antioxidants are markedly decreased in stroke patients (Cherubini, et al., 2000). The study concluded that most antioxidants evaluated were immediately reduced after stroke, possibly due to the detriment of the oxidative stress present in the microenvironment (Cherubini, et al., 2000). However, unexpectedly, our data showed the opposite direction;

38

TRAP was positively associated with HHcy, even without a concomitant increase in lipid or protein oxidative stress markers.

Interestingly, sex has been shown to affect tHcy concentration (Yang et al., 2014), and some previous studies reported a sex difference in the relationship between homocysteine and risk of cardiovascular disease (Bertoia et al., 2014; Zhong Et al., 2017). Our results showed that there were more male patients in the HHcy group, and that sex was associated to HHcy, independently of the possible confounding variables. Our findings are in agreement with other studies which also reported higher levels of plasma homocysteine in men than in women (Fukagama et al., 2000; Wang et al., 2015). This result could be explained mainly by the differences in estradiol and homocysteine metabolism pathway such as remethylation or transmethylation (Zhong Et al., 2017; Fukagama et al., 2000). Blom et al. (1988) suggested that a higher rate of methionine transamination in premenopausal women may contribute to lower homocysteine concentrations and hence protect against vascular disease, whereas Fukama et al. (2000) reported that changes rates of remethylation and transmethylation were higher in women than in men after an oral methionine load.

Some studies have shown that higher homocysteine levels are associated with diabetes type 2, and that homocysteine level is worsened by increasing insulin resistance, dyslipidemia and poor glucose control (Ala et al., 2017). Differently from the literature, in this study the patients with hyperhomocysteinemia had lower plasma glucose levels than healthy controls. Our data also showed an inverse association between HHcy and glucose; nevertheless, the majority of the patients of both group used hypoglycemic agents what deeply limits the validity of this measurement.

Our present study has certain limitations. First, this cross-sectional study does not allow inferences on causal relationships. Second, homocysteine level is influenced by several factors which were not considered in this study, such as dietary habits, folate and vitamin B12 levels, which may potentially cause bias. Third, in the current study, the majority of the ischemic stroke patients had moderate to severe disability (Rankin grade 4) with incapacity to walk and to attend their physiologic needs without assistance. Therefore, our data and association may be limited to ischemic stroke patients with this score.

In conclusion, this is the first study in which homocysteinemia and oxidative and nitrosative stress markers were investigated in stroke patients. HHcy were associated with male sex, decreased glucose and increased TRAP. More studies are necessary to elucidate the complex relationship between homocysteine and status redox in these patients.

References

Ala OA, Akintunde AA, Ikem RT, Kolawole BA, Ala OO[4], Adedeji TA. Association between insulin resistance and total plasma homocysteine levels in type 2 diabetes mellitus patients in south west Nigeria. Diabetes Metab Syndr. 2017 Dec;11 Suppl 2:S803-S809.

André C. Manual de AVC. 2 ed. Rio de Janeiro: Revinter, 45p., 2006.

Anniwaer J, Liu M, Xue K, Maimaiti A, Xiamixiding A. Homocysteine Might Increase the Risk of Recurrence in Patients Presenting with Primary Cerebral Infarction. Int J Neurosci. 2018. Sep 14:1-16.

Babu MS, Kaul S, Dadheech S, Rajeshwar K, Jyothy A, Munshi A.
Serum albumin levels in ischemic stroke andits subtypes: correlation with clinical outcome. Nutrition 29(6):872-5, 2013.
Bertoia ML, Pai JK, Cooke JP, JoostenMM, MittlemanMA, Rimm EB, Mukamal KJ (2014) Plasma homocysteine, dietary B vitamins, betaine, and choline and risk of peripheral artery disease. Atherosclerosis 235:94–101

Blom HJ, Boers G, van den Elzen J, van Roessel J, Trijbels J, Tangerman A. Differences between premenopausal women and young men in the transamination pathway of methionine catabolism, and the protection against vascular disease. Eur J Clin Invest 1988;18:633–8.

Bonita R, Beaglehole R. Recovery of motor function after stroke. Stroke 19:1497–500, 1988.
Cherubini A, Polidori MC, Bregnocchi M, Pezzuto S, Cecchetti R, Ingegni T, Di Iorio A, Senin U, Mecocci P. Antioxidant Profile and Early Outcome in Stroke Patients. Stroke. 2000;31:2295-2300.

Faraci FM, Lentz SR (2004) Hyperhomocysteinemia, oxidative stress, and cerebral vascular dysfunction. Stroke 35:345–347.

Feng PN, Liang YR, Lin WB, Yao ZR, Chen DB, Chen PS, Ouyang J. Homocysteine induced oxidative stress in human umbilical vein endothelial cells via regulating methylation of SORBS1. Eur Rev Med Pharmacol Sci. 2018 Oct;22(20):6948-6958

Fukagawa NK, Martin JM, Wurthmann A, Prue AH, Ebenstein D, O'Rourke B. Sex-related differences in methionine metabolism and plasma homocysteine concentrations. Am J Clin Nutr. 2000 Jul;72(1):22-9.

Global burden of disease study. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990- 2013: a systematic analysis for the Global Burden of Disease Study. Lancet. 2015. V. 386: 743–800.

Gonzalez Flecha B, Llesuy S & Boveris A (1991) Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle. Free Radic Biol Med 10, 93–100.

Guevara I, Iwanejko J, Dembin´ska-Kieć A, et al. (1998) Determination of nitrite/nitrate in human biological material by the simple Griess reaction. Clin Chim Acta 274, 177–188.

