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Serum Vitamin B12 Levels in Patients of Ischaemic Stroke: A Cross-Sectional Study

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Abstract

***Background:** Ischaemic stroke is the most common cerebrovascular disease and is one of the leading causes of death and long-term disability in the world. Vitamin B12 (B12) deficiency, by virtue of causing hyperhomocysteinaemia may be implicated as an acquired risk factor of ischaemic stroke, which is also easily modifiable. There is a scarcity of data from India regarding the prevalence of B12 deficiency and hence the correlation with ischaemic stroke patients. The objective of this study was to evaluate the relationship between serum vitamin B12 levels and ischaemic stroke, including folic acid and homocysteine levels.*

***Material and methods:** The study was a matched case-control study. 50 cases of ischaemic stroke were compared with an equal number of age and gender matched controls. Serum vitamin B12, folate and homocysteine levels were analysed as continuous data (Student's t test) as well as categorical data (Chi square test) using Statistical Package for Social Sciences (SPSS) version 21.0. Correlation of serum B12, folate and homocysteine in ischaemic stroke was studied using Pearson's co-efficient.*

***Results:** Mean levels of vitamin B12, folic acid and homocysteine in cases were 187.25 pg/mL, 7.95 ng/mL and 31.12 µmol/L respectively while in controls it was 463.84 pg/mL, 13.42 ng/mL and 6.63 µmol/L respectively and the differences were statistically significant, pvalue < 0.001. There was a negative correlation of vitamin B12 (-0.424) and folic acid (-0.355) levels and positive correlation of homocysteine (0.304) with ischaemic stroke.*

***Conclusions:** Vitamin B12 and folic acid deficiency and hyperhomocysteinaemia appear to be important risk factors for cerebrovascular accidents. It is therefore important to assess vitamin B12 folate and homocysteine levels in all cases of cerebrovascular accidents.*

Introduction

Stroke is defined as a clinical syndrome of sudden onset rapidly developing symptoms or signs of focal, and at times global, loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin[1]. Acute ischaemic stroke is the most common cerebrovascular disease and is one of the leading causes of death (10.55%) and long-term disability (daily 4.88%) throughout the world[2].

Management of the disease is largely conservative. Several risk factors for stroke have been identified which are the target of both primary and secondary preventive strategies[3]; these risk factors include hypertension, diabetes mellitus, cardiac diseases, sickle cell anaemia, cigarette smoking, other emerging risk factors include hyperhomocysteinaemia, hypovitaminosis B12, and low folic acid levels, etc.[4-7].

Vitamin B12 is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of erythrocytes. Vitamin B12 deficiency leads to abnormality in methylene-tetrahydrofolate reductase resulting in impaired ability to form methyl-tetrahydrofolate from methylene-tetrahydrofolate. This causes functional folate deficiency, resulting in failure to remethylate homocysteine to methionine leading to hyperhomocysteinaemia[8-10]. Hyperhomocysteinaemia is a known risk factor for ischaemic stroke.

However there are no clear cut recommendations for supplementing vitamin B12 for the prevention of stroke. A recent metaanalysis by David J A indicated that B vitamin combinations and folic acid reduced the risk of stroke; the authors recommended folic acid for this purpose in countries where folate fortification does not exist[8].

Hence, this study was undertaken to evaluate the serum level of vitamin B 12 in patients of ischaemic stroke.

Aims and objectives

Aim

The aim of this study was to evaluate the relationship between serum vitamin B12 levels and ischaemic stroke.

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Objective

To determine levels of serum vitamin B12 in patients of ischaemic stroke.

Material and Methods

Study setup: The study was conducted in the Department of Medicine, Haematology and Biochemistry, VMMC and Safdarjung Hospital, New Delhi.

Collaborative department: Department of Haematology and Biochemistry, Safdarjung Hospital, New Delhi.

Study period: 18 months.

Study design : Case-control study.

Sample Size: Considering the prevalence of vitamin B12 deficiency in general population as 6%[9] and in stroke patients 40%[10], keeping confidence interval 95% and power of study 80% sample size selected was 50 cases, with 50 healthy age and sex-matched controls by using software Open Epi version 3.0.

Sample collection : 10 mL venous blood sample was taken for investigation. Vitamin B12 including folate and homocysteine levels were done by automated chemiluminiscent immunoassay in Beckman coulter access 2 machine.

