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Research Article

SUPPLEMENTATION OF VITAMIN E IMPROVES COGNITIVE STATUS AND OXIDATIVE STRESS IN TYPE 2 DIABETES MELLITUS

Anand Vijayakumar PR*, Bhatt Jayesh Kumar, Varghese Jenny M, Das Sushanta Kr.
Department of Pharmacy Practice, JSS College of Pharmacy (JSS University), Ootacamund, Tamilnadu, India 643001

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*Email: pranadvijayakumar@jsscpooty.org

ABSTRACT
Oxidative stress has been suggested as a major contributing factor for initiation and progression of cognitive impairment along with various other complications in type 2 diabetes mellitus. The present study was, therefore, designed to assess the effect of vitamin E supplemention along with oral hypoglycaemic agents on cognitive functions and oxidative stress in patients with type 2 diabetes mellitus. A total of 74 type 2 diabetes mellitus patients were enrolled in this randomized open label controlled study from out-patient department of secondary care Government headquarters hospital, Ooty, India. Patients were randomised into two groups viz., control (n=36) and intervention (n=38). Control group received OHA's i.e, Glibanclamide and/or Metformin. Patients in intervention group received OHA's along with vitamin E supplementation (600mg/day) for a period of three months. Cognition functions were assessed for control and intervention group by using Mini Mental State Examination. Blood samples were analysed for the level of thiobarbituric acid reactive substances, superoxide dismutase, catalase, reduced glutathione and HbA1C by using specific assay methods at the baseline and by end of three months. Intervention group patients showed significant improvement in the orientation (9.22±0.27), $p \leq 0.001$ and in total MMSE score (28.00±0.43), $p \leq 0.001$ after three months of vitamin E supplementation. There was a significant decreased in lipid peroxidation and HbA1C level. Significant increase in enzymatic and non enzymatic antioxidant levels was observed in intervention group when compared to control group. Vitamin E supplementation is found to be effective in improving orientation, total MMSE score and oxidative stress in type 2 diabetes mellitus.
KEY WORDS: Type 2 diabetes mellitus; Cognition; Oxidative stress; Vitamin E; Human

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a common, serious, chronic and currently incurable metabolic disorder characterized by hyperglycaemia that results from a deficiency in production or action of insulin. The estimated Diabetes mellitus (DM) prevalence for 2010, worldwide is 285 million and by 2030 the number is set to increase 438 million. India is identified to be the diabetes capital of the world with current estimated 50.8 million diabetic's patients[1].

T2DM is associated with a high risk for microvascular and macrovascular complications which leads to profound changes in the peripheral and central nervous system. Impaired cognition function and slowly progressive end-organ damage in the brain are reported to occur in diabetic patients[2,3].

The pathogenesis of the central nervous system changes associated with T2DM includes several factors, i.e., inflammation, microvascular dysfunction, and oxidative stress[4]. Persistent hyperglycemia may cause high production of free radicals and reactive oxygen species (ROS)[5,6] which react with cellular components contributing to increased neuronal death through protein oxidation, DNA damage, and peroxidation of membrane lipids[7]. In addition to enhanced ROS production, intracellular antioxidant mechanisms (i.e., glutathione peroxidase activity and manganese dismutase protein content) are reduced and brain mitochondrial nitric oxide (NO) levels are increased[6]. Decreased brain glutathione levels coupled with oxidative stress have been suggested to be responsible for the induction of cognitive deficits often observed in T2DM condition[8].

Common mechanism in type 2 DM that contribute to increase oxidative stress through, increased free radical production during glucose autoxidation and hyperglycemia-induced reduction in the levels of protective endogenous antioxidants (vitamins A, E and C) and decreased activity of antioxidant enzymes such as glutathione peroxidase[5]. Upset in the oxidant/antioxidant equilibrium lead to a condition so called oxidative stress, which is known to be a component of molecular and cellular tissue damage[7].

However, at present no specific treatment options are available for the management of cognitive deficits induced by diabetes[9]. Recently, interest has increased in the use of natural nootropic agents for the amelioration of cognitive deficits[10,11]. Antioxidant like, vitamin C can enhance learning and memory and prevent memory deficits in various experimental conditions[12-15]. Vitamin E has been also reported to prevent aging induced memory impairments in rats[16]. Additionally, clinical studies suggest that vitamin E may be a complementary intervention for patients with cognitive dysfunction[17,18].

Information regarding the benefits of supplemental antioxidant use for the prevention of incident cognitive impairment in healthy individuals or cognitive decline is limited to epidemiologic evaluations that have yielded conflicting results[19-24]. Some prospective studies have reported that the use of vitamins C and E prevent cognitive decline[19,20,23]. The present study was designed, therefore, to evaluate the effect of vitamin E on cognitive function and oxidative stress in type 2 diabetic patients.

METHODS AND MATERIALS

Subjects

A total of 74 patients with type 2 diabetes mellitus aged between 35 to 65 years, either sex, without co-morbidities, on oral hypoglycaemic agents (metformin, glibenclamide and combination) and duration of disease ≤10 years with HbA1C level > 9% were included in the study. None of the patients were on antioxidant supplement. Patient with history of dementia, on treatment with antidepressant therapy, type 1 diabetes, juvenile diabetes, pregnant women and lactating mothers, voluntary withdrawal and significant hepatic & renal dysfunction were excluded. Written consent was obtained from all participants after a clear explanation of its experimental nature. The project protocol was approved by Institutional Ethics Committee, JSS College of pharmacy, Ootacamund, The Nilgiris, Tamil Nadu, India.

