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Original Article

Role of Vitamin-D in the prevention and treatment of Alzheimer's disease

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Abstract

Alzheimer's disease is the most common form of age related cognitive impairment. Aim of the present study was to see the effect of vitamin D on cognitive function in elderly. The study was conducted in Department of Physiology, King George's Medical University (KGMU). A total of 80 subjects were enrolled based on Mini Mental State Examination (MMSE) score < 24 and vitamin D deficiency. They were divided into two groups as Group A (case) and Group B (control), each group having 40 subjects. Intervention (Vitamin D supplementation) was given in Group A. The assessment of dementia was done by Mini Mental State Examination (MMSE) score. Every subject was clinically evaluated and estimation of vitamin D was done by direct ELISA kit. Gender, weight, height, BMI, residence and education were also similar between two groups. A significant (p=0.0001) change in MMSE score was observed in both Group A and Group B from baseline to 3 & 6 months and from 3 to 6 months, however, mean change was higher in Group A than Group B. In conclusion, vitamin D supplementation caused significant improvement in the cognitive performance in subjects with senile dementia.

Introduction

Senile dementia is a global phenomenon with extensive economic and social consequences. It is associated with decrease in efficiency of physiological system. In addition to this, the social consequences may lead to higher morbidity rate (1). Prevalence of dementia in India ranges from 0.6% to 3.5% in rural areas and 0.9% to 4.8% in urban areas (2). Constitution of India provides special privileges for older persons. Article 41 provides effective rights to public assistance in case of old age group persons (3).

Breakdown of joint family system and modern lifestyle

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have changed the approach of younger generation towards older people and they have become a sort of burden for the younger generation. Since older people feel difficulty to change their ideas and views according to younger generation thus they are unable to adjust with the young generation. This type of family environment leads to development of various psychological problems like cognitive impairment, depression, sleeplessness, anxiety, insecurity etc. (4, 5, 6). Ignorance by family leads to development of stress and nutritional impairment in older people. Nutritional impairment causes deficiency of various macro and micronutrients in the body like various vitamins. Over 80% of Indian population aged 60 years or above have deficiency of vitamin D (7, 8).

Alzheimer's disease is the most common form of age related cognitive impairment (9, 10). It is characterized by decreased brain cholinergic activity that ascertains the use of acetyl-cholinesterase inhibitors. Alzheimer's disease is associated with glutamine induced excite-toxicity (11, 12). Glutamine induced excite-toxicity cause neuronal death through intra-neuronal calcium influx (13, 14). Memantine, a voltage dependent, low affinity, non-competitive N-methyl-D-aspartate receptor antagonist, is used for the treatment of Alzheimer's disease. Memantine provides symptomatic relief but it does not offer preventive or curative treatment, thus subjects are still exposed to oxidative stress (15, 16). So coupling an antioxidant with treatment regimen provides solution to the neural apoptosis induced by glutamine excess.

Vitamin D is a steroid hormone. It crosses the blood brain barrier and binds with receptors present in neurons and glial cells of various parts of central nervous system like hippocampus, cortex, sub-cortex etc. (17, 18, 19). It controls intra-neuronal calcium homeostasis by regulating the voltage gated calcium channels thus prevents neuronal necrosis (20). Being a steroid hormone it also has antioxidant property. It facilitates cellular functions that reduce oxidative stress induced by glutamate and dopaminergic toxins. Thus it prevents apoptosis of dopaminergic neurons (21). Due to its antioxidant property and regulating intra-neuronal calcium homeostasis, it can be hypothesized that vitamin D has a role to play in preventing age related cognitive decline.

Methods

Study was conducted in Department of Physiology, King George's Medical University (KGMU), Lucknow in collaboration with Departments of Geriatric Mental Health (GMH) and Biochemistry, KGMU, Lucknow, India. Subjects were enrolled from outpatient department (OPD) of GMH after taking ethical clearance from ethics committee of KGMU, Lucknow and informed consent from patient's guardian.

Prevalence of dementia using survey diagnosis or clinical diagnosis of DSM IV or ICD 10 reported from Indian studies range from 0.6% to 3.5% in rural areas and 0.9% to 4.8% in urban areas (2). As present study included both rural and urban subjects so an average of rural and urban prevalence of dementia that is 2.7% was taken for calculation of sample size. Sample size was calculated by following formula,

$$n = Z^2PQ / e^2$$

where P = Prevalance (2.7%), Q = (1-P), e = Estimated error (5%), n = Number of samples, Z= Differential coefficient (1.96). Total 80 subjects were enrolled based on MMSE score and serum vitamin D level. Subjects with MMSE score < 24 were considered as having cognitive impairment. Subjects with serum vitamin D level < 30 ng/ml were considered as having vitamin D deficiency. After that all the subjects were randomized into two groups, Group A (cases) and Group B (controls).

Intervention (Vitamin D supplementation) was given in Group A. The assessment of dementia in present study was done by Mini Mental State Examination (MMSE) score. MMSE is useful for evaluating the subjects having dementia syndrome because these subjects cooperate well only for short duration (22). It includes few questions and takes only 5-10 minutes to apply (23). It is more sensitive to dementia than routine clinical judgement and can be used in OPD (24).

