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HOMOCYSTEINE LOWERING EFFECT OF DONEPEZIL AND VITAMIN B_{12} SUPPLEMENT IN ALZHEIMER'S DISEASE

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Abstract

Background: Association of homocysteine (Hcy) metabolism with cognitive dysfunction in AD pathogenesis has been revealed by many studies. The aim of this study was to investigate serum levels of vitamin B_{12} (VB_{12}) and Hcy with response to donepezil and VB_{12} supplement therapy in AD treatment.

Methods: Study comprised of 71 AD patients and 70 healthy controls above 60 years of age. Blood serum samples were analyzed for VB_{12} and Hcy levels by the chemiluminescence method. AD patients were treated with donepezil (5mg/day) and nutritional supplement containing VB_{12} (1.5mg/day) and was observed at intervals of 3 and 6 months. Statistical evaluation was done by using IMB SPSS statistics version 25.

Results: The serum levels of Hcy were significantly higher and VB_{12} were significantly low in AD patients as compared to baseline. The therapy did not show any improvement at 3 months interval but after 6 months the serum levels of VB_{12} increased and Hcy decreased as compared to 3 months. Hcy and VB_{12} show a negative correlation with each other in AD groups. ($p < 0.01$)

Conclusion: We conclude that the combination of donepezil and VB_{12} not only render symptomatic reliefs in AD patients, but also helps to revive from the metabolic consequences caused due to HHcy. It lowers the serum Hcy levels in AD, thereby reducing the progressive neurodegeneration and further vascular complications of AD. Research on efficacy of this combination therapy for a longer duration will help in better understanding of the disease pathogenesis and thus develop novel therapeutic target drugs.

Keywords: Homocysteine, Vitamin B_{12}, Alzheimer's Disease, Donepezil

Introduction

Alzheimer's disease (AD) is a degenerative disorder of the central nervous system (CNS) with pathological conditions involving the formation of neurofibrillary tangles and amyloid plaques within neuronal tissue. The cholinergic hypothesis has been proposed to be an etiology of AD, based on the presynaptic deficits found in diseased brains. (1) Association between B-group-vitamin deficiency and cognitive dysfunction appeared in the literature long before homocysteine (Hcy) gained prominence as a cardiovascular disease (CVD) risk factor in the 1990s. Early reports noted a high frequency of Vitamin B_{12} (VB_{12}) deficiency in psychogeriatric populations and a high prevalence of hyperhomocysteinaemia (HHcy) in elderly patients with dementia. (2) HHcy has proved to be an independent risk factor with significant strength for both vascular dementia and AD. (3)

B vitamins, including folate, vitamin B_2, B_6, and B_{12} are involved in one-carbon transfer reactions such as methylation, which is necessary for the production of

monoamine neurotransmitters, phospholipids, and nucleotides in the brain. VB_{12} and folate are cofactors necessary for the methylation of Hcy, which acts as a risk factor having a direct neurotoxic effect resulting in brain atrophy, cognitive impairment, and dementia. (4) Thus, fluctuations in the VB_{12} levels may explain the relationship between Hcy and AD. However, an elevated serum Hcy level might also suggest a VB_{12} deficiency. Hence, there is some debate regarding differing levels of serum Hcy and VB_{12} among healthy controls, and patients with AD.

Although elevated Hcy levels have consistently been reported as an independent risk factor for AD and a predictor of cognitive decline in AD patients, it remains controversial whether HHcy is an independent causal risk factor for AD or is a consequence of an inadequate dietary intake or just occurs as secondary epiphenomenon of neurodenegeration. Therefore, it is necessary to determine whether the association between Hcy and AD is causal and reversible in response to treatment. (4)(5)(6)

Currently, the most common drugs used for AD treatment are cholinesterase inhibitors (ChEI) including donepezil which provides symptomatic relief in AD patients. Donepezil hydrochloride is an acetylcholinesterase inhibitor (AChEI) which inhibits the action acetylcholinesterase (AChE) in neuronal synapses, rendering increased acetylcholine action for better cognition. (7)(8) Since, AD patients are also found to be deficient for Vitamin B_{12} which has a striking effect on cognitive dysfunction, a combination of donepezil and methylcolabamin, an active form of VB_{12} might be beneficial in AD treatment. (9)

The aim of the present study was to investigate the serum levels of VB_{12} and Hcy in AD patients. The effect of donepezil and vitamin B_{12} supplement combination therapy on serum levels of VB_{12} and Hcy was also observed at intervals.

