ejpmr, 2016,3(6), 159-167

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

HYPERHOMOCYSTEINEMIA IN CEREBRAL VASCULAR ACCIDENTS AND ITS ASSOCIATION WITH DEFICIENCIES IN VITAMIN B GROUPS.

Amrane Mounira[1], Boussouf Khaira[2], Attalah Salah[3], Chekour Mouhamed Chahine[4], Touabti Abdelrezek[1]

[1]Research Laboratory of Genetic and Nutritional Cardiovascular Disease - Biochemistry Laboratory CHU Setif, Algeria.
[2]Research Laboratory of Genetic and Nutritional Cardiovascular Disease – Service of Cardiology CHU Setif, Algeria.
[3]Department of Animal Biology, Ethnobotany Palynology and Ethnopharmacology- toxicology Laboratory; Faculty of Natural Science and Life, Montouri University Constantine, Algeria.
[4]Service of Vascular Neurology CHU Setif, Algeria.

***Corresponding Author: Attalah Salah**
Department of Animal Biology, Ethnobotany Palynology and Ethnopharmacology- Toxicology Laboratory; Faculty of Natural Science and Life, Montouri University Constantine, Algeria.

Article Received on 13/04/2016 **Article Revised on 03/05/2016** **Article Accepted on 23/05/2016**

ABSTRACT

Cerebral vascular accidents constitute a public health problem and a cause of mortality and morbidity over the world. The hyperhomocysteinemia is an independent atherothrombotic risk factor for stroke. The B12 vitamin and folic acid are important determinants in homocysteinaemia levels. Our objective is evaluated the presence and / or absence of the association of hyperhomocysteinemia as well as vitamin B12 and vitamin B9 in cerebral vascular accidents in a population of the town of Setif, Algeria. This is a transversal study that includes 60 stroke patients hospitalized at the pavilion in vascular neurology emergencies and 60 controls. The blood in these patients was taking in 24-48 hours after cerebrovascular accident diagnosed by angiography scanner. We have dosing the homocysteine, vitamin B12, B9, total cholesterol, LDL and HDL, glucose, creatinine, creatinine clearance, uric acid, CRP, ferritenemie and researched other risk factors including diabetes, hypertension, smoking, obesity and metabolic syndrome according to the criteria of ATPIII. **SPSS18 statistical analysis:** The average homocysteinaemia in patients is significantly elevated compared to controls (14.14 ± 5.83µmol / l vs. 9.97 ± 2.57µmol / l). Hyperhomocysteinemia is correlated to vitamin B12 deficiency; 52.63% which presents a B12 deficiency and decreased vitamin B9. Stroke (AVC) in our patient was correlated with metabolic syndrome and the hyperhomocysteinemia, hyperglycemia and deterioration of renal function. **Conclusion:** The high frequency of deficiencies in Group B vitamins in our patients associated to the hyperhomocysteinemia found in diabetes obliges us to take charge emergency these deficiencies in the dietary plan as well as the therapeutic level.

KEYWORDS: Cerebral Ischemic Stroke, homocysteine, vitamin B12, vitamin B9.

INTRODUCTION

Stroke is a public health problem and a cause of mortality and morbidity in the world. Stroke is the third leading cause of death in industrialized countries (after heart attack and cancer). It concerns first the affection of the elderly subject, 75% of patients older than 65 years.

Strokes are divided into two types; 80% are ischemic (ischemic stroke or AIC, or cerebral infarction) and 20% haemorrhagic (Intraparenchymal haemorrhaging or HIP in 75% of cases and meningeal haemorrhage in 25% of cases.[1]

The most frequent pathology are either thromboembolic stroke atherosclerosis or thromboembolic cardiogenic etiologies conventional risk factors do not explain the high incidence of stroke. The identification of modifiable risk factors may help reduce mortality from stroke. Among the modifiable risk factors, hypertension, diabetes, obesity and smoking all contribute to the incidence and stroke events.

