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Research Article

Hyperhomocysteinaemia and Folates Deficiency in Ischemic Stroke Victims in Developing Country

Kombate D*, Amouzou K, Assogba K Gbandi Fa Md, Belo M, Balogou Aak and Grunitzky Ek

Department of Neurology, Teaching Hospital Campus of Lomé, Lomé-Togo

***Corresponding Author:** Kombate Damelan, Teaching Hospital Campus of Lomé, Department of Neurology, Lomé-Togo.

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Abstract

Background: The hyperhomocysteinaemia is an independent and modifiable vascular risk factor. The metabolism of the homocysteine involves the folates and cobalamin. These vitamins intake reduces the hyperhomocysteinaemia.

Objective: To evaluate the prevalence of the hyperhomocysteinaemia associated with the folates and cobalamin deficiency among victims of ischaemic stroke.

Patients and Methods: The diagnosis of the ischaemic stroke was made on clinical and brain CT scan criteria among 65 victims. The measurement out of homocysteine was made by the Fluorescence Polarizing Immunoassay (FPIA) technic. The SimulTRAC-SNB box of Inc Pharmaceuticals was used for the quantitative and simultaneous radio-immunological measurement out of the B12 vitamin and the folates in the serum.

Results: The average age was of 60.51 years. The prevalence of the moderate hyperhomocysteinaemia was of 40% and that of the intermediate hyperhomocysteinaemia of 10.70%. The average homocysteinemia was of 18.2 µmol/l among hypertensive and 21.2 µmol/l in diabetics. Five (7.7%) patients presented the hyperhomocysteinaemia as only vascular risk factor. All the patients presented a deficit in folates with rates of folates lower than 41 nmol/l.

Conclusion: The hyperhomocysteinaemia was probably caused by the folates deficiency.

Keywords: Hyperhomocysteinemia; Folates; Ischaemic Stroke

Introduction

The hyperhomocysteinaemia is an independent and modifiable vascular risk factor. Several studies established a relative risk to develop an ischaemic stroke among hyperhomocysteinaemia patients, [1,2].

Homocysteine is an intermediate sulphur amino-acid metabolism of methionine. The metabolism of the homocysteine involves B6 (pyridoxin), B9 (folates) and B12 (cobalamin) vitamins. The plasmatic concentration of these vitamins is inversely proportional to the homocysteinemia. The hyperhomocysteinaemia has several causes (metabolic, nutritional, and genetic). The genetic cause of hyperhomocysteinaemia undergoes several polymorphisms. The most important polymorphism is the 677C → T mutation of the gene encoding for the synthesis of Methylen Tetrahydrofolate Reductase (MTHFR). The hyperhomocysteinaemia can be associated with folates, pyridoxin and cobalamin deficiency, [3]. The intake of these vitamins reduces the hyperhomocysteinaemia and the vascular risk, [4]. A high prevalence of hyperhomocysteinaemia (20%) with rates of folates lower than 6.75nmol/l with a MTHFR CT/TT genotype was recently reported in the coastal regions of West Africa, specifically in Togo and Benin, both being low income countries, [5]. This hyperhomocysteinaemia is prevalent due to metabolic, nutritional or genetic causes. Our objective was to determine the average homocysteinemia, the rate of cobalamin and folates among ischaemic stroke victims of the Teaching Hospital, CHU Campus in Togo and the associated vascular risk factors.

Materials and Methods

The department of neurology of the Teaching Hospital CHU Campus of Lomé has been used as framework of study. This department welcomes an average of 400 victims of stroke per year; of which 35% victims of hemorrhagic stroke and 65% of ischaemic stroke. The study concerned 72 consecutive patients, victims of ischaemic stroke between May 5, 2011 and November 5, 2012. The study had the assent of the Togolese ethics committee and the patients agreed to belong to the study by giving their written assent after a thorough presentation of the purpose of the study. All the patients underwent a rigorous clinical examination with research of the vascular risk factors. The diagnosis of the ischaemic stroke

