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Study of Serum Folic Acid and Vitamin B12 levels in Patients of Vascular Dementia

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Abstract: ***Background: Dementia is a major health problem worldwide and the number of people affected is expected to rise considerably, especially in developing countries. Vascular dementia is a degenerative cerebrovascular disorder that results from ischemic or haemorrhagic brain damage. Vitamin B12 and folate represent modifiable risk factors for dementia. They may increase the risk of vascular dementia as their deficiency can increase the homocysteine(vascular risk factor). Hence, it is important to explore the status of serum vitamin B12, folate in vascular dementia to evolve the treatment strategies for the same. Objectives: To determine the levels of serum folic acid and vitamin B12 in cases of vascular dementia and to compare these with age and sex matched controls. Methodology: In this case control study, 30 vascular dementia cases and 30 age and sex matched controls were selected. Serum levels of Folic acid and vitamin B12 were estimated by chemiluminescence method. Results: The vitamin B12 levels in vegetarians and mixed diet patients showed p value of 0.079 suggestive of significance. In Pearson correlation between MMSE score and vitamin B 12, r = 0.463 and p = 0.010 showing significant positive correlation indicating that cases with low vitamin B12 levels had more cognitive deficit. Conclusion: Vitamin B12 represents the modifiable risk factor for vascular dementia, hence B vitamin supplementation should be considered as preventive and therapeutic intervention.***

Keywords: Vascular dementia, folic acid, vitamin B12, chemiluminescence method, MMSE score

1. Introduction

The term dementia denotes a deterioration of intellectual or cognitive function with little or no disturbance of consciousness or perception. Dementia is a syndrome characterized by loss of or decline in memory and other cognitive abilities and reduces the lifespan of affected people.[1,2] Vascular risk factors are involved in causation of both vascular dementia and Alzheimer's disease, which accounts for 90% of all dementias. Vascular dementia (VaD) is a degenerative cerebrovascular disorder that results from ischemic or haemorrhagic brain damage.[3,4]

Abnormality in the methylene tetrahydrofolate reductase [MTHFR] enzyme results in 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 leading to hyperhomocysteinemia and trapping folate as methyl-tetrahydrofolate.[5]

Low vitamin B12 concentration is common in Indian men, particularly in vegetarians.[6] Supplementation of vitamin B12 and folic acid is recommended for elderly by many studies, to prevent dementia and associated morbidity. The present study is undertaken to compare the serum levels Folic acid and Vitamin B12 in patients of vascular dementia with controls.

2. Materials and Methods

A study of serum folic acid and vitamin B12 levels in patients of vascular dementia was conducted in patients admitted to hospital. The diagnosis of vascular dementia was established by NINDS-AIREN[7] criteria. Each gave informed consent and the study was approved by ethical and research committee to use human subjects in the research study. Duration of study was from November 2011 to March 2013.

2.1 Inclusion and Exclusion criteria

a) Inclusion criteria
Patients above 18 years of age, fulfilling criteria of probable vascular dementia according to NINDS-AIREN criteria.[7]

b) **Exclusion criteria:** Cases of Endocrine abnormalities, Chronic infectious diseases like HIV/Neurosyphilis, Toxin induced cognitive dysfunction, Primary neoplasm/metastasis.

c) **Controls** - Normal healthy age and sex matched individuals were included. Cognitive deficit in cases was assessed on the basis of mini-mental state examination (MMSE)[1] score.

2.2 Materials

Method of sample collection:

Informed consent was taken from patients and control subjects. Selected subject's blood samples were collected with all aseptic precautions. 5 ml of blood was collected from the median cubital vein. It was allowed to clot for 30 minutes in a clean dry test tube and was subjected to centrifugation for 20 minutes to separate the serum. The serum samples were stored at -80^{o}C till they were analyzed.

Sample size
A total number of sixty subjects participated in the present study. Thirty clinically diagnosed cases of vascular dementia patients were selected. Thirty age and sex matched control subjects, who were healthy at the time of study were selected.

Sample analysis

The separated serum was used to estimate

- Folic acid - A quantitative measurement was done by fully automated ADVIA Centaur Folate assay - a competitive immunoassay using direct chemiluminescent technology.[8,9]
- Vitamin B12- A quantitative measurement was done by fully automated Chemiluminescent immunoassay method. [8,9]
- Estimation of serum fasting blood sugar [FBS] and postprandial blood sugar [PPBS] by Hexokinase method, blood urea by Urease method & serum creatinine by Jaffe's method, serum lipid profile by Enzymatic method and HIV test by ELISA method were also performed.

The chemicals and reagents used for the procedures were of analytical grade. Normal reference range - Folic acid: > 5.38ng/mL and Vitamin B12: 211-911pg/mL.