Study population: 50 ischaemic stroke patients attending, the department of Internal Medicine and Neurology after fulfilling inclusion and exclusion criteria with 50 age and sex-matched controls, who gave consent, after fulfilling inclusion and exclusion criteria.

Stastistical analysis: The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0. Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non-parametric tests were used.

Statistical tests were applied as follows:-

1. Quantitative variables were compared using unpaired t-test/Mann-Whitney Test (when the data sets were not normally distributed) between the two groups.
2. Qualitative variables were compared using Chi-square test/Fisher's exact test.
3. Logistic regression was used to find the risk factors of vitamin B12 deficiency in ischaemic stroke patients.

A p value of < 0.05 was considered statistically significant.

Consent and ethical clearance

Written and informed consent was taken from all subjects participating in the study. Ethics clearance was taken from the Ethics Committee of Safdarjung Hospital, before conducting the study. The study was performed according to the principles of Declaration of Helsinki.

Inclusion criteria

Patients presenting with ischaemic stroke aged 18 - 70 years within 7 days of onset.

Exclusion criteria

1. Patient on vitamin B12 or folic acid supplementation within last 3 months.
2. Patients with haemorragic stroke and previous history of ischaemic stroke.
3. Patient with chronic illness, liver or renal disease.
4. Chronic alcoholic.
5. Patients with major gastrointestinal surgery or malabsorption syndrome.

Results

Most patients were in the age group of 40 - 60 years; 33 (66%) in cases and 34 (68%) in controls. Males were 32 (64%) in cases and 35 (70%) in controls, while females were 18 (36%) and 15 (30%) respectively. Smoking was present in 32 (64%) cases and 31 (62%) controls. There were 14 (28%) diabetic patients in cases and 12 (24%) in controls. Dyslipidaemia was present in 17 (34%) cases and 11 (22%) controls. Hypertension were present in 18 (36%) patients in both the groups. In cases, 21 (42%) patients were vegetarian while 12 (24%) patients in controls were vegetarians (Table I).

Table I: Demographic profile of patients.

Factors		Cases	Controls	Total	p value
Age (years)	1) <= 40	6 (12%)	7 (14%)	13 (13%)	
	2) 41 - 50	15 (30%)	18 (36%)	33 (33%)	0.881
	3) 51 - 60	18 (36%)	16 (32%)	34 (34%)	
	4) >60	11 (22%)	9 (18%)	20 (20%)	
Gender	F	18 (36%)	15 (30%)	33 (33%)	0.523
	M	32 (64%)	35 (70%)	67 (67%)	
Smoking	Y	32 (64%)	31 (62%)	63 (63%)	0.836
	N	18 (36%)	19 (38%)	37 (37%)	
Diabetes	N	36 (72%)	38 (76%)	74 (74%)	0.648

	Y	14 (28%)	12 (24%)	26 (26%)	
Dyslipidaemia	N	33 (66%)	39 (78%)	72 (72%)	0.181
	Y	17 (34%)	11 (22%)	28 (28%)	
Hypertension	N	32 (64%)	32 (64%)	64 (64%)	1
	Y	18 (36%)	18 (36%)	36 (36%)	
Diet	M	29 (58%)	38 (76%)	67 (67%)	0.056
	V	21 (42%)	12 (24%)	33 (33%)	

Mean value of serum vitamin B12 in cases was 187.26 ± 138.74 pg/mL whereas in controls it was 463.84 ± 251.75 pg/mL. Median value was 145.5 pg/mL in cases and 368 pg/mL in controls, (p value < 0.0001) (Fig. 1).

Mean value of serum folic acid in cases was 7.95 ± 5.1 ng/mL and 13.42 ± 6.59 ng/mL in controls. Median value was 6.8 ng/mL in cases and 12.5 ng/mL in controls. (p value < 0.0001) (Fig. 2).

Mean value of serum homocysteine in cases was 31.12 ± 14.98 µmol/L and 6.63 ± 3.86 µmol/L in controls. Median value was 32.23 µmol/L in cases and 6.14 µmol/L in controls (Fig. 3).