was made on clinical and brain scanning criteria (Brain CT scan interpreted by a radiologist) among patients admitted after the beginning of the symptoms and not having any treatment before that admission. Seven (7) patients with antiepileptic treatment, kidney failure (creatinine higher than 106 µmol/l) and under treatment with the B vitamins were excluded from our study, [6]. Sixty-five (65) patients were considered for the study. For the dosage of homocysteine, blood samples were collected before the patients took their breakfast in a tube with Ethylen Diamin Tetra Acetate (EDTA). Plasma was separated from red blood cells in less than 4 hours and the samples were frozen at - 4 degree Celsius. The dosage of the homocysteine was made by the Fluorescence Polarizing Immunoassay (FPIA) technique. We considered for the 2 sexes (men and women) the threshold of 15 µmol/l as threshold of normal homocysteinemia, the moderated hyperhomocysteinaemia between 15 and 30 µmol/l, the intermediary between 30 and 100 µmol/l and severe, the concentrations higher than 100 µmol/l, [7]. The dosage of the B12 vitamins and folates: The SimulTRAC-SNB box of Inc Pharmaceuticals was used for the quantitative and simultaneous radio-immunological proportioning of the B12 vitamin and the folates in the serum. The calibration curve and the results were calculated starting from the counting of Cobalt 57 for the B12 vitamin and the counting of iodine 125 for the folates. The concentration of folates that was lower than 41 nmol/l and B12 vitamin inferior to 147 pmol/l was used as criterion to define the deficit in these vitamins, [8]. The data were collected and processed using the software EXCEL 2007 of Microsoft Office, then transferred on statistical software SPSS 12. Windows in view of the tabulation and the tests of association and correlation. The test of Khi2 was used with the threshold of 5% to compare the variables.

Results

Among the 65 patients, there were 44 and 21 women (sex- ratio of 2.09). The average age was 60.51 years (34 - 82). Eleven percent (11%) were less than 45 years, 37% between 45 and 60 years old, whereas 51% were above 60 years. Twenty percent (20%) were diabetics, 83% were hypertensive; seven-point seven percent (7.7%) were found with hyperhomocysteinaemia as only vascular risk factor.

The ct scan displays 21 cases of large ischemic stroke, 16 of large ischemic associated with multiple infarcts and 28 cases of multiple infarcts. The electrocardiogram did not display any atrial fibrillation. The neck Doppler did not display any stenosis of carotid arteries. The average homocysteinemia was of 18 µmol/l (4.2 - 84.6); 49.3% patients had a normal homocysteinemia, 50.7% had a hyperhomocysteinaemia and among those, 40% with a moderate hyperhomocysteinaemia and 10.7% with an intermediate hyperhomocysteinaemia. We did not find a correlation between the homocysteinemia and the age (p = 0,088). The average homocysteinemia among men was 17.9 µmol /l and 17,4 µmol/l among women (p = 0.84). Among the diabetics, the average rate of homocysteinemia was far higher in diabetics (21.2 µmol/l) than non-diabetics (16.9 µmol/l); p = 0.05. The average homocysteinemia among hypertensive was 18.2 µmol/l and 15.4 µmol/l among non-hypertensive patients (p = 0.38). All the patients had folates deficiency. The average rate of folates was 6.98nmol/l (1.70 - 40). It was 7.08 nmol/l (max 40 nmol/l) in men and 6.78 nmol/l (max 20 nmol/l) among women (p = 0.06); 60% patients had a rate of folates lower than 6.3 nmol/l and 40%, between 6.30 and 40 nmol/l. We did not find any cobalamin deficiency. The average rate of cobalamin was 883.88 pmol/l (195 - 4700). There was, a discrepancy, though not statistically significant, between the homocysteinemia on the one hand, and the folates (R = -0.57, p = 0.65) and cobalamin (R = -0.60, p = 0.63) on the other hand, between the cobalamin and the folates (R = -0.54, p = 0.67). For the rates of folates < 6.30 nmol/l (Table), the average of homocysteinemia was 18.8 µmol/l with a maximum of 48.2 µmol/l. For rates of folates between 6.30 and 40 nmol/l, the average homocysteinemia was 16.6 µmol/l with a maximum of 49µmol/l. The mean value of LDL cholesterol was of 3.3 mmol/l (1.1 - 6.5), 30% of subject had a hyperLDLcholesterolemia.

	< 6,3 nmol/l	6,3 - 40 nmol/l
< 15 µmol/l	19 (48, 7%)	13 (50%)
15 - 30 µmol/l	15 (38, 4%)	11 (42, 3%)
> 30 µmol/l	5 (12, 8%)	2 (7, 7%)
Total	39	26

Table: Homocysteinemia Level According to Folates Deficiency.