Inclusion criteria of Group A and Group B:

Age ≥ 60 years, mini mental state examination score < 24, Serum vitamin D level ≤ 30 ng/ml and cognitive impairment due to aging.

Exclusion criteria of Group A and Group B:

Age < 60 years, uncooperative subjects, all non-degenerative dementia, uncontrolled diabetes, hypertension and other chronic illnesses, smokers, drug addiction and non-availability of informed consent.

Clinical evaluation of subjects:

Every subject was clinically evaluated by taking the history of present illness, past history, family history of medical and surgical illnesses, socio-economic status, drug, nutritional, occupational, menstrual (if applicable) and drug addiction history.

Blood sample collection and storage:

4 ml fasting venous blood sample was drawn from each subject by trained staff nurse for estimation of serum vitamin D and stored in –80°C deep freezer.

Estimation of vitamin D:

Estimation was done by direct enzyme linked immune sorbent assay(ELISA) kit (Immunodiagnostik AGstubenwald- Allee).

Principle of test:

Assay utilizes a competitive ELISA technique with a selected monoclonal antibody recognizing vitamin D. For a reliable determination of vitamin D, it is necessary to release it from the vitamin D - vitamin D binding protein (DBP) complex. A dose response curve of the absorbance unit (optical density at 450 nm) vs. concentration was generated using the values obtained from the standard. Concentration of vitamin D in sample was determined from this curve. For evaluation of test results, a single- point calibration via a standard curve described by a four parameter curve fit was used. The parameters as well as the optical density value of calibrator were given on the quality control certificate of the kit. Results were read from the constructed calibration curve.

Vitamin D supplementation:

Both Group A and Group B were on same line of medical treatment. Only Group A subjects were supplemented with vitamin D apart from the routine medical treatment. It was given in the form of calcirol granules orally. 100 IU of vitamin D raises serum vitamin D concentration by 0.7 ng/ml. Daily dose of 4000 IU was expected to raise the serum vitamin D concentration by at least 28 ng/ml, with a concentration ultimately reached > 30 ng/ml. Chosen dose did not reach to the toxic level and raised serum concentration within nontoxic limit (25, 6), so serial serum vitamin D monitoring was not necessary.

Assessment:

Assessment of improvement in cognitive performance was done after every 3 months by MMSE score.

Results

Data were summarized as Mean±SD. Groups were compared by independent Student's t test. Categorical groups compared by chi-square(x^2) test. A two sided (α=2) $p<0.05$ was considered statistically significant. All analysis was performed on SPSS (version 17.0) software.

(A) Basic characteristics

The study evaluates effect of vitamin D supplementation on cognitive performance in subjects of cognitive impairment. There was no statistically significant difference in basic parameters among subjects of two groups (Table I). In other words, subjects of two groups were demographically matched and comparable

(B) Primary outcome measures

I. Biochemical parameter

The mean level of serum vitamin D was found lower in Group A compared to Group B but difference was

TABLE I: Basic characteristics of the cases and controls.

		Group A (n=40) No. (%)	*Group B (n=40) No. (%)*	*P-value*
Age in years	60-70	24(60%)	30(75%)	0.15[a]
	71-80	16(40%)	10(25%)	
	Mean±SD	69.68±6.45	66.68±4.80	
Gender	Male	27(67.5%)	31(77.5%)	0.31 [a]
	Female	13(32.5%)	9(22.5%)	
Height in cms		165.38±5.29	164.00±4.5	0.21[b]
Weight in kg		52.88±6.67	51.55±6.80	0.38 [b]
BMI (kg/m^2)		19.37±2.77	19.16±2.47	0.72 [b]
Residence	Rural	15(37.5%)	13(32.5%)	0.63 [a]
	Urban	25(62.5%)	27(67.5%)	
Education	Up-to 10th	32(80.0%)	29(72.5%)	0.43 [a]
	Above 10th	8(20.0%)	11(27.5%)	

[a]Chi-square test, [b]Unpaired t-test.

not statistically significant (p>0.05) (Table II).

II. Baseline MMSE score (Pre-treatment MMSE score)

The baseline (pre-treatment) mean MMSE score of Group A was higher than the baseline mean MMSE score of Group B but statistically not significant (p>0.05) (Table III).

(C) Secondary outcome measures

Post MMSE score (after supplementation)

The after 3 months of supplementation increase in MMSE score was higher in Group A than Group B

TABLE II: Biochemical parameter levels (Mean±SD) of two groups.

Parameters	*Group A (n=40)*	*Group B (n=40)*	*% change*	*t value (DF=78)*	*P value*
Vit. D (ng/ml)	8.22±6.23	9.34±5.77	11.9%	0.83	0.409[b]

[b]Unpaired t-test.

TABLE III: Changes in MMSE score (Mean± SD) from baseline to 3 and 6 month.