Biochemical parameters modifiable risk factors are abnormalities of lipid metabolism.[2] hypercholesterolemia, hyperhomocysteinemia found in diabetes increases the frequency of stroke.[3]

Homocysteine is a sulfur amino acid derived from an amino acid prevalent in our body that is methionine.[4]

The values of homocysteine varied between 5- 15 mg / dL in the normal population.[5] The introduction for the first time of homocysteine in the pathogenesis of atherosclerosis by Mc Cully when he found children with a hyperhomocysteinemia found in diabetes with severe arteriosclerosis and deficient in β synthetase cysthationine.[6] The hperhomocysteinémie is considered as a modifiable risk factor in cardiovascular or

cerebrovascular atherosclerotic pathologies and the hypercoagulable states.[7]

The increase in homocysteine has an important role in the pathogenesis of atherosclerosis, thromboembolism, endothelial dysfunction and the increase of the frequency of ischemic stroke.[8]

Other associations have observed in stroke between homocysteine and other risk factors especially smoking, renal impairment, deficits in vitamins B6 and B12.B9.[9]

Other mechanisms of hyperhomocysteinemia in the development of the thrombus, decreased thrombolysis, increasing the production of hydrogen peroxide, the increase in LDL oxidation all these factors contributed to the development of endothelial dysfunction.[10] In the cerebral vascular accident, there is liberation of NO by the vascular endothelium and increased homocysteine level in this first resulting in the formation of a complex with blood vessels and an oxidative stress.

Homocysteine, increasing the oxidative stress; decreases the expression of the activity of antioxidant enzymes and increase in enzymes that lead to the formation of superoxide anions.[11]

Homocysteine is also associated to high blood pressure, to hypercholesterolemia.[12] The vitamin B12 deficiency is a major cardiovascular risk factor.[13.14] Homocysteine or methylmalonic acid are indicators of the status of intracellular vitamin B12.[15] Another function of the homocysteine is a marker of folate status as the MTHFR mutations and other pathology as well as kidney dysfunction, cerebral vascular accidents, coronary and peripheral arterial disease.[16]

The metabolites of homocysteine as homocysteic acid entails a toxicity of glutamatergic receptors N-methyl-D-aspartate receptors was affecting their turns glutamate and intracellular Ca 2+ as activating protein proaptotiques and cell death.[17]

Hyperhomocysteinemia can also increased the SAM which in turn entails a neurotoxicity by inhibiting methylation monoamines which are neurotransmitters and methylation of the phospholipids.[18.19]
The hyperhomocystenémie associated with a stroke can affect the etiology and progression of stroke.

MATERIALS AND METHODS

Our work is a prospective, concerning of 60 patients with a cerebrovascular accident, recruited at the neuro vascular Service of the University Hospital of Setif in the period December 2014 to May 2015.

The 60 witnesses are recruited at the blood transfusion Center, they are blood donors presenting no pathology. We has performed the assay in patients the following parameters 48-72 H after hospitalization and among controls, folic acid, vitamin B12, homocysteine on (the Immulite 2000), the ferritenemie (Cobas e 411) blood sugar, cholesterol T, triglycerides, HDL cholesterol, LDL cholesterol, creatinine, urea, uric acid, bilirubin T, the ionogramme (Na +, K +, CL), the phospho-calcium balance and serum iron on the (COBAS INTEGRA 400), the haematological evaluation; hemoglobin, MCV, MCHC and IDVE on hematological PLC (Cobas).

We also looked for the age, sex, hypertension, diabetes, heart disease, obesity, smoking, metabolic syndrome according to the criteria of ATPIII, inflammatory syndrome by accelerated VS or CRP positive, clearance creatinine using the equation of Cockcroft. The cerebral vascular accident was objectified in patients by Tomodensitométric Test (CT) scan requested in the neurorovasculaire service CHU Setif Town.

Statistical analysis was performed with SPSS Version 20 software; a descriptive analysis (mean, standard deviation, variance and extended) and an analytical analysis (normality test, analysis of variance, mean comparison test, correlation test, Chi-square and odds ratio) Statistical significance is defined by a value p-value <0.05.

RESULTS

Table 1: Type AVC in our population

Stroke type patients	Patients	
	Effectif	Pourcentage%
Ischemic stroke	30	50
Hemorrhagic stroke	18	30
Central venous hrombosis	12	20
Total	60	100

Table 2: Distribution of the population according to various biochemical and hematological parameters.