Discussion

Some insufficiencies were identified with our work. In fact, we did not study the genetic polymorphisms of homocysteine among our patients. However, several homocysteine genetic polymorphisms have been identified. The 677C→T mutation of the gene encoding for the synthesis of the MTHFR involves a moderate hyperhomocysteinaemia. It is the most important polymorphism, [9]. The 1298 A→C mutation and 1317 T→C (silent mutation) were also identified in gene MTHFR. The 1298 A→C polymorphism is associated with hyperhomocysteinaemia in partnership with the 677 C→T polymorphism, [1]. Another polymorphism, the 2756A→G was identified in the gene of methionine synthase (MTR), but its association with the hyperhomocysteinaemia was not established, [10]. The prevalence of hyperhomocysteinaemia was 50.7%; 40% patients presented a moderate hyperhomocysteinaemia. The average plasmatic homocysteinemia was 17.9 µmol/l among patients who are above 45 years old and 16.2 µmol/l (no significant difference, p = 0.088) among patients of less than 45 years. Although homocysteinemia increases with age in both sexes, it is higher among men than women, [11]. The average homocysteinemia was of 18µmol/l among hypertensive patients. Forty three percent (43%) associated a hyperhomocysteinaemia with an arterial hypertension. There was no significant difference (P = 0.38) in homocysteinemia between the hypertensive subjects and non-hypertensive. But according to some authors, this correlation, likely, exists, [12]. The hyperhomocysteinaemia constitutes an additional risk factor of the ischaemic stroke. It would, in addition, have a neurotoxic incidence/impact on the nervous system but the mechanism of action remains hypothetical, [13]. The hyperhomocysteinaemia by its effects (vascular and neurotoxic) is implied in the cognitive decline and in the occurrence of the vascular dementia, [14]. A significant difference exists in homocysteinemia among diabetics and non-diabetics subjects (p = 0.05).

The plasmatic homocysteinemia probably increases in the case of diabetes because, there might be a potentiation with pathogenic effects of hyperhomocysteinaemia in the genesis of an atherosclerosis in case of diabetes 2, [15]. Three theories explain the atherogenicity of the hyperhomocysteinaemia. The atherogenicity of homocysteine is caused by the peroxidation of the low-density lipoproteins (LDL) inducing a fragmentation of their apolipoprotein (apo) B100 at the origin of an increase in their capture by the receiving scavengers of the macrophages. According to the inflammatory assumption, the atherogenicity of hyperhomocysteinaemia could also result from a primitive activation of the vascular endothelial cells, leading to their functional deregulation, followed by platelets and leucocytic activation and a proliferation of the smooth muscular cells, [16]. For the third theory, the two previous theories lead together to the atherogenicity of homocysteine. Despite the fact that all the patients had the folates deficiency, only 50.7% patients had hyperhomocysteinaemia. We did not find any deficit in B12. The average rate of B12 vitamin was 883.88 pmol/l. This implies that, not all deficit in folates can lead to hyperhomocysteinaemia, and that there is a complex metabolic relation between the homocysteinemia, the folates and the cobalamin, [17]. Apart from the genetic polymorphism and metabolic cause like kidney failure, the main cause of hyperhomocysteinaemia is nutritional, [5]. The Nutritional origin of hyperhomocysteinaemia would depend more on a folates deficiency than cobalamin, [18]. We found an inverse correlation statistically uninfluential between the homocysteinemia, the folates and cobalamin.

The common metabolic way where the folates and the cobalamin interact is in the reaction of methionine synthetase. In this reaction, the 1C corresponding to methyl of the 5-methyl tetrahydrofolate (5 CH 3-THF) is transferred on Cobalamin to form the methyl cobalamin; then after the methyl is transferred on the homocysteine to produce methionine. The serum rates of Cobalamin and folates are affected by a deficiency in one of these two nutrients. In the cobalamin deficiency, the serum rate of folates rises, with regard to folates deficiency, the rate of Cobalamin decreases, [19]. The hyperhomocysteinaemia by vitamin deficiency is generally moderate. The folates, cobalamin and pyridoxin intake reduce it, [4].