	Group A (n=40)	*Group B (n=40)*	*P-value (n=40)*
Baseline	17.75±2.91	17.42±2.89	0.61[1]
3 month	21.20±3.01	20.12±2.67	0.09[1]
6 month	23.58±2.27	21.85±1.80	0.0001*

[1]Unpaired t-test, *Significant (Paired t-test).

but statistically not significant (p>0.05). However, improvement in MMSE score became significantly (p=0.0001) higher in Group A compared to Group B at 6 month (Table III). A significant (p=0.0001) mean change was observed in both Group A and Group B from baseline to 3 & 6 month and from 3 to 6 month, however, mean change was higher in Group A than Group B (Table IV).

TABLE IV: Mean changes in MMSE score (Mean±SD).

	Group A (n=40)	*Group B (n=40)*
Baseline to 3 month	3.45±1.10 (p=0.0001*)	2.70±0.85 (p=0.0001*)
Baseline to 6 month	5.82±1.23 (p=0.0001*)	4.42±1.58 (p=0.0001*)
3 month to 6 month	2.37±1.33 (p=0.0001*)	1.72±1.30 (p=0.0001*)

*Significant (Paired t-test).

Discussion

Dementia is a common cognitive disorder reflective of a wide spread chronic progressive degenerative disease and may be a part of normal aging process (26). Dementia has become one of the leading public health problems facing our society. Since burden of dementia inflict on individual, families and societies, so more emphasis has been placed on the study of these diseases (27).

Number of old age people increased in both developed and developing countries due to longer life expectancy and declining fertility (28). Women seem to be affected more frequently by dementia when different patterns of mortality took into consideration however gender distribution of the dementia varies according to the cause of dementia and in some cases males out number females (29). Dementia presents with a slow loss of cognitive function and is characterized by impairment in language, memory, visuo-spatial skills, emotions and personality (30). It is a part of group of illnesses that cause a progressive decline in person's mental functions. Criteria used for the dementia are developed by the World Health Organization for the International Classification of Disease (ICD), tenth

revision, 1989 (31) and the American Psychiatric Association, third and fourth editions (32, 33). According to ICD- 10 dementia must occur in the presence of clear consciousness, must be of such degree that it impairs activities of daily living and symptoms must be present for at least six months (34).

Subjects with MMSE score < 24 are considered as having cognitive impairment (23). All subjects enrolled for study had MMSE score < 24 that is all the subjects had cognitive impairment. MMSE score had been used for cognitive assessment because it is easy to administer and takes minimal training.

Role of micronutrients (vitamins and minerals) in normal functioning and growth of neural tissue is well known. Loss of cell function could be due to deficiency of vitamin D. Vitamin D acts like a neuro-steroid hormone in areas of neurotransmission and neuro-immunomodulation. Hypovitaminosis D has been associated with neuromuscular disorders, dementia and Parkinson's disease. Thus, prophylactic vitamin D supplementation may be protective against these neurological disorders (18). Serum vitamin D level > 30 ng/ml is considered as sufficiency, 20-30 ng/ml insufficiency and if its serum level is less than 20 ng/ml than this condition is called vitamin D deficiency (35).

All the subjects in present study had a mean value of vitamin D < 20 ng/ml. During study we found that all the subjects with vitamin D deficiency did not have cognitive impairment but all the subjects with cognitive impairment had vitamin D deficiency. Previous studies show that relative risk of cognitive decline in subjects who had severe vitamin D deficiency in comparison with those who had sufficient level of vitamin D was 1.6 (36). In a study done on cohort of community-dwelling older women, it was found that serum vitamin D deficiency was associated with cognitive decline (37). In a meta-analysis using random effects models for the weighted mean difference and Hedge's and it was found that lower serum vitamin D concentration was associated with poor cognitive function and higher risk of Alzheimer's disease (38). Cognition was evaluated with the Abbreviated Mental Test Score. Low serum vitamin D level was associated with balance and cognitive functions (39).

In present study, after 3 months of supplementation, increase in MMSE score was higher in Group A than Group B but statistically not significant. However, improvement in MMSE score was significantly higher in Group A as compared to Group B at 6 month. A significant change in MMSE score was observed in both Group A and Group B from baseline to 3 & 6 month and from 3 to 6 month, however, mean change was higher among Group A than Group B. Vitamin D modulates age related increase in pro-inflammation and amyloid burden (40). There was significant association between weekly vitamin D dietary intake and Short Portable Mental Status Questionnaire (SPMSQ) score. Inadequate weekly vitamin D dietary intake was also associated with cognitive impairment (41). All the above studies corroborate with the findings of present study.

Hence, the present study clearly indicates that Vitamin D supplementation has an additional effect on the cognitive functions in elderly who are on medical therapy for senile dementia. Most probable explanation is that vitamin D regulates the intra-neuronal calcium homeostasis via the regulation of voltage gated calcium channels thus preventing necrosis. It has also exhibited neuro-protective properties against glutamate toxicity through antioxidant effects thus preventing apoptosis. The present study is one of on-going steps in the direction of establishing role of Vitamin D in prevention and treatment of senile dementia. However to achieve more confirmatory results further study on larger sample size is needed.

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