MATERIAL AND METHODS:

The study comprised of 71 clinically diagnosed AD patients and 70 normal healthy controls. Recruited subjects were of both genders in the age group above 60 years. Patients admitted and those visiting Out Patient Department at Sir J. J. Group of Hospitals, Mumbai were included in the study. Subjects not willing to participate in the study and those suffering from a head injury, cerebral stroke, chronic diabetes, poisoning, schizophrenia, chronic alcoholism or any other mental illness were excluded. There was no history of thyroid disease or of any other major ailment in any of the patients. Informed consent was obtained from the representatives of diagnostically confirmed AD patients recruited in the study. Ethical Clearance approval was taken from the Institutional Ethics Committee of Grant Government Medical College and Sir J. J. Group of Hospitals, Mumbai (IEC approval letter no.: IEC/pharma/328/15). Informed consents along with details of patients were taken prior to the study.

Venous blood samples of all the study participants were collected by venipuncture and serum was separated. Serum samples were analyzed for the VB_{12} and Hcy as baseline investigation by using the chemiluminescence method on Siemens Fully Automated Immulite 1000 Chemiluminescent Immunoassay. AD patients were treated with Donepezil (5mg/day) and nutritional supplements containing Methycolabamin (1.5mg/day) tablets for 6 months. Serum levels of VB_{12} and Hcy were analyzed again at intervals of 3 months and 6 months. Socioeconomic status of AD patients was categorised based on modified Kuppuswamy socioeconomic status scale. (10) Statistical evaluation was done by using IMB SPSS Statistics version 25.

RESULTS:

Table 1: Age and Sex Wise Distribution in Control and AD

Sex	Age (years)			
	Mean ± SD	Median	Minimum	Maximum
Control				
Male (n=40)	70.13 ± 5.59	69	60	80
Female (n=30)	66.7 ± 6.24	65	60	86
Total (n=70)	68.65 ± 5.98	68	60	86
Alzheimer's Disease				
Male (n=46)	71.19 ± 7.52	70	60	89
Female (n=25)	67.8 ± 5.47	68	60	80
Total (n=71)	70 ± 7.02	69	60	89

Table 2: Serum levels of Vitamin B_{12} (VB_{12}) and Homocysteine (Hcy) in Control and AD at Baseline (B), 3 Months (3M) and 6 Months (6M).

Parameters	Groups	Mean ± SD	Variance	Median	Minimum	Maximum
VB_{12} (pg/ml)	Control (B)	749.27 ± 131.09	17186.78	743.5	428	1016
	AD (B)	380.89 ± 177.28	31427.35	371	11.68	794
	AD (3M)	337.42 ± 162.32	26347.64	319	51.8	736
	AD (6M)	342.48 ± 163.68	26792.77	328	52.2	741.4
Hcy (µmol/l)	Control (B)	8.25 ± 2.34	5.48	7.95	3.62	15.4
	AD (B)	23.29 ± 3.81	14.49	23.3	14.6	31.1
	AD (3M)	21.32 ± 3.87	14.97	21.6	13.1	28.4
	AD (6M)	19.36 ± 3.81	14.49	19.7	12.6	25.7

Table 3: Pearson's correlations between VB_{12} and Hcy in Control and AD groups at Baseline, 3 Months and 6 Months.