Risk factors like diabetes, hypertension, smoking, vitamin

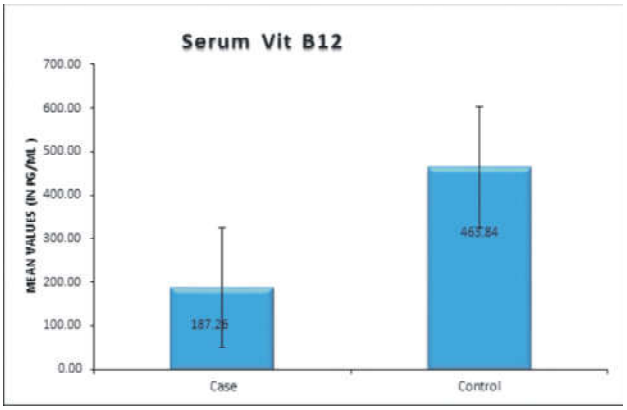

Fig. 1: *Levels of serum vitamin B12 in study population.*

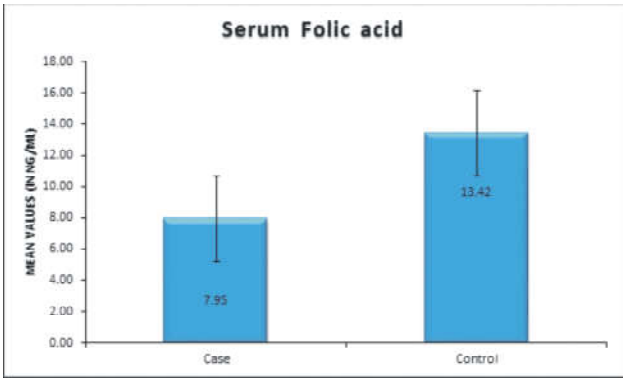

Fig. 2: *Levels of serum folic acid in study population.*

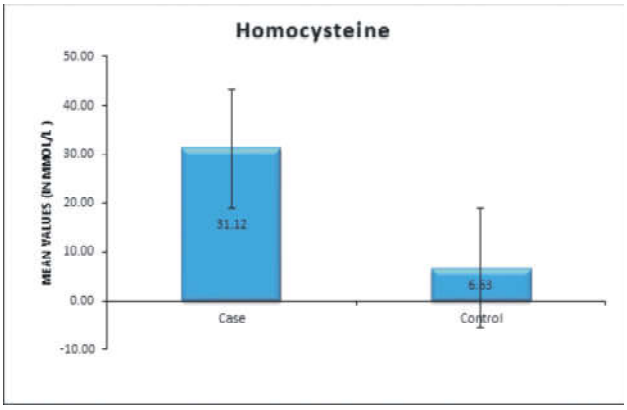

Fig. 3: *Levels of serum homocysteine in study population.*

B_{12} and folic acid levels were low and serum homocysteine levels were high (Table II).

Table II: Association of serum vitamin B12, folic acid and homocysteine in cases and controls with risk factors.

		Vitamin B12		Folic Acid		Homocysteine	
		Mean Values (in pg/mL)	P value	Mean Values (in ng/mL)	p value	Mean Values (in µmol/L)	p value
Diabetes	Cases	205.14 ± 158.78	**<0.001**	7.93 ± 4.62	**<.0001**	45.56 ± 11.79	**<.0001**
	Control	370.58 ± 131.93		13.71 ± 5.33		8.64 ± 2.11	
Hypertension	Cases	164.61 ± 151.99	**<0.005**	7.27 ± 4.42	**<.0001**	37.29 ± 16.41	**0.034**
	Control	462.67± 131.5		12.82 ± 5.47		7.33 ± 3.13	
Smoking	Cases	166.11 ± 147.71	**<0.001**	6.59 ± 3.32	**<.0001**	39.85 ± 13.82	**0.001**
	Control	452.53 ± 134.36		12.27± 5.78		7.4 ± 3.44	
Dyslipidaemia	Cases	154.53 ± 91.84	**<0.001**	7.63 ± 4.9	**<.0001**	45.24 ± 8.56	**<.0001**
	Control	420.27 ± 156.19		12.5 ± 5.26		9.72 ± 2.1	
Diet	Cases	153 ± 20.28	**<0.0001**	8.07 ± 5.39	**<.0001**	22.54 ± 11.34	**<.0001**
	Control	337.6 ± 138.03		13.28 ± 4.8		9.7 ± 1.75	