Conclusion

This study cannot prove the real vascular impact of hyperhomocystaenemia because of the association of many vascular risk factors in our stroke victims, but suggests that folates deficiency prevalence is probably high among stroke victims. Although the folates deficiency is not known as a vascular risk factor, this deficiency could be the main cause of hyperhomocystaenemia among stoke victims in developing countries. The folates deficiency could be nutritional.

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Conflict of Interest and Source of Fundings

No conflict of Interest.

Bibliography

1. Kwon HM., *et al.* "Homocysteine as a predictor of early neurological deterioration in acute ischemic stroke". *Stroke* 45.3 (2014): 871-873.

2. Kumral E., *et al.* "Association of Hyperhomocysteinemia with Stroke Recurrence after Initial Stroke". *Journal of Stroke and Cerebrovascular Diseases* 25.8 (2016): 2047-2054.

3. Manolescu BN., *et al.* "Homocysteine and vitamin therapy in stroke prevention and treatment: a review". *Acta Biochimica Polonica* 57.4 (2010): 467-477.

4. Shklovsky VM., *et al.* "A role of genetic factors and related disorder of the folate cycle in ischemic stroke". *Zh Nevrol Psikhiatr Im S S Korsakova* 116(8 Vyp 2. Insult) (2016): 30-32.

5. Amouzou EK., *et al.* "High prevalence of hyperhomocyteinemia related to folate deficiency and the 677C→T mutation of the gene encoding methylenetetrahydrofolate reductase in coastal West Africa". *The American Journal of Clinical Nutrition* 79.4 (2004): 619-624.

6. Ye Z., *et al.* "High Prevalence of Hyperhomocysteinemia and Its Association with Target Organ Damage in Chinese Patients with Chronic Kidney Disease". *Nutrients* 8.10 (2016): E645.

7. Malinow MR, *et al.* "Homocysteine, diet and cardiovascular disease. A health care statement for health care professionals from the nutrition committee, American heart association". *Circulation* 99.1 (1999): 178-189.

8. Ni J., *et al.* "Association between the MTHFR C677T polymorphism, blood folate and vitamin B12 deficiency, and elevated serum total homocysteine in healthy individuals in Yunnan Province, China". *Journal of the Chinese Medical Association* 80.3 (2017): 147-153.

9. Wang X., *et al.* "Geographical and Ethnic Distributions of the MTHFR C677T, A1298C and MTRR A66G Gene Polymorphisms in Chinese Populations: A Meta-Analysis". *PLoS One* 11.4 (2016): e0152414.

10. Spence JD. "Homocysteine lowering for stroke prevention: Unravelling the complexity of the evidence". *International Journal of Stroke* 11.7 (2016): 744-747.

11. Huo Y., *et al.* "Efficacy of Folic Acid Therapy in Primary Prevention of Stroke Among Adults with Hypertension in China the CSPPT Randomized Clinical Trial". *JAMA* 313.13 (2015): 1325-1335.

12. Liu J., *et al.* "Relationship between cardio-ankle vascular index and homocysteine in hypertension subjects with hyperhomocysteinemia". *Clinical and Experimental Hypertension* 38.7 (2016): 652-657.

13. Bonetti F., *et al.* "Cognitive Status According to Homocysteine and B-Group Vitamins in Elderly Adults". *Journal of the American Geriatrics Society* 63.6 (2015): 1158-1163.

14. Whalley LJ., *et al.* "Homocysteine, antioxidant micronutrients and late onset dementia". *European Journal of Nutrition* 53.1 (2014): 277-285.

15. Kundi H., *et al.* "Association between plasma homocysteine levels and end-organ damage in newly diagnosed type 2 diabetes mellitus patients". *Endocrine Research* 42.1 (2017): 36-41.

16. Minet JC., *et al.* "Assessment of vitamin B12, folate and vitamin B6 status and relation to sulfur amino acid metabolism in neonates". *The American Journal of Clinical Nutrition* 72.3 (2000): 751-757.

17. Hainsworth AH., *et al.* "Homocysteine, hyperhomocysteinaemia and vascular contributions to cognitive impairment and dementia (VCID)". *Biochimica et Biophysica Acta* 1862.5 (2016): 1008-1017.

18. Smith AD and Refsum H. "Homocysteine, B Vitamins, and Cognitive Impairment". *Annual Review of Nutrition* 36 (2016): 211-239.

19. Blom HJ and Smulders Y. "Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects". 34.1 (2011): 75-81.

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