Gungor L, Polat M, Ozberk MB, Avci B, Abur U. Which Ischemic Stroke Subtype is associated with hyperhomocysteinemia? J Stroke Cerebrovasc Dis. 2018 Jul;27(7):1921-1929. Han L, Wu Q, Wang C, et al. Homocysteine, ischemic stroke, and coronary heart disease in hypertensive patients. A population-based, prospective cohort study. Stroke 2015;46:1777-1786.

Hou HT, Wang J, Zhang X, Wang ZQ, Chen TN, Zhang JL, Yang Q, He GW. Endothelial nitric oxide synthase enhancer AVE3085 reverses endothelial dysfunction induced by homocysteine in human internal mammary arteries. Nitric Oxide. 2018 Dec 1;81:21-27.

Iso H, Moriyama Y, Sato S, et al. Serum total homocysteine concentrations and risk of stroke and its subtypes in Japanese. Circ 2004;109:2766-2772. Kwon HM, Lee YS, Bae HJ, et al. Homocysteine as a predictor of early neurological deterioration in acute ischemic stroke. Stroke 2014;45:871-873.

Lotufo PA. Stroke in Brazil: a neglecteddisease. São Paulo Med. 2005. J. 123 (1): 3-4.

Lu SS, Xie J, Su CQ, Ge S, Shi HB, Hong Xn.Plasma Homocysteine Levels And Intracranial Plaque Characteristics: Association And Clinicalrelevance In Ischemic Stroke. BMC Neurol. 2018 Dec 6;18(1):200.

Moretti R, Caruso P. The controversial role of homocysteine in neurology: from labs to clinical practice. Int J Mol Sci. 2019 Jan 8;20(1). pii: E231.

Navarro-Gonzálvez JA, García-Benayas C & Arenas J (1998) Semiautomated measurement of nitrate in biological fluids. Clin Chem 44, 679–681.

Oh MS, Yu KH, Chu MK, Ma HI, Kim YJ, Kim JY, Lee BC. Mi SO, Kyung-Ho Y, Min-Kyung C, Hyeo-Il M, Yun JK, Joo YK, Byung-Chul L. Long-term prognosis of symptomatic isolated middle cerebral artery disease in Korean stroke patients. BMC Neurology, 2011.

Oikonomidi A, Lewczuk P, Kornhuber J, Smulders Y, Linnebank M, Semmler A, Popp J. Homocysteine metabolism is associated with cerebrospinal fluid levels of soluble amyloid precursor protein and amyloid beta. J Neurochem. 2016. Oct;139(2):324-332.

Panunzio MF, Pisano A, Antoniciello A et al. (2003) Supplementation with fruit and vegetable concentrate decreases plasma homocysteine levels in a dietary controlled trial. Nutr Res 23, 1221-1228

Repetto M, Reides C, Gomez Carretero ML, Costa M, Griemberg G, Llesuy S. Oxidative stress in blood of HIV infected patients. Clin Chim Acta. 1996;255:107-17.

Serrano-Ponz M, Rodrigo-Gasqué C, Siles E, Martínez-Lara E, Ochoa-Callejero L, Martínez A. Temporal profiles of blood pressure, circulating nitric oxide, and adrenomedullin as

predictors of clinical outcome in acute ischemic stroke patients. Molecular Medicine Reports 13: 3724-3734 2016.

Sharma, M.; Tiwari, M.; Tiwari, R.K. Hyperhomocysteinemia: Impact on neurodegenerative diseases. Basic Clin. Pharamcol. Aand. Toxicol. 2015, 117, 287–296.

Venturini D, Simão ANC, Scripes NA, et al. (2012) Evaluation of oxidative stress in overweight subjects with or without metabolic syndrome. Obesity 20, 2361–2366

Wang C, Han L, Wu Q et al. (2015) Association between homocysteine and incidence of ischemic stroke in subjects with essential hypertension: a matched case-control study. Clinical and experimental hypertension (New York, NY: 1993):1–6

Weiss N. Mechanisms of increased vascular oxidant stress in hyperhomocys-teinemia and its impact on endothelial function. Curr Drug Metab. 2005. 6(1):27-36.

Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, NguyenKhoa T, Nguyen AT, Zingraff J, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int, 49:1304-13, 1996.

Wu W, Guan Y, Xu K, Fu XJ, Lei XF, Lei LJ, et al. Plasma homocysteine levels predict the risk of acute cerebral infarction in patients with carotid artery lesions. Mol Neurobiol. 2016;53:2510–7.

Yang B, Fan S, Zhi X, Wang Y, Wang Y, Zheng Q, Sun G (2014) Prevalence of hyperhomocysteinemia in China: a systematic review and meta-analysis. Nutrients 7:74–90

Ye Z, Zhang Z, Zhang H, et al. Prognostic value of C-reactive protein and homocysteine in large-artery atherosclerotic stroke: a prospective observational study. J Stroke Cerebrovasc Dis 2017;26:618-626.

Zhong C, Xu T, Xu T, Peng Y, Wang A, Wang J, Peng H, Li Q, Geng D, Zhang D, Zhang Y, Zhang Y, Gao X, He J; CATIS Investigation Groups.
Plasma Homocysteine and Prognosis of Acute Ischemic Stroke: a Gender Specific Analysis From CATIS Randomized Clinical Trial. Mol Neurobiol. 2017 Apr;54(3):2022-2030.