There were 36 vitamin B12 deficient patients (vitamin B12 < 211 pg/mL), 19 folic acid deficient patients (folic acid < 5.38 ng/mL) and 42 hyperhomocysteinaemic patients (homocysteine >15 µmol/L). There were 14 vitamin B12 and folic acid deficient patients; 19 folic acid deficient and hyperhomocysteinaemic patients; 34 vitamin B12 deficient and hyperhomocysteinaemic patients. There were 15 vitamin B12 and folic acid deficient and

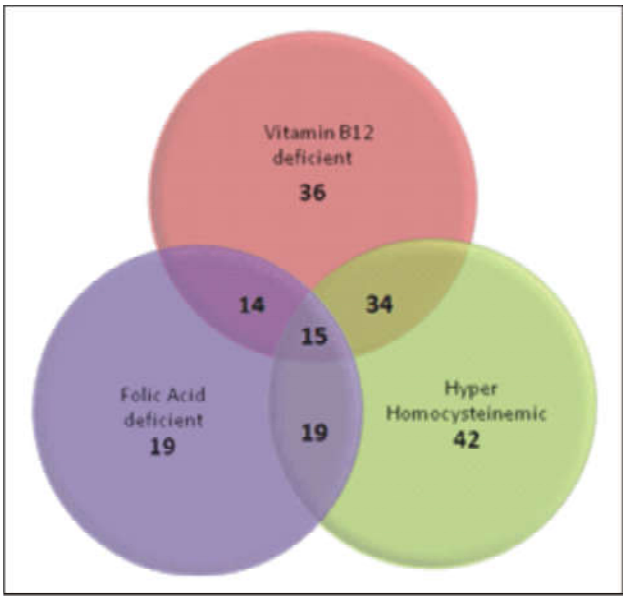

Fig. 4: *Combined result of vitamin B12 and folic acid deficient and hyperhomocysteinaemic patients.*

hyperhomocysteinaemic patients (Fig. 4). There was positive correlation between vitamin B12 and folic acid (pearson co-efficient value 0.355, logistic regression -0.077); and both of them had negative correlation with homocysteine (pearson co-efficient value -0.343, logistic regression value 0.025). It showed that lower levels of vitamin B12 and folic acid are associated with hyperhomocysteinaemia.

Discussion

Stroke is the major cause of death and disability worldwide. Each year, about 4.4 million people die of stroke globally, of whom almost three million are from developing countries. Decreased level of vitamin B12 and folic acid in blood may be an important factor associated with ischaemic stroke. There are very limited number of studies which compare the levels of vitamin B12, folate and homocysteine and their combined or independent effects on the risk of acute ischaemic stroke in India.

Our findings were similar to the findings of Gajbhare *et al*[11], Narang *et al*[12], Biswas *et al*[13] and Modi *et al*[14] who concluded that hyperhomocysteinaemia as an important risk factor for ischaemic stroke. But in contrast to these studies, relatively higher values of homocysteine was observed in both cases and controls in our study. These findings might be reflective of a higher prevalence of hyperhomocysteinaemia in the population our hospital caters to. This assumption can however, only be confirmed by a large scale community based study in this particular part of the country. Wadia *et al*[15] also concluded that vitamin B12 deficiency leads to raised serum homocysteine levels which is common in India and is a major risk factor for strokes. Ahmed *et al*[16] also showed vitamin B12 deficiency and hyperhomocysteinaemia in patients of stroke or transient ischaemic attack.

Limitations

Our study had the following limitations. First, the design was cross-sectional, done in a tertiary care hospital based population. Second, though study duration was long, relatively small number of patients were included in this study. Third, metabolic deficiency of vitamin B12 was not taken into account along with various genetic factors for vitamin B12 and folic acid deficiency and hyperhomocysteinaemia. Fourth, iatrogenic vitamin B12 deficiency like patients on proton pump inhibitors metformin was not excluded. Fifth, number of vegetarian patients were more in cases which could be the confounding factor. Sixth, patients with cardio-embolic stroke were not excluded. Hence, further large scale studies are required including a large number of cases and control in a normal based population, taking into account various environmental, iatrogenic, confounding and genetic factors.

Conclusion

We conclude that in patients of ischaemic stroke levels of vitamin B12 were low, along with low levels of folic acid and high homocysteine levels. This showed negative correlation of vitamin B12 and folic acid levels and positive correlation of homocysteine levels in patients of ischaemic stroke. However, there were few limitations of this study. Further large scale studies including various other risk factors like environmental and genetic factors are needed to confirm the findings.

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