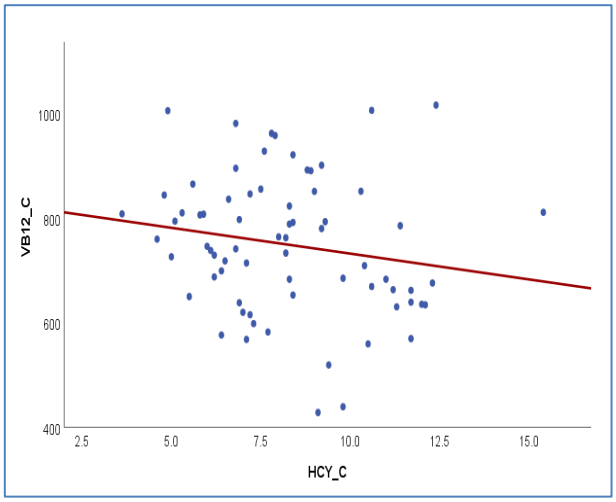
Groups	Parameters	r-value	P value
Control / Control	VB_{12} (B) / Hcy (B)	-0.176	0.141
Control / AD	VB_{12} (B) / VB_{12} (B)	0.076	0.530
	VB_{12} (B) / VB_{12} (3M)	0.094	0.437
	VB_{12} (B) / VB_{12} (6M)	0.097	0.423
	Hcy (B) / Hcy (B)	-0.256*	0.031
	Hcy (B) / Hcy (3M)	-0.268*	0.024
	Hcy (B) / Hcy (6M)	-0.286*	0.016
AD / AD	VB_{12} (B) / VB_{12} (3M)	0.911**	0.000
	VB_{12} (B) / VB_{12} (6M)	0.912**	0.000
	VB_{12} (3M) / VB_{12} (6M)	0.998**	0.000
	Hcy (B) / Hcy (3M)	0.964**	0.000
	Hcy (B) / Hcy (6M)	0.943**	0.000
	Hcy (3M) / Hcy (6M)	0.966**	0.000
	VB_{12} (B) / Hcy (B)	-0.252*	0.034
	VB_{12} (3M) / Hcy (3M)	-0.307**	0.009
	VB_{12} (6M) / Hcy (6M)	-0.340**	0.004

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

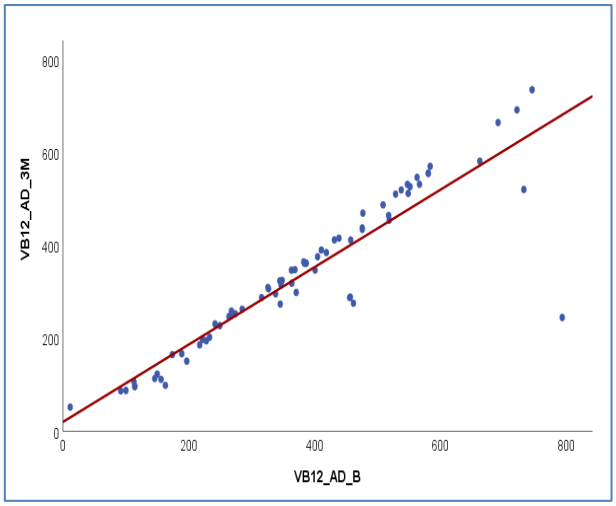
Table 4: Categorization of AD patients by socioeconomic status.

Category	I	II	III	IV	V
Socioeconomic status	Upper class	Upper Middle class	Middle class	Lower Middle Class	Lower class
No. of AD patients	2	5	32	28	4

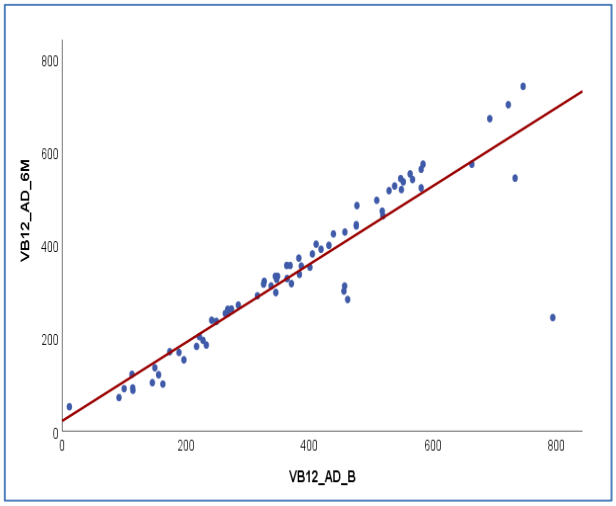
Figure 1: Linear regression graphs representing correlations between Hcy and VB_{12} in AD at Baseline, 3 Months and 6 Months:



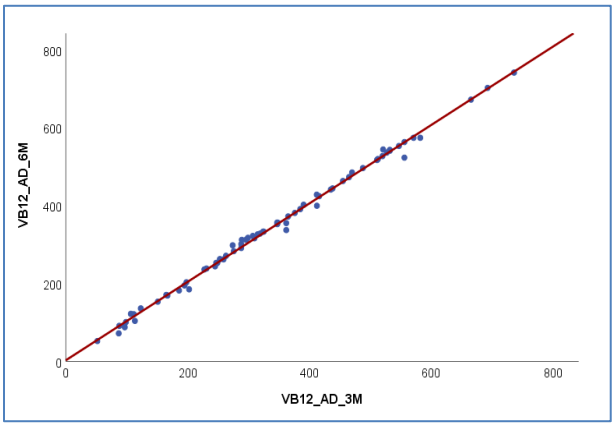
(a)C - VB_{12} (B) and C - Hcy (B)



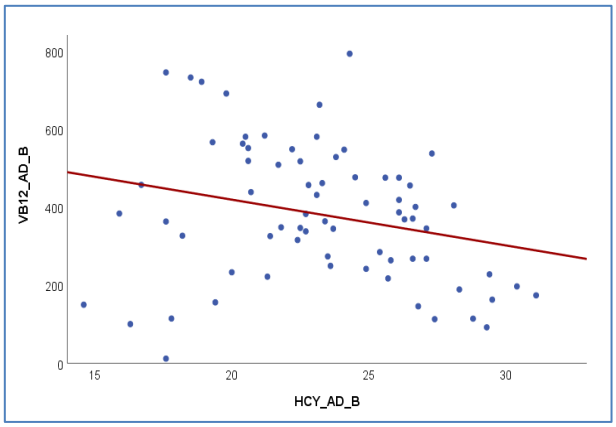
(b) AD - VB_{12} (B) and AD - VB_{12} (3M)



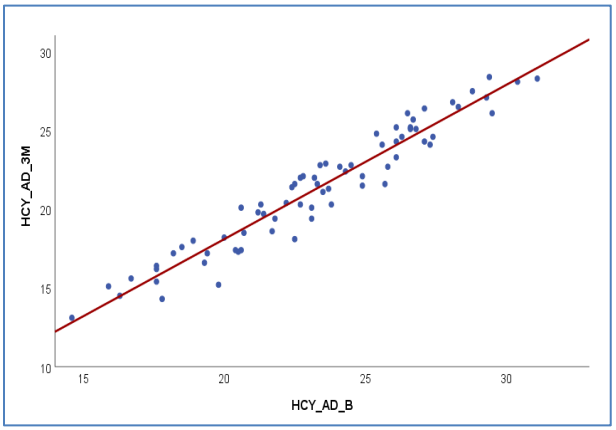
(c) AD - VB_{12} (B) and AD - VB_{12} (6M)



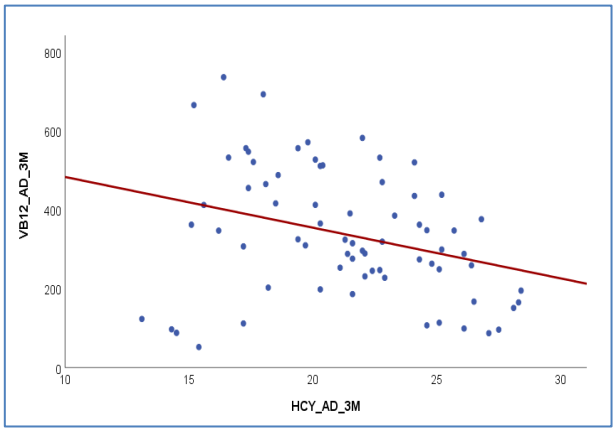
(d) AD - VB_{12} (3M) and AD - VB_{12} (6M)