	Patients	Controls	Average comparison
Age (years)	55,54±18,89	48.9±5.76	DS
Sex M / F%	46.9/53.1	49.2/50.8	DNS
Holate(ng/l)	11,31±6,17	13,61±6,19	DNS
Vitamins B12(pg/l)	270.98±189.35	274.80±150.23	DNS
Homocysteine(µmol/l)	14.14±5.83	9,97±2,57	DS
Triglycerides(g/l)	1.47±0.77	1.16±0.42	DNS
Cholesterol T(g/l)	1.71±0.34	1.61±039	DNS
Chol LDL(g/l)	1.06±0.66	1.23±0.32	DNS
Chol HDL(g/l)	0.50±0.40	0.55±0.07	DS
Blood sugar(g/l)	1.43±0.38	1,04±0,05	DS
Calcemia(mg/l)	84±20.22	90.12±	DNS
Uric acid(g/l)	50.96±16.72	55.23±	DNS
Creatinine(mg/l)	7,92±3,53	6,25±2,89	DNS
Biluribine T(mg/l)	12,44±4,02	8,54±0,96	DS
NA+(meq/l)	133.43±19.63	140± 6.47	DS
K+(meq/l)	3.65±0.54	3.85±0.56	DNS
serum iron(mg/l)	0.63±0.35	1.02±	DS
Ferritinimia(ng/l)	234.31±154.49	258±125	DS
Hemoglobin(g/dl)	13.47± 1.87	13.10±2.01	DNS
VGM fl/l	89.75 ± 10.12	90.45± 4.45	DNS
CCMH g/dl	33.11± 1.31	35.25± 1.96	DNS
IDVE%	14.36± 3.39	13.47±2.35	DNS

Table 3: Pearson Correlation different parameters in strokes (AVC)

	AVC	AGE	chol	TG	LDL	HDL	Glu	Urea	Creat	Clcrea	BILI	Hb	homocys	VB12	VB9
AVC		**0.33***													
Age											0.44**	0.46**		**0.35***	
Chol				**0.67****	**0.83****										
TG					**0.43****										
LDL															
HDL															
Glu								**0.41***							**0.25***
Urea															
Creat										**0.71****					
Clcreat															
BILI												**0.34***			
HB															
Homocyst														0.43**	0.23*
VitB12		**0.35***											**0.43****		
VitB9							**0.25***						**0.23***		

Table 4: Association of different risk factors of stroke (AVC) in patients.

Factors associated to strokes risk	%
Diabetes	70,3
hypertension	51,4
cardiopathy	2,7
Obesity >40%	36.8
Cholesterol T >2 g/l	24.3
Cholesterol HDL<0,40g/l	56,7
Cholesterol LDL>1g/l	48,6
TG >1,5g/l	35,1
Metabolic syndrome (ATP III)	65
Smoking %	27
inflammatory syndrome (CRPpositif)	60
IR(cl <60ml/mn) %	13,5
hyperhomocysteinemia >15 µmol/l	40.90
Deficiency of Vit B12 (<200)	31.25
deficiencies of VitB9	9.37
hyponatremia <135mmol/l	28.12
ferritinemia >300ng/l	31.25

Table 5: OR brute different parameters in stroke

	OR and confidence interval to 95%	Signification P
Homocysteine	4,18(0,34-11,24)	< 1‰
Metabolic syndrome	2,66(0,34-10,70)	1%
Deficiency of Vit B12	6.56(1.09-39.32)	<1‰
deficiencies of VitB9	1.33 (0.32-5.43)	<5%
hyperglycemia	1.35(0.29-6.18)	1%
hypertriglyceridemia	1.35(0.32-5.69)	1%
Chol LDL	2.66(0.66-10.70)	1%
CholHDL	1.35(0.34-5.36	1%
Cl creat	10.22(1-104.31)	1‰

A clear predominance of ischemic stroke than hemorrhagic stroke and venous thrombosis central.

The AVCH represent an important fraction, 50% is represented by the meningeal haemorrhage and 50% with intracerebral hemorrhage (Table1).

The average age of patients was 55.54 ± 18.89 and is significantly higher as compared to controls Without Predominantly of sex.

In controls homocysteine is 10.29 ± 2.44 µmol/l in males and 9.66 ± 2.27 µmol/l in females and 9.97 ± 2.57 µmol/l for the two sexes.

The superior limit of normal of homocysteinaemia can be fixed at the average + 2 standard deviations or (2 x 9.97 + 2.57 µmol/l) = 15.11 µmol/l.

