

DETERMINATION OF SERUM B₁₂ VITAMIN AND FOLIC ACID LEVELS IN PATIENT WITH STROKE

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Summary: The aim of this study is to evaluate whether any relationship between stroke and vitamin B₁₂, folic acid concentration. We examined 50 patients with stroke (male: 26 and female: 24) aged between (X±SD, year) 59±14 and 16 control subjects (male: 6 and female: 10) aged between (X±SD, year) 53±11. Patients were classified into two groups as infarct and hemorrhagic by using Computerised Brain Tomography and Cranial Nuclear Magnetic Resonance Imaging techniques. The percentage of patients with infarct was 47.5 % in female and 52.5 % in male while it was 40 % in female and 60 % in male with hemorrhagic. The vitamin B₁₂ levels of patients with infarct, hemorrhagic and control group were found as X±SEM, 355±47 pg/ml, 313±58 pg/ml, 1569±258 pg/ml and folic acid levels 13.4±2.6 ng/ml, 7.7±1.0 ng/ml, 14.8±4.0 ng/ml, respectively. The significant difference was found between subgroups of patients with stroke and control group in both folic acid and B₁₂ vitamin levels (p<0.001 and p<0.0001, respectively). But, there was no significant difference between vitamin B₁₂ and folic acid levels of patients with hemorrhagic and infarct. In order to well understand the effects of these vitamin in patients with stroke, more detailed follow up studies with long period are needed.

Key words: Vitamin B₁₂; Folic acid; Stroke

Introduction

Homocyst(e)ine refers to the sulfur-containing amino acids homocysteine, homocystine and homocysteine-cystine mixed disulfide, which is normally exist in plasma in the both free and protein bound forms (3). B₁₂, folic acid replace as a cofactor B₆ and coenzyme in homocysteine metabolism (16). Homocysteine can be metabolised by two pathway, either catabolised by the transsulfuration pathway to cysteine or remethylated to methionine mainly by the folate and vitamin B₁₂ dependent enzyme methionine synthase (7). Both these vitamins occupy a key position in the remethylation and synthesis of S-adenosylmethionine (SAME). Deficiency in either of these vitamin lead to decrease in SAME and increase in homocysteine. Therefore, while hyperhomocysteinemia increase the risk of stroke, it is unlikely to be a primary initiating factor (4,13). It has been found an inverse relationship between the status of the relevant B vitamins and homocysteine blood concentration. Supplementation of these vitamin results in a significant reduction of the homocysteine level. Nutritive amounts seem to be significant to obtain this reduction, even in the case of elevated homocysteine levels (9,14). Plasma homocysteine concentrations are increased in carotid artery diseases, lacunar stroke and also in hemorrhagic or embolic strokes (12). Folate deficiency is frequently observed in elderly people, smokers, alcoholics and oral con-

traceptive users (1). Total homocysteine concentration of approximately 15-40 % of patients with coroner insufficiency, cerebral, peripheral arterial diseases were found highly (2). A linear coefficient suggested a stroke risk increase by 6% to 7% for every 1-μmol/L increase in total homocysteine (17). Hyperhomocysteinemia is frequently present in cases of stroke is independent of other stroke risk factors or the type of stroke, and is partly related to renal function and the concentrations of homocysteine metabolism (8,10). A number of investigations support the theory that the decreased vitamin B₁₂ and folic acid levels are associated with stroke. This study was done to examine whether decreased vitamin B₁₂ and folic acid concentrations in patient with stroke.

Material and Method

Study subjects

In this study, we conducted a hospital-based-case-control study with 16 controls (male:6, female:10) and 50 patients (male: 26, female: 24) with stroke aged between (X±SD) 53±11 and 59±14, respectively. The baseline characteristics of study group is given in Tab. 1. Eighteen percentage of the patients were suffering from diabetes mellitus. Patients formerly having diagnosis of vitamins B₁₂ and folic acid deficiency and the patients using drugs that would change serum vitamin B₁₂ and folic acid level were

not included. Patients were diagnosed using Computerised Magnetic Resonance Imaging techniques. Patients were classified into two groups as hemorrhagic and infarct with these techniques.

