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A Study of Vitamin B12 Level as a Risk Factor for Ischemic Stroke

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ABSTRACT

Introduction: Hyperhomocysteinemia is an independent risk factor for Ischemic stroke. Data from prospective studies on the associations between Vitamin B-12 plasma levels and the risk of stroke is limited. We conducted a Case control study to establish the relationship between Vitamin B12 levels and acute ischemic stroke.

Aims: 1.To study the prevalence of Vitamin B12 levels in normal healthy Individuals

2. To estimate the serum levels of Vitamin B12 in Ischemic Stroke and compare it with normal age and sex matched control population

3. To study Vitamin B12 level as an independent risk factor of Ischemic Stroke.

Methods: This study was conducted involving 60 patients of acute ischemic stroke with 40 age and sex matched healthy controls. Data collected involved age, sex and risk factors. Vitamin B12 and Homocysteine levels were sent within 48 hours of the onset of symptoms and analysed by Chemiluminescent Immunoassay.

Results: Mean ± SD age of the patients was 54.7±14.7 years. 23.3 % of patients were of young stroke (≤ 45 years).73.3% were male while 23.7 % were female. Hypertension was observed in 43.3% while Diabetes was present in 23.3%

patients. Mean vitamin B-12 levels were 316.5±218.9 pg/ml in the cases while controls had a mean level of 334.1±190.5 pg/ml. Mean Vitamin B12 levels in stroke patients were lower than the control Group, but were not statistically significant. There was no correlation between vascular risk factors and Low Vitamin B12 levels.

Conclusions: Low Vitamin B12 levels were not associated with an increased risk of Ischemic Stroke.

Key-words: Vitamin B-12, Homocysteine, Acute Ischemic Stroke.

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INTRODUCTION

Stroke remains a major cause of mortality and morbidity worldwide. The burden of stroke arises largely from the elderly population. However, there remains a small but significant subset of younger patients with ischemic stroke, in whom conventional vascular risk factors play a smaller role. Unusual causes of stroke, such as arterial dissection or thrombophilia, are more common than elderly, although the final cause may remain undetermined in 21% to 31%.[1]

Abnormality in the methylene-tetrahydrofolate reductase results in an impaired ability to form methyl-tetrahydrofolate from methylene-tetrahydrofolate. This causes functional folate deficiency, resulting in failure to remethylate homocysteine to methionine leading to hyperhomocysteinemia.

Vitamin B12 deficiency leads to impairment of methionine synthase, a vitamin dependent enzyme, resulting in accumulation of homocysteine and trapping folate as methyl-tetrahydrofolate.[2]

Several prospective and intervention studies[3] have demonstrated

that increased levels of folate and vitamin B12 lower plasma homocysteine levels, an established risk factor for myocardial infarction and stroke. These findings formed the basis for large-scale secondary prevention trials[4,5] designed to investigate whether the risk of recurrent myocardial infarction, stroke, and other cardiovascular events could be reduced through supplementation with these B vitamins.

Few prospective studies have investigated the associations between folate or vitamin B12 plasma levels and the risk of stroke and results have been inconsistent. The present study was therefore undertaken to compare the, vitamin B12 and homocysteine levels in patients of stroke with controls.

SUBJECTS AND METHODS

The study was conducted at a Tertiary care Hospital in Kota, Rajasthan during period 2014-2015 after obtaining written informed consent. Sixty Patients Presenting with Acute Ischemic

Stroke within 7 days were included. Forty healthy age and sex matched controls were taken. Risk factors for Ischemic Stroke like Hypertension, Diabetes, Smoking, Dyslipidemia were included in both cases and controls.

CASES
Inclusion Criteria: Patients presenting with acute ischemic stroke aged 18 to 80 yrs within 7 days of onset were included in this study.

Exclusion Criteria: Patients with ischemic stroke <18yrs &>80 yrs of age, Patients on vitamin B12, folic acid supplements within last 3 months, Patients with hemorrhagic stroke or venous sinus thrombosis, Patients with liver and renal disease.

CONTROLS
Inclusion Criteria: Age and sex matched healthy individual attending the hospital for health check-ups were included in the study.

Exclusion Criteria: Subjects with history of previous Cerebrovascular disease, Transient Ischemic Attack (TIA), Myocardial Infarction, Subjects taking vitamin B12 or folic acid supplements within last 3 months.

Selected cases in the study were interviewed about risk factors: Hypertension, Diabetes, Smoking, Dyslipidemia, diet, Onset and duration of Symptoms, Clinical Examination with blood pressure measurements were done. Healthy volunteers were interviewed about various risk factors.

METHODOLOGY
Following selection of subjects of both cases & controls and after obtaining informed consent about the proposed study, blood samples of about 5.0 ml were collected from them by venepuncture under all aseptic precautions.

For Vitamin B12 (Cyanocobalamin) level estimation; 3 ml serum was taken from a Serum Seperating Tube (SST), the Sample was Frozen and Analysed by Chemiluminescent Immunoassay. Homocysteine levels were similarly estimated using 2ml serum by chemiluminescent Immunoassay.