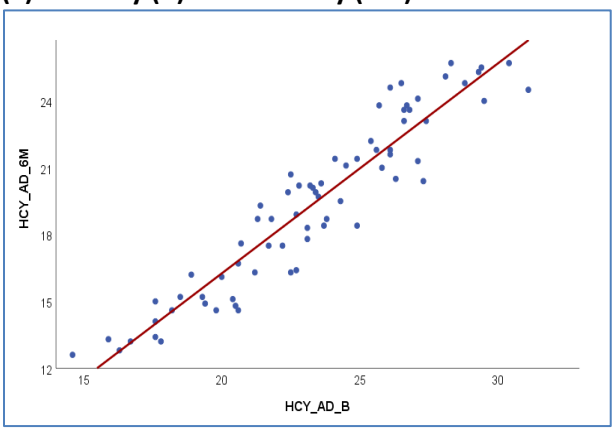
(h) AD - Hcy (B) and AD - VB_{12} (B)



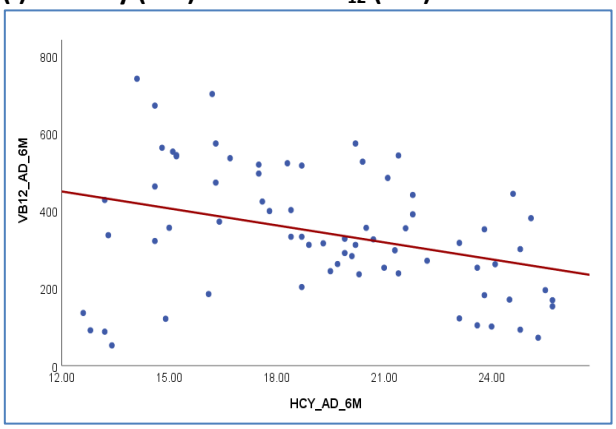
(e) AD - Hcy (B) and AD - Hcy (3M)



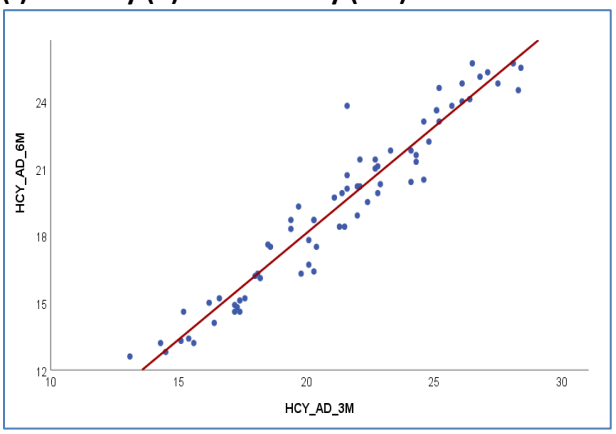
(i)AD - Hcy (3M) and AD - VB_{12} (3M)



(f) AD - Hcy (B) and AD - Hcy (6M)



(j) AD - Hcy (6M) and AD - VB_{12} (6M)



(g) AD - Hcy (3M) and AD - Hcy (6M)

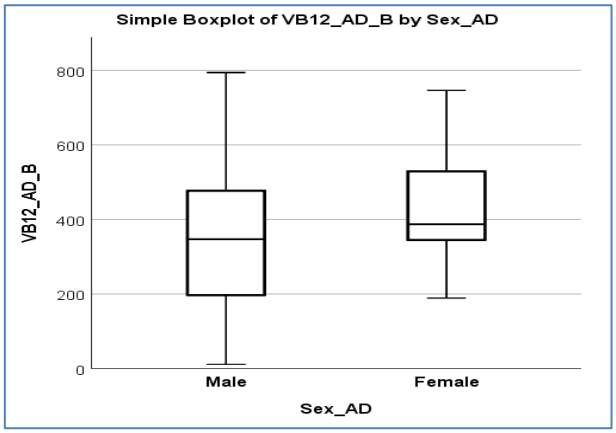


Fig 2: (A)

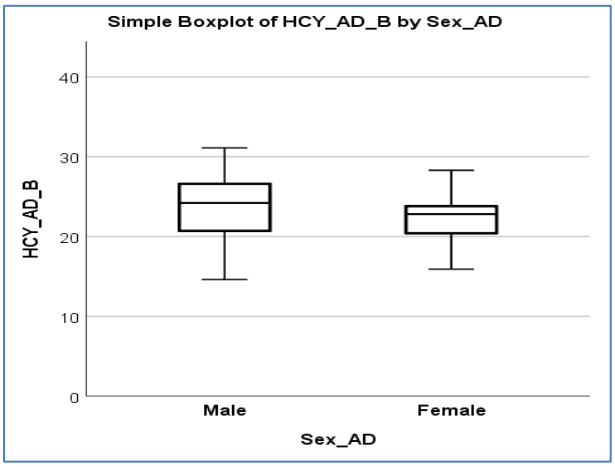


Fig 2: (B)

Figure 2: Sex wise distribution of serum levels of **(A)** VB_{12} and **(B)** Hcy in AD patients.