Tab. 1: The baseline characteristics of study group.

n:50	Patient (XSD±D)	Control (X±SD)	
Age (year)	59±14	53±11	
Female/Male %	48/52	37/63	
Patients with diabetes mellitus n: 9	% 18	-	
Kind of stroke	Infarct	F: % 47.5	-
		M: % 52.5	-
	Hemorrhagic	F: % 40	-
		M: % 60	-

Specimen handling and storage

10 ml venous blood samples were taken into vacutainer tubes then left to coagulate in one hour at room temperature and the centrifuged at 2500-3000 rpm for 15 minutes. Sera was removed from sample. Biochemical routine analysis were performed the same day whereas other aliquots stored at -20°C in dark for the determination of vitamin B₁₂ and folic acid until analysis.

Analysis

The levels of glucose, urea, creatinine, cholesterol, triglyceride, HDL, LDL and VLDL in both patients and control groups were analysed by Technicon DAX 24 auto-analyser. The level of vitamin B₁₂ and folic acid in serum were evaluated with IMx analyser, (Abbott Diagnostics, North Chicago, USA), microparticle enzyme Immunoassay, with IMx Kits according to recommendation of manufacturer's producer.

Statistical Analyses

Data were usually reported as Mean standard error (SEM). Paired differences and the relations of the analytes are evaluated with paired samples t test and Pearson's bivariate correlation analysis. Comparison of subgroups were performed with Mann Whitney U test. Two tailed p<0.05 values were taken into consideration. Figures were drawn in multiple variables graph as box-and-whisker plots and dots. All statistical analyses and illustrations were obtained with MedCalc® statistic programme (Mariakerke-Belgium).

Results

Vitamin B₁₂, folic acid and some biochemical parameter levels are given in Tab. 2. The vitamin B₁₂ levels of infarct, hemorrhagic and control group were as X±SEM, 355±47 pg/ml, 313±58 pg/ml, 1569±258 pg/ml while folic acid levels were 13.4±2.6 ng/ml, 7.7±1.0 ng/ml, 14.8±4.0 ng/ml, respectively. Blood glucose level 135.6±15.0 mg/dl,

Tab. 2: Vitamin B₁₂, folic acid and some biochemical parameter levels of patients with stroke.

Number of patients n: 50	Kind of stroke	X±SEM
Vitamin B ₁₂ (pg/ml)	Infarct (A)	355±47
	Hemorrhagic (B)	313±58
	Control (C)	1569±258*
Folic acid (ng/ml)	Infarct (A)	13.4±2.6
	Hemorrhagic (B)	7.7±1.0
	Control (C)	14.8±4.0**
	<u>X±SEM</u>	<u>Reference Range</u>
Glucose (mg/dl)	135.6±15.0	70-110
Creatinine (mg/dl)	1.6±0.4	0.6-1.5
Urea (mg/dl)	43.5±9.7	25-40
Cholesterol (mg/dl)	191.2±8.4	120-240
Triglyceride (mg/dl)	112.5±10.7	44-175
HDL (mg/dl)	40.3±3.8	35-65
LDL (mg/dl)	125.3±2.7	60-230

*A and B versus C p<0.001

**A and B versus C p<0.0001

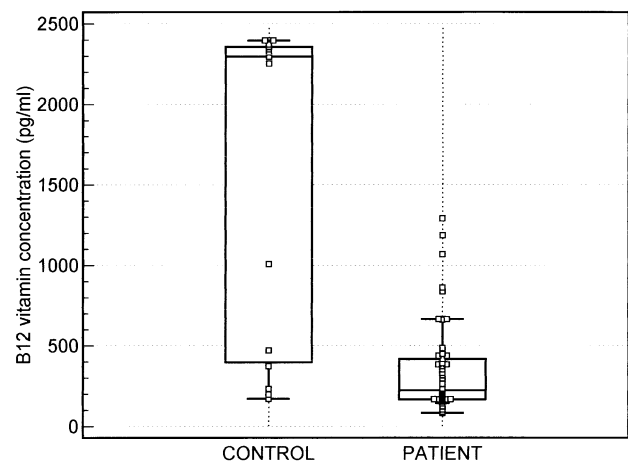


Fig. 1: Distribution of B₁₂ vitamin concentration of patients with stroke and control group.

