



## MICRONUTRIENTS STATUS IN ALZHEIMER'S DISEASE IN RURAL REGION OF VIDARBHA, MAHARASHTRA, INDIA

### Biochemistry

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### ABSTRACT

Alzheimer disease is a multifarious neurodegenerative disorder which is characterised by deposition of intracellular neurofibrillary tangles and extracellular amyloid protein which contributes to senile plaques. Our aim is to see serum homocysteine, vitamin D, vitamin B12 and folic acid level in the patients of Alzheimer's disease. Total sample size was 100, Age group between 60-80 years, which was divided into 50 study group with the diagnosed Alzheimer's disease cases who attended the Psychiatry OPD of AVBRH Hospital and 50 age and sex matched healthy controls included in the study. The serum vitamin D, folate, vitamin B12 level was lower in cases as compared to controls. Serum homocysteine level was higher in cases compared to the controls. Our study concluded that higher serum homocysteine levels, lower vitamin D, vitamin B12 and folate levels are risk factor for the disease progression.

### KEYWORDS

Alzheimer's disease, Vitamin B12, Vitamin D, Folic acid.

### INTRODUCTION

Alzheimer's disease is one of the most common neurodegenerative disease and accounts for more than 80% of dementia cases worldwide in elderly people which leads to the progressive loss of memory and cognitive decline and ability to learn.<sup>1</sup> Studies suggest that metabolically significant vitamin B12 (cobalamin) and folate (folic acid) deficiency in the elderly are much more common.<sup>2</sup> These deficiencies in the elderly are important because these vitamins are crucial for proper brain function and play an important role in mental and emotional health. Metabolism of folate and vitamin B12 is interconnected, deficiency of either commonly produces megaloblastic anaemia and sometimes severe neurological consequences.<sup>3</sup> Folate acts as a cofactor in many different biochemical reaction also in one carbon metabolism.<sup>4</sup>

Humans cannot synthesize folate and therefore we must obtain it in diet. Major sources of folate are green vegetables, citrus fruits, liver and whole grains. Consequence of folate deficiency in humans is an increased risk of cancers. Evidence suggests that low vitamin D levels also play a role in the pathogenesis of a wide range of non skeletal age-associated diseases such as various cancers<sup>5</sup>, type 2 diabetes<sup>6</sup>, cardiovascular disease<sup>7</sup>, hypertension<sup>8</sup> and stroke<sup>9</sup>. Low serum 25-hydroxyvitamin D levels are associated with increased risk of prevalent cognitive dysfunction and dementia in various studies.<sup>10,11</sup> The role of plasma homocysteine, folate and vitamin B12 levels in the Alzheimer's disease patient have not been evaluated in the Vidarbha region of Maharashtra. Hence, this study will help in throwing light on the preventive aspect of dementia in terms of nutritional supplementation like vitamin B12, folate. Our study is a rural hospital based study and it will provide the necessary insight into the situation. Hence, in present study an attempt was made to assess correlation between plasma homocysteine, folate and vitamin B12 levels with cognitive status of patient suffering from Alzheimer's disease. The study was carried out in the Department of Biochemistry in association with Department of Psychiatry, Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha, Maharashtra, India.

### MATERIALS AND METHOD

A comparative and cross-sectional study was conducted. Institutional Ethics Committee approved the study. The study was done from August 2018 to February 2019, total sample size 100 including males and females and divided into two groups. Informed written consent was taken for the study purpose. 50 study group with Alzheimer's disease who attended the outpatient clinic of the Psychiatry Department of AVBRH Hospital, Sawangi (Meghe), Wardha, India and 50 age and sex matched healthy controls. All patients with known history of Alzheimer's disease within the age group of 60-80 years included in the study. Information about subject's age, sex, lifestyle, hypertension, family history of diabetes and other chronic diseases/disorders were written in pre-design format.

**Estimation of serum vitamin B12 and folate:** Quantitative measurement of serum vitamin B12, folate, and vitamin D was done by competitive ELISA.

**Estimation of serum homocysteine:** ELISA based on the Biotin double antibody sandwich technology.

**Estimation of vitamin D:** Kit for Vitamin D uses competitive-ELISA as the method.

### Sample Collection

Blood samples were collected from patient upon admission taking all aseptic precautions, about 5 mL of blood was drawn by venipuncture from a peripheral vein, with a disposable syringe and thereafter transferred to a clean dry glass tube where it was allowed to stand for 30 minutes for retraction of clot. This was centrifuged at 3000 rpm for 10 minutes to separate the serum. The serum sample was stored at -20°C in the refrigerator for analysis. Care was taken to avoid haemolysis of sample.