STATISTICAL ANALYSIS
Data was analysed using Statistical Package for Social Science (SPSS) version 16 software. Continuous data of age, Vitamin B12, Homocysteine concentration was compared in between the two groups by Unpaired t test after testing for homogeneity of variance in the groups. Association of Proportion of patients in between the study and control group was analysed for discrete data using Pearson chi square test. P value of less than 0.05 was considered significant to evaluate the outcome.

RESULTS
The present analysis is based on a case-control study consisting of 60 confirmed cases of ischemic stroke and 40 subjects without any signs of cerebral ischemia. Mean±SD baseline ages were 54.7±14.7years for cases and 51.7±12.1years for controls. Baseline characteristics of the study are listed in Table 1. Hypertension was present in 43.3%, Diabetes in 23.3% ,Smoking in 30 % and chronic alcohol consumption in 20 % and Hypercholesterolemia in 28.3% in the study group.

Mean ± SD levels of Vitamin B12 in the study group were 316.5±218.9 pg/ml whereas controls had Mean±SD level of 334.1±190.5pg/ml (Table 2). The difference in the mean levels was not statistically significant (p>0.05).

In study group 41.7% patients had Vit B 12 deficiency while in control group VIt B12 deficiency was found in 30% patients. Even though the study group had higher percentage of patients having vitamin B12 deficiency (<211pg/ml), it was not statistically significant (p>0.05)(Table 3)

Mean difference of Homocysteine concentration between study group and control group was 6.0μmol/L. The higher concentration difference of study group patients compared to control group was statistically significant (p<0.05).(Table 4)

There was very highly significant (p<0.001) negative correlation of Vitamin B 12 and Homocysteine level in study and control group. (Table 5)

Table 1: Association of Risk factors of patient in Study and control group.

		STUDY GROUP (N=60)	CONTROL GROUP (N=40)	TOTAL
HT	PRESENT	26(43.3%)	13(32.5%)	39(39.0%)
	ABSENT	34(56.7%)	27(67.5%)	61(61.0%)
DM	PRESENT	14(23.3%)	5(12.5%)	19(19.0%)
	ABSENT	46(76.7%)	35(87.5%)	81(81.0%)
Smoking	PRESENT	18(30.0%)	8(20.0%)	26(26.0%)
	ABSENT	42(70.0%)	32(80.0%)	74(74.0%)
Alcohol	PRESENT	12(20.0%)	6(15.0%)	18(18.0%)
	ABSENT	48(80.0%)	34(85.0%)	82(82.0%)
Dyslipidemia	PRESENT	17(28.3%)	7(17.5%)	24(24.0%)
	ABSENT	43(71.7%)	33(82.5%)	76(76.0%)
Other Risk Factors	PRESENT	54(90.0%)	-	54(54.0%)
	ABSENT	6(10.0%)	40(100.0%)	46(46.0%)

Table 2: Comparison of Vitamin B12 concentration of patient in between groups.

Vitamin b12 (pg/ml)	Study Group (n=60)	Control Group (n=40)
Mean ±S.D	316.5±218.9	334.1±190.5
Mean difference	-17.57	
t value	-.414	
P value	.680	

Table 3: Association of Vitamin B12 level deficiency of patient in Study and control group.

Vitamin B12 level	Study Group (n=60)	Control Group (n=40)	Total
Normal	35	28	63
	58.3%	70.0%	63.0%
Deficiency (<211pg/ml)	25	12	37
	41.7%	30.0%	37.0%
Total	60	40	100
	100.0%	100.0%	100.0%
Chi-Square Tests			
	χ² Value	df	P value
Pearson Chi-Square	1.401	1	.236

Table 4: Comparison of Homocysteine concentration of patient in between groups.

Homocysteine (µmol/L)	Study Group (n=60)	Control Group (n=40)
Mean ±S.D	21.3±16.2	15.3±6.1
Mean difference	6.0	
t value	2.239	
P value	.027	

Table 5: Correlation of Vit B12 and Homocysteine levels in Study and control group.

		Homocysteine	
		Study Group	Control Group
Vitamin B12	Pearson Correlation	-0.569	-0.640
	Sig.	<0.001	<0.001
	N	60	40

Table 6: Vitamin B12 level relationship with risk factors in Study Group (n=60)

Risk factor	Status	Vitamin b12	Mean difference	P value
Age(years)	<45(n=14)	364.9±223.0	63.1	0.349
	≥45(n=46)	301.8±217.9		
Sex	Male(n=44)	344.8±232.4	106.2	0.097
	Female(n=16)	238.7±157.2		
Hypertension	Present(n=26)	270.5±192.7	-81.2	0.156
	Absent(n=34)	351.7±233.6		
Diabetes Mellitus	Present(n=14)	265.4±216.2	-66.8	0.322
	Absent(n=46)	332.1±219.7		
Smoking	Present(n=18)	305.2±206.1	-16.2	0.795
	Absent(n=42)	321.4±226.4		
Alcohol	Present(n=12)	320.8±225.3	5.3	0.941
	Absent(n=48)	315.5±219.7		
Dyslipidemia	Present(n=17)	387.9±241.4	99.6	0.113
	Absent(n=43)	288.3±205.5		
Other risk factors	Present(n=54)	315.1±222.2	-14.2	0.881
	Absent(n=6)	329.3±204.3		
Diet	Mixed(n=26)	312.3±203.1	-7.5	0.896
	Veg (n=34)	319.8±233.2		