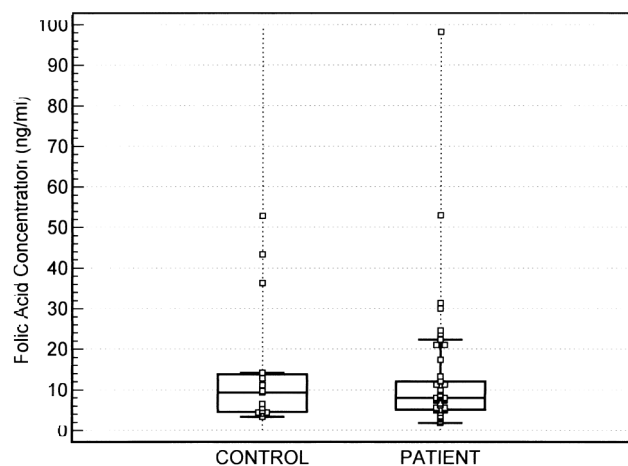


Fig. 2: Distribution of folic acid concentration of patients with stroke and control group.

creatinine 1.6 ± 0.4 mg/dl, urea 43.5 ± 9.7 mg/dl, cholesterol 191.2 ± 8.4 mg/dl, triglyceride 112.5 ± 10.7 mg/dl, HDL 40.3 ± 3.8 mg/dl, LDL 125.3 ± 2.7 mg/dl were found. All these parameters were in reference limits except glucose.

There was no significant difference between vitamin B₁₂ and folic acid levels patients with hemorrhagic and infarct subgroups ($p < 0.05$) while vitamin B₁₂ and folic acid levels of both subgroup were significantly higher than the control group ($p < 0.001$ and $p < 0.0001$, respectively). This relation was shown in Fig. 1 and Fig. 2. There was not any significant correlation between routine biochemical parameters, vitamin B₁₂ and folic acid concentrations.

Discussion

Hyperhomocysteinemia has recently been identified as a risk factor for stroke and other vascular diseases. But, pathophysiology of stroke is poorly defined. The frequency of vitamin B₁₂ and folic acid deficiency in elderly people was higher than younger ones and stroke is also seen in elderly people (18). In our study, vitamin B₁₂ and folic acid concentrations were unrelated with kind of stroke, some biochemical parameters, consumption of alcohol and/or cigarette, hypertension, hypercholesterolemia, diabetes which have causative effect on stroke (8,11,15). A number of investigations show that correlation of total homocystein concentration of control group and different patients groups (coronary artery diseases, peripheral vascular and cerebrovascular diseases) were high (3,5,6,12,13,16). Data from in vitro and in vivo experiments clearly indicate that folic acid deficiency results in a high plasma concentration of homocysteine due to an increase in extracellular flux. Furthermore, numerous epidemiological studies performed in healthy subjects have reported an inverse correlation between dietary and/or plasma folate status and homocysteinemia. These relationship have also been observed in atherosclerotic patients and linked to the incidence of cardiovascular disorders (1,5). The significant relation between serum vitamin B₁₂ and folic acid concentration of patients with stroke and control group was found in this study ($p < 0.0001$, $p < 0.001$, respectively). This relation was supported in a number of investigation (6,7,8,9,12,13,14). Hultberg et al. noted a significant decrease of blood folate concentrations in a subgroup of stroke patients who had increased plasma homocysteine concentrations (7). Supplementation of these vitamins results in a significant reduction of the homocysteine level. Nutritive amounts seem to be sufficient to obtain this reduction, even in the case of elevated homocysteine levels (9,14).

It can be concluded that dietary intake of vitamin B₁₂ and folate could be protective agent and medicament. The decreased level of B₁₂ and folic acid could be a risk factor

for stroke. But, acute phase variations are also likely to effect the serum level of these vitamins. This idea was supported by lower serum level of vitamin B₁₂ and folic acid in hemorrhagic subjects. Other specific studies should be performed to document and characterise the clinical efficacy of such treatment in these subjects.

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