DISCUSSION:

The sex and age wise distribution of AD patients and the serum levels of VB_{12} and Hcy in both genders is shown in **Table 1 and Figure 2**. We observe that there were more male AD patients than females in this urban population. The serum levels of VB_{12} and Hcy were also found to be more disturbed in males than in females, rendering males more prone to the risk of AD and CVD development **(Figure 2a and 2b)**.

The association between elevated plasma levels of Hcy and nutritional status has been shown in AD patients. Several cross-sectional and longitudinal studies have proposed that HHcy levels may be an independent risk factor for impaired cognitive function or AD, although other studies found no significant association between Hcy and cognitive function. (4) In our study, AD patients showed a significantly increased serum Hcy levels and decreased VB_{12} levels in AD patients **(Table 2)**, similarly to the results shown by Grzegorz Raszewski et al. 2016. (3)

A longitudinal study of ageing and AD, found that dementia was worse in the presence of brain infarcts, suggesting contribution of HHcy to AD dementia by induction of vascular changes. However, Hcy was directly excitotoxic to cortical neurons in cell culture, which showed a causal role in the cholinergic deficit characteristic of AD. (2)(11)(12) In **Table 2, 3 and Figure 1(a), 1(h), 1(i) and 1(j)**, we can see that the serum levels of Hcy and VB_{12} are inversely correlated with each other in both control and AD groups. Previous studies have shown that the relation between a high Hcy level and low vitamin B levels in AD patients is due to biochemical damage, rather than a nutritional deficit. (4)(6) Fei Ma et al. 2017 concluded that increased levels of Hcy are associated with low vitamin B plasma levels were found only in AD patients as compared to vascular dementia. Thus, vitamin B metabolism does not represent the direct consequence of the nutritional status and suggests that neuronal damage results in a functional vitamin B deficiency. Hcy may exert its neurotoxic effects by activating the N-methyl-D-aspartate receptor, leading to cell death, or by being converted into homocysteic acid, which also has an excitotoxic effect on neurons. (4) HHcy inducing AD, can behave oneself both as risk factor and marker simultaneously. (6)

In our study, the AD patients treated with a combination therapy of donepezil and VB_{12} supplement showed no significant improvement in AD patients at 3 months interval. Serum levels of VB_{12} were reduced and those of Hcy were increased as compared to the baseline values. But consequently, the continued treatment upto 6 months showed a significant improvement in the disease status. We observed a remarkable increase in the VB_{12} serum levels along with a decrease in Hcy levels which were initially raised in AD patients. **(Table 2)**

Moreover, the serum levels of VB_{12} **(Figure 1b, 1c and 1d)** and Hcy **(Figure 1e, 1f and 1g)** showed a positive correlation along their follow-ups at 3 and 6 months intervals respectively and negative correlations within levels of each other **(Table 3 and Figure 1**). Thus, we observed a positive effect of the treatment on the AD patients with respect to the Hcy metabolism in a period of 6 months. The AD patients involved in the study were categorized according to their socioeconomic status to predict the role of nutrition in disease incidence **(Table 4)**. We observed that the occurrence of disease had no significant relation to the socioeconomic status of the subjects. However, the number of AD patients was more in levels below middle class. Nutrition therefore plays a role in the rate of disease progression.

CONCLUSION:

We conclude that the combination of donepezil and VB_{12} not only render symptomatic reliefs in AD patients, but also helps to revive from the metabolic consequences caused due to HHcy. It lowers the serum Hcy levels in AD, thereby reducing the progressive neurodegeneration and further vascular complications of AD. Research on efficacy of this combination therapy for a longer duration will help in

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