3. Results and Statistical Analysis

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc**.**

Statistical Methods: Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented as Mean ± SD [Min-Max] and results on categorical measurements are presented as Number [%]. Significance is assessed at 5 % level of significance.

Student t test [two tailed, independent] has been used to find the significance of study parameters. Pearson correlation between MMSE and study variables is performed to find the relationship.

Significant figures

+ Suggestive significance [p value: 0.05< p <0.10]
* Moderately significant [p value: 0.01< p ≤ 0.05]
** Strongly significant [p value: p ≤ 0.01]

Among 30 cases in this study, 3 were in 32 – 40 years age group [10%], 7 were in 41 – 50 years age group [23.3%], 8 were in 51 – 60 years age group [26.7%], 8 were in 61 - 70 years age group [26.7%] and 4 were in 71 – 80 years age group [13.3%]. Among 30 cases, 21 were men [70%] and 9 were women [30%]. Among 30 controls, 21 were men [70%] and 9 were women [30%].

In the present study, 12 [40%] out of 30 cases were vegetarians and 18 [60%] were on mixed diet. Among controls, 8 [26.7%] were vegetarians and 22 [73.3%] were on mixed diet. Among 30 cases, 8 [26.7%] had diabetes mellitus, 22[73.3%] had hypertension, 15 [50%] were smokers and 7 [23.3%] were alcoholic.

Comparison of levels of Serum Folic acid, Vitamin B12 in cases and controls

The mean and SD of serum folic acid [FA] in the controls were 8.70 ng/mL and 5.46 ng/mL respectively and the mean and SD in the cases were 11.47 ng/mL and 8.87 ng/mL respectively with p value of 0.151. The mean and SD of serum vitamin B12 in the controls were 300.57 pg/mL and 163.93 pg/mL respectively. The mean and SD in the cases were 522.93 pg/mL and 419.14 pg/mL respectively with p value of 0.009, shown in table 1. The values of vitamin B12 are within normal range but there is significant difference between cases and controls.

Table 1: Comparison of levels of Serum Folic acid, Vitamin B12 in cases and controls

Variables	Cases	Controls	P value
Folic acid [ng/mL]	11.47±8.87	8.70±5.46	0.151
Vitamin B12[pg/mL]	522.93±419.14	300.57±163.93	0.009**

Mean Levels of Folic Acid, Vitamin B12 According to Diet in Cases

The mean ± SD of folic acid in vegetarians and mixed diet patients was 14.40 ± 9.23 ng/mL and 9.51 ± 8.30 ng/mL respectively with p value 0.142. The mean ± SD of vitamin B12 in vegetarians and mixed diet patients was 358.75 ± 337.19 pg/mL and 632.39 ± 440.92 pg/mL respectively with **p value 0.079** suggestive of significance. Results are shown in table 2.

Table 2: Mean Levels of Folic acid, Vitamin B12 according to Diet in cases

Variables	Diet		P value
	Veg	Mixed	
Folic acid [ng/mL]	14.40±9.23	9.51±8.30	0.142
VitaminB12 [pg/mL]	358.75±337.19	632.39±440.92	0.079+

Pearson Correlation of MMSE Score With Parameters Studied In Cases

In Pearson correlation between MMSE score and folic acid, r value was -0.192 and p value was 0.310. For vitamin B 12 **r = 0.463** and **p = 0.010** showing significant positive correlation.

Results are shown in table 3.

Table 3: Pearson correlation between MMSE score and levels of Folic acid, Vitamin B12 in cases

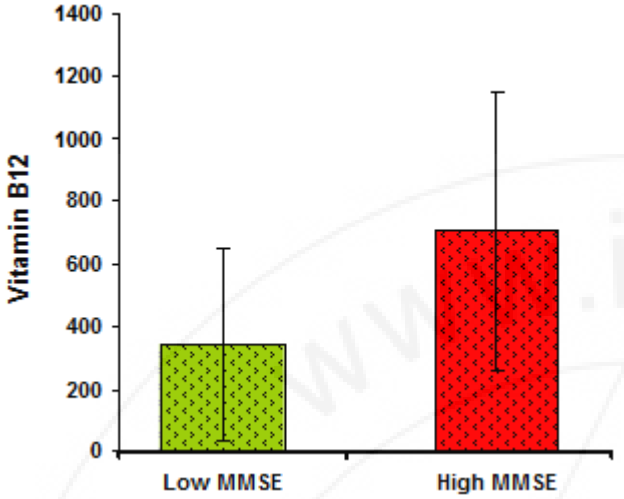
Pair	*Cases [n=30]*	
	r value	*p value*
MMSE vs FA	-0.192	0.310
MMSE vs Vitamin B12	0.463	0.010*