**Inclusion criteria:** All the study subjects were diagnosed by the consultant psychiatric according to DSM IV and NINCDS-ADRDA criteria.<sup>12</sup>

**Exclusion criteria:** Patient suffering from any significant comorbid physical illness, clinically diagnosed DSM V psychiatric illness, any nutrition deficient status or patient not accompanied by any care giver.

### Statistical Analysis

Statistical analysis was done between patients of Alzheimer's disease and age matched controls using SPSS-20.0. The data were expressed as mean  $\pm$  SD,  $p < 0.005$  was considered significant and mean  $\pm$  SD,  $p < 0.001$  was highly significant.

### RESULTS

The present study was carried out to evaluate the role of homocysteine, folate, vitamin B12 and vitamin D and its effect on cognitive status of patient suffering from Alzheimer's disease. Serum vitamin B12 level was lowered in cases as compared to the controls  $1.25 \pm 1.61$ . Serum folate level was lowered in cases as compared to the controls  $16.59 \pm 22.74$ . Serum vitamin D level was lowered in cases as compared to the controls  $20.01 \pm 34.68$ . Serum homocysteine level was higher in cases as compared to the controls  $12.65 \pm 8.0$ , which is statistically significant ( $p < 0.001$ ).

Parameters	Cases (50) Mean $\pm$ SD	Controls (50) Mean $\pm$ SD
Vitamin B12 (ng/mL) $p > 0.05$	$1.25 \pm 1.61$	$1.45 \pm 0.75$
Folate (ng/mL) $p > 0.05$	$16.59 \pm 22.74$	$26.66 \pm 27.26$
Vitamin D (ng/mL) $p > 0.05$	$20.01 \pm 34.68$	$42.75 \pm 70.49$
Homocysteine (nm/mL) $p < 0.001$	$12.65 \pm 8.0$	$6.28 \pm 6.77$

A correlation between serum folate and homocysteine and serum homocysteine and vitamin B12 shows that there was no correlation between these parameters and it was observed that these parameters are not related with each other and was statistically insignificant. No correlation in between vitamin D and homocysteine.

## DISCUSSION

In the present study, 50 Alzheimer's disease patients and 50 age matched controls were taken. It was observed that serum levels of homocysteine are significantly higher in patients in comparison to age matched controls. Vitamin D was significantly lower among the patients in comparison to controls. Studies suggested that metabolically significant vitamin B12 and folate deficiency in elderly is commonly present.<sup>13</sup> These vitamins are required for proper brain function. These are interdependent and essential component of the one carbon metabolism. Homocysteine is produced from methionine by demethylation. Homocysteine levels are normally maintained low. The mechanism in which homocysteine is remethylated to form methionine by a reaction that requires folate and vitamin B12.<sup>14</sup> Therefore, deficiency of vitamin B9 and vitamin B12 increases homocysteine level.

In present study, the serum homocysteine levels in patients with Alzheimer's disease were significantly higher than controls ( $p < 0.001$ ). As vitamin B12 and folic acid deficiency also increase the homocysteine levels in our body, these vitamins help in conversion of homocysteine to cysteine. There is familial form of vitamin B12 deficiency induced Alzheimer's disease which is not associated with other sign and symptoms of vitamin B12 deficiency which include macrocytosis and anaemia.<sup>11</sup> Vitamin B12 and folate levels in patients and controls were studied but both the levels were statistically insignificant ( $p > 0.005$ ). Vitamin D deficiency is a risk factor for cardiovascular diseases so it may be associated with increased risk with dementia and Alzheimer's disease. Its deficiency is associated with increased plaque and tangles formation in the brain, so increased risk of Alzheimer's disease. In present study, vitamin D levels were significantly decreased in cases in comparisons to the controls ( $p < 0.001$ ). A correlation between these parameters was observed that these parameters are not related with each other and that was statistically insignificant. The above study is important in its attempt to correlate the vitamins B12, folate and vitamin D with the cognitive status. The homocysteine levels have also been correlated with the cognitive status. We have used well standardised procedures to assess the cognitive status and also the biochemical levels.

## CONCLUSION

From this study, we have concluded that vitamin B12 and serum folate levels significantly decreased in cases as compared to controls but there is no association between these parameters. Higher serum homocysteine levels and lower vitamin D levels are risk factor for the Alzheimer's disease. Homocysteine is reduced by supplementing the body with vitamin B12.

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