The plasma concentrations of folate in the controls are 13.61 ± 6.19 ng / ml, which vary from 2.9 to 24 ng / ml and vitamin B12 is 274.8 ± 150.23 pg / ml. and which varies between 106-810 and the difference is not significant with patients.

The average homocysteine in patients is 14.14 ± 5.83 µmol / l varies between 5.4-23.5µmol / l. The presence of hyperhomocysteinemia is 40.90% in the stroke and is significantly higher compared with controls without predominance of sex.

Blood glucose and total bilirubin were significantly higher in patients compared to controls. HDL cholesterol and natremia were significantly lower than in controls (Table 2). The age is associated with a stroke by Pearson correlation (r = 0.33, p <5%).

Homocysteine was correlated to vitamin B12 (r = 0.43, p <1%) and vitamin B9 (r = 0.23, p <5%).

Vitamin B12 is correlated with age (r = 0.35, p <1%) and vitamin B9 is correlated to blood glucose levels (r = 0.25, p <1%) (Table 3).

Hyperhomocysteinemia on one hand explained by Vit B12 deficiency which 31.25% have a level <200 pg / ml, 25% have a rate between 200-270 pg / ml, 28.12% between 270-370 pg / ml and 15.62% > 370pg / ml.

On the other hand hyperhomocysteinemia is explained by the decrease in folate and 9.37% for a rate <3 ng / ml, 59.38% a rate between 3.1-8ng / ml and 31.25% rate> 8 ng / ml.

Deficiencies in vitamins B12 are most important however anemia for hemoglobin <12 g / l of 18.8% and in most cases is a normochromic normocytic anemia, and megaloblastic anemia is almost totally absent.

Diabetes is the leading cause of strokes and is more de70.3% of which 37.5% are treated by glucophage and obesity was present in 70% of which 36.8% have severe obesity.

Metabolic syndrome according to the criteria of ATP III was present in 65%, 60% tobacco, hypertension in 51.4% 46.87% which are treated with diuretics.

The inflammatory syndrome with VS accelerated and / or positive CRP is present in 60% the deterioration of renal function with a clearance <60ml / min was present in 13.5%.

More than 56.7% of our patients have a rate of HDL chol <0.40 g / l and HDL cholesterol mean patients was significantly decreased than controls; is it results of unprotected patients?.

More than 15.6% of our patients were treated for a period extended by omeprazole and pertain the concept of gastritis. Microcytic normochromic anemia was present in 10.7% and only 31.25% have a ferritenemia levels> 300 ng / l. Hyponatremia <135 mmol / l was present in 28.12%.

Our patients have a creatinine clearance using the equation of Cockroft <60ml / min in 13.5%, for a clearance between 60-89ml / min in 21.6% and clearance ≥90ml / min in 64.9% (**Table 4).**

DISCUSSION

Stroke is one of major cause of mortality in the general population and is a public health problem in the world.[20] The identification of risk factors that are associated with the development of this affection is very important The homocysteine was higher in our males controls than in women.

This finding is consistent with the general literature. Usually, at the same age, homocysteine is higher than 1 µmol/l in males than in women. The possible causes could be interference of female hormones with the homocysteine metabolism, kidney function better and a smaller muscle mass in women.

Several studies converges towards our study confirms the association of hyperhomocysteinemia to stroke and the odds ratio was OR = 4.18 (0,34-11,24).[21.21.23.24]

Homocysteine causes significant endothelial aggression by different mechanisms leading to the formation of atherosclerotic plaque; inhibition of growth factors in endothelial cells, inducing an imbalance between O.- and NO causing an alteration of the normal vascular physiology, induction of expression of various adhesion molecules, increased lipid peroxidation by increasing the oxidized LDL.