COMPARISON OF MEAN LEVELS OF FOLIC ACID, VITAMIN B12 WITH MMSE SCORE IN CASES

Cases with MMSE score <20 had mean folic acid levels of 12.92 ng/mL and SD 8.96 ng/mL. Cases with MMSE score >20 had mean folic acid levels 10.01ng/mL and SD 8.86 ng/mL with p value 0.379. For low MMSE [<20] group, mean ± SD of vitamin B12 was 341.40 ± 309.19 pg/mL and for high MMSE [>20] group, mean ± SD of vitamin B12 was 704.47 ± 445.32 pg/mL with significant **p value [0.015]**, shown in table 4 and graph 1.

Table 4: Comparison of Mean Levels of Folic acid, Vitamin B12 and Homocysteine with MMSE score in cases

Variables	*Low MMSE [<20]*	*High MMSE [>20]*	*P value*
Folic acid [ng/mL]	12.92±8.96	10.01±8.86	0.379
Vitamin B12 [pg/mL]	341.40±309.19	704.47±445.32	0.015*



Graph 1: Comparison of Mean Levels of Vitamin B12 with MMSE score in cases

4. Discussion

In present study, the mean value for vitamin B12 in cases found to be 522.93±419.14 pg/ml and for controls 300.57 ± 163.93pg/ml, with p value of 0.009. These values were within normal range, but there was significant difference between the groups [cases and controls]. The mean value of folic acid for cases was 11.47 ± 8.87 ng/ml and for controls, 8.70 ± 5.46 ng/ml with p value of 0.151 which was not significant; may be because of small sample size. Total vitamin B_{12} levels in the plasma may not signify the deficiency state of vitamin B_{12} at the tissue level and finally, to establish such correlation, sample size should be much larger.[10]

In our study, 15 patients [50%] had MMSE score < 20 with mean vitamin B12 levels 341.40 ± 309.19 pg/ml and 15 patients [50%] had MMSE score > 20 with mean vitamin B12 levels 704.47 ± 445.32 pg/ml with significant **p value of 0.015**. The Pearson correlation between MMSE and vitamin B12 showed significant association, **r = 0.463** and **p = 0.010** with positive correlation. This indicates that vitamin B12 deficient patients had more cognitive dysfunction. J. Kalita et al[11] studied 36 patients of vitamin B12 deficiency neurological syndromes and found that MMSE was abnormal in 47% patients which improved following treatment.

There seems to exist a link between oxidative stress involved in the pathogenesis of stroke and the depletion of B-vitamins. Oxidative stress resulting from immune activation indeed could represent the cause for moderate hyperhomocysteinemia.[12]

The mechanism by which B-12 deficiency affects the nervous system may be due to the role B-12 plays in creating the components necessary to build the myelin sheath. Vitamin B-12 may also be involved in the production of neurotransmitters - brain chemicals that bridge the synapses between nerves, allowing the transmission of impulses from one nerve to the next.[13]
Deficiency of B12 has a role in neuronal damage either directly or via increasing homocysteine concentration thus contributing to the pathogenesis of cognitive impairement.[14,15]

Karin Nilsson et al[16] evaluated cobalamin-folate status and the presence of vascular disease in 1,982 psychogeriatric patients and concluded that cobalamin-folate deficiencies do not play an important role in cognitive dysfunction in psychogeriatric patients. A prospective study done by Jae-Min Kim et al[10] showed that lower folate levels predicted cognitive decline over a 2.4 year follow-up period, but no associations were found between baseline cognitive decline and the levels of vitamin B12.

A study conducted by Merrill F.Elias et al among 812 participants concluded that higher folate, vitaminB6 and B12 concentrations have been positively associated with cognitive performances.[17] A prospective study done by Jae-Min kim et al among 607 elderly individuals concluded that folate & homocysteine are involved in the etiology of cognitive decline.[10]

Vitamin B12 is implicated in myelin rarefaction and periventricular white matter leucencies(WML). Because vitamin B12 has been implicated in maintaining the integrity of the blood-brain barrier, deficiency may lead to blood-brain barrier damage which has been suggested to be early phenomena in the development of small vessel disease leading to WML.[18] In present study low vitamin B12 levels were significantly associated with low MMSE score indicating its role in cognitive deficit.

Vitamin B supplementation is effective in reducing oxidative damage. Based on the findings of the present study, it can be concluded that the levels of vitamin B_{12} are relevant to the clinical course of VaD and should be considered for preventive and therapeutic intervention. Large scale study over long duration is required to observe the more significant changes.

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