There was statistically no significant (p>0.05) relationships between Vitamin B12 levels and cerebrovascular risk factors like Age, sex, hypertension, diabetes mellitus, Smoking, alcohol consumption, Dyslipidemia, other risk factors and type of diet of patients in study group.(Table 6)

DISCUSSION

There are very limited number of studies which compare the effects of Vitamin B12, Folate levels and their combined or independent effects on the risk of acute Ischemic Stroke. In our study 41.7% patients and 30 % controls had vitamin B12 deficiency.

The main findings of this population-based, case control study was that

(i) Low vitamin B12 levels were not significantly associated with increased risk of cerebral ischemia.

(ii) Serum homocysteine levels were significantly higher in ischemic stroke patients.

(iii) There was no relationship between B12 vitamin serum levels with risk factors

A study done by Nigel Choon-Kiae Tan et al. recruited 109 consecutive young [<50years] first-ever Asian ischemic strokes patients and 88 age/gender-matched hospital based controls during a period of 18 months and examined vitamin B12, folate and homocysteine levels. They found vitamin B12 levels significantly lower in cases than controls. Folate levels were not significantly different.[1]

Weikert et al. in 2007 investigated the individual and combined effects of plasma folate, vitamin B12 levels on the risk of ischemic stroke & transient ischemic attack [TIA] in a large, prospective case-cohort study comprising 779 subjects free from cardiovascular disease & 188 incident cases of cerebral ischemia. Their data suggest that low vitamin B12 plasma levels particularly in combination with low folate levels, increases the risk of cerebral ischemia.[6]

Wadia R S et al. in 2004 studied 147 cases of ischemic stroke of which exact dietary intake was known in 137 cases. Fifty eight were vegetarians, 54 were occasional non-vegetarians and 25 were frequent non-vegetarians of which 94.8 %, 85.2 % & 44 % had serum homocysteine>16 umol /L respectively. Among these patients 75.8 %, 51.8 % & none had serum vitamin B12 < 200 pg /ml and 8.6 %, 22.2 %, 24 % had serum vitamin B12 between 200 to 300 pg/ ml respectively. With these findings they concluded that vitamin B12 deficiency leads to raised serum homocysteine levels which is common in India & is a major risk factor for strokes. The important predisposing factor is vegetarian diet.[7]

Carlos Cantu et al. in 2004 through a case control study examined the potential association of folate, vitamin B12, homocysteine levels and common C 677 – T mutation in methylene tetrahydrofolate reductase gene in patients with cerebral venous thrombosis [CVT]. They concluded that low plasma folate levels & high plasma concentration of homocysteine were associated with an increase in CVT, in which low socioeconomic conditions & deficient nutritional status may contribute to its relatively high incidence.[8]

In a prospective study, Perini et al. measured homocysteine plasma levels in stroke patients in order to investigate possible correlations of homocysteine with stroke severity and clinical outcome. The plasma level of Homocysteine was neither an independent determinant for stroke severity nor for patient's outcome by the Barthel index. Mean plasma homocysteine of both ischemic and haemorrhagic stroke was significantly higher than in controls (P<0.05).

Homocysteine in the acute phase of stroke was not associated with stroke severity or outcome. Elevated plasma homocysteine in the acute phase of stroke was associated with both ischemic and hemorrhagic stroke.[9]

A case control study conducted in Iranian patients by Hoselani et al[10] involved 93 consecutive ischemic stroke patients analysed total homocysteine, vitamin B12 and folate levels suggested that mild hyperhomocysteinemia is confirmed to have a significant role as risk factor for all etiological subtypes of stroke, No significant differences in B12 vitamin and folate serum levels were observed between cases and controls.

Our study in comparison with the some of the studies listed above showed Low Vitamin B12 levels were not significantly associated with ischemic stroke, however mean plasma homocysteine was significantly higher in study group than controls (p<0.05). Serum Vitamin B12 and homocysteine levels showed a strong negative correlation. (p<0.001)

There were some limitations to the study. We could not rule out the possibility of acute phase response being responsible for the elevation of serum Homocysteine level in acute stroke patients. Folate and Vitamin B6 (pyridoxal5-phophate [PLP]), other B vitamins involved in homocysteine metabolism may have contributing effects to cerebrovascular ischemia .These were not studied due to financial constraints.

CONCLUSION

We conclude that Low Vitamin B12 levels are not associated with an increased risk of ischemic Stroke, however increased homocysteine levels are significantly associated with increased risk.

More prospective and population based studies are needed to define whether low vitamin B12 is an independent risk factor for cerebrovascular diseases or stroke by itself it is the cause for hyperhomocysteinemia.

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