These mechanisms explain part of the association of hyperhomocysteinemia and stroke.[25]

Homocysteine can produce hydroxyl radicals and lipid peroxidation by auto-oxidation and the formation of thiol group sulfidryl.[26]

Homocysteine increases peroxynitrite who activate their towers poly ADP-ribose polymerase PARP. These latter potentiate vascular dysfunction in stroke and in case of diabetes. In addition peroxynitrite can induce oxidative stress by the nitration of tyrosine residues of proteins, inhibition of NO synthase and production of superoxide ions all contributing to the reduction of NO.[27]

The hyperhomocysteinemia is associated with increased prothrombotic factors such as β-thromboglobulin, plasminogen activation (tissue plasminogenactivator) and factor VII.[28]

Several studies have established the relationship of hyperhomocysteinemia with the stroke of the big and small blood vessels.[29.30.31]

The hyperhomocysteinémieassocié has an ischemic cerebrovascular accident is a risk of mortality after adjustment for other risk factors, age, sex, smoking, Chole LDL, blood sugar, diabetes, hypertension, the obesity and inflammation.[32.33] Perini and neck have not observed correlation between hyperhomocysteinemia and severity of cerebral vascular accident.[34]

The hyperhomocysteinemia is explained by much of the vitamineB12 deficiency in the elderly and folate deficiency.

The frequency of vitamin B12 deficiency by taking the threshold 200 pg / ml in the stroke was higher than that found in other studies, which vary between 8-22% according to studies done.[35.36.37.38.39.40] and the odds ratio was OR = 6.56 (1.09-39.32).

Vitamin B12 deficiency is very common elderly subjects and it is also consistent with other studies.[41.42.43]

However, the prevalence of folate deficiency was near relative to the frequencies found in other studies, which vary between 9.8-20% in different studies.[44.45] and (odds ratio OR = 1.33 is (0.32- 5.43).

n elderly subjects, decreased folate and vitamin B12 are common. It results from an absorption disorder that could affect a achlorhydrique gastritis and / or imbalance of the intestinal flora and / or villous atrophy or secondary to decreased intake.

Several studies incriminates the advanced age as a risk factor for vitamin B12 deficiency and folate.[46.47] and the increase in cardiovascular disease and homocysteine in the same age.[48.49.50.51]

However, this vitamin B12 deficiency was observed in our population diseased and even witnesses who were younger.

The folate deficiency were frequent in patients with hyperglycemia or diabetes, they increase renal elimination of hydrosoluble B group vitamins.[52.53.54]

In spite of that there are deficiencies in vitamin B12 and B9 but no megaloblastic anemia this may be explained by the IDVE which is an index of the heterogeneity of erythrocytes (anisocytosis) is a prognostic factor for cardiovascular diseases, 'IDVE predicts mortality independent of hemoglobin levels and hematocrit.

The biological mechanism for increasing the IDVE (index deviation erythrocyte) can be explained by the delay in the maturation of red blood cells; Clinically disorders IDVE is suggested by iron deficiency anemia or increases IDVE.

When the VGM anemic patients <80 FL the systemic search of a vitamin B12 deficiency and B9 generally not helpful, one must hold that macrocytosis can be masked by a related microcytes iron deficiency or thalassemia condition. Obesity was significantly linked to stroke in our study and several studies. This association was stronger when it is associated with high blood pressure and high levels of blood glucose and cholesterol.[55.56]

Our study reports the association of stroke to the decrease in HDL cholesterol and increased LDL cholesterol and in agreement with other studies that found this association.[57.58] and the odds ratio is to decrease Chol HDL OR = 2.66 (0.66-10.70) and increased LDL Chol OR = 1.35 (0.34-5.36) but the odds ratio may be exaggerated because more than half of our population were on diuretics, these contribute to the

disruption of lipid profile. and finally the deterioration of renal function that can be a cause and consequence in hyperhomocysteinemia.[59.60]

CONCLUSION

Our study was made on a small group of patients with extensive atherosclerotic field shows the association of homocysteine had the stroke. The modifiable risk factor is associated with a high frequency of deficit in vitamin 12 and vitamin B9 despite the absence of megaloblastic anemia suggesting that homocysteine is a cause of ischemic pathophysiology.

Must we searched for front of all anemia associated to cardiovascular disease the assay homocysteinaemia, used as a prognostic marker of ischemia?.

In the majority of cases, cerebral vascular accident plays the principal role in the alteration of endothelial walled, however, his association with other risk factors makes it unpredictable prognosis.

Therapeutic prevention of neurovascular risk largely through cholesterol lowering especially fibrates and statins, however, the effectiveness of this prevention mark these limits. Homocysteine opens up the outlook detection, prevention and treatment of neurovascular diseases in the knowledge that these determinants are vitamin B12, B6 and B9. Of intervention trials with vitamin therapy is being planned in our region of Setif and tests measuring the impact of vitamin therapy on vascular events.

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