Study design

A randomized open label controlled trial include 74 study patients divided into two groups control (n=36) and intervention (n=38). The study was carried out at outpatient department of secondary care Government headquarters hospital, Ootacamund, The Nilgiris, Tamil Nadu, India, during the period of June 2008 to January 2009. At the screening visit after explaining the objective of the study and verifying inclusion and exclusion criteria, each patient signed a written informed consent. Enrolled patients were randomized by using computer assisted randomization procedure and assigned to

control group and intervention group. Initially demographic data and general health characteristics include height, body weight, body mass index, social habit, smoking status and food habits were collected on standard structured data collection form by face to face interview.

Control group patients received oral hypoglycaemic agents (Glibenclamide-5mg, Metformin- 500mg) treatment and intervention group patients received vitamin E (600mg OD) supplementation along with Oral hypoglycaemic agents (OHA's) for a period of three months.Cognitive functions were measured by using Mini Mental State Examination (MMSE) in Tamil version. Copyright permission to use Mini Mental State Examination (MMSE) in Tamil version was obtained from Psychological Assessment Resources Inc USA. Biochemical parameters that include oxidative stress, HbA1C were measured at the baseline and at the end of three months.

Mini Mental State Examination Scale (MMSE)

The MMSE[25] is a brief, quantitative measure of cognitive status in adults. It is used to screen for cognitive impairment, to estimate the severity of cognitive impairment at a given point of time, to follow the course of cognitive changes in an individual over time, and to document an individual's response to treatment. It is an 11 questions measure that tests five areas of cognitive function: orientation, registration, attention and calculation, recall, and language. The maximum score is 30. A score of 24-30 indicates no cognitive impairment, score of 18-23 indicates mild cognitive impairment and score of 0-17 indicates severe cognitive impairment.

Biochemical parameters

Fasting blood samples (5ml) were collected from each patient at baseline visit and at the end of 3 months trial. Blood samples were collected by venous puncture in heparinized sample collection tubes and the plasma was separated by centrifugation at 1000 rpm for 20 min. After the collection of plasma, the buffy coat was removed and the packed cells were washed with cold physiological saline. A known volume of the erythrocytes was lysed with hypotonic phosphate buffer. The hemolysate was separated by centrifugation at 2,500 rpm for 10 min followed by biochemical analysis.

The glycated haemoglobin (HbA1C) was determined by using semi-autoanalyzer (Microlab 200 Merck). Superoxide dismutase (SOD) was determined using spectrophotometric method by Kakkar, et al.[26]: based on inhibition of the formation of nicotine amide adenine dinucleotide, phenazine methosulfate and amino blue tetrazolium formazan. Lipid peroxides were estimated by measurement of thiobarbituric acid reactive substances (TBARs) in plasma by the method of Yagi [27]: the pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde, a secondary product of lipid peroxidation was estimated. The absorbance of clear supernatant was measured against reference blank at 535 nm. Catalase was assayed colorimetrically at 620 nm as described by Sinha[28]: the reaction mixture (1.5 ml) contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of erythrocyte lysate and 0.4 ml of 2 M H_2O_2 . The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3). Reduced glutathione (GSH) was determined by the method of Ellman[29]. 1.0 ml of supernatant was treated with 0.5 ml of Ellmans reagent and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

Statistics

Data obtained from the study were analysed by using the Graph Pad "Instat". Student's t-test was performed to compare between mean of two continuous variables and the "p" value of ≤ 0.001 was considered as extremely significant and "p" value of ≤ 0.05 was considered as statistically significant. The results are expressed as mean ± SD.

RESULTS

Characteristics of included subjects

Table 1 shows the demographic characteristics of the enrolled study patients. The mean age was found to be 56.61±8.17 years in control and 54.65±8.44 years in intervention group. Body mass Index was found to be 5.83±1.99 in control and 6.56±1.91in intervention patients, which was not significant.

Effect of vitamin E supplementation on cognitive function

Table 2 shows the comparison of cognitive status by using MMSE in control and intervention group. Patients who were administered vitamin E supplement along with OHA's showed extremely significant improvement in orientation scores (9.22±0.27), $p\leq 0.001$ and in total MMSE scores (28.00±0.43), $p\leq 0.001$ at the end of three months follow up in comparison with control group.

Effect of vitamin E supplementation on oxidative stress and glycaemic control

Table 3 illustrate the biochemical parameter before and at the end of three months of vitamin E supplementation. The extent of lipid peroxidation (TBARs) was significantly decreased (6.35±0.28), $p\leq 0.05$ in intervention group when compared to control group. The activities of enzymatic antioxidant SOD (2.60±0.05), $p\leq 0.05$& CAT (63.81±3.48), $p\leq 0.05$ and the level of non-enzymatic antioxidant GSH were significantly increased(34.92±1.71), $p\leq 0.05$ in intervention group when compared to control patients. Significant change was also observed in HbA1C level (10.35±0.86), $p\leq 0.05$ in intervention group with respect to control group.

DISCUSSION

The present study evaluated the effect of vitamin E supplementation along with oral hypoglycaemic agents on cognitive function and oxidative stress in patient with type 2 diabetes mellitus. The results of this study suggest that vitamin E supplementation along with OHA's significantly improved the cognitive function and oxidative stress in patient with T2DM.

In this study among the enrolled subjects female population was higher compared to male population and majority of the study populations were coming under age group of 40-50 or above 50 years. In older patients the oxidative damage will also be more due to the destruction of antioxidant defence system. Most of population were non-vegetarian and overweight, since the Nilgiris is situated above 2400 meter; the climatic condition with the low atmospheric temperature affects the life style of the residents. This is the important factor which may affects the basal metabolic rate of the populations, forced to have the carbohydrate rich and non-vegetarian diet which may be one of the epidemic cause for incidence of type 2 diabetes[30,31].

In the present study majority of enrolled patients had less than 5 years of disease duration. The antioxidants supplementation could be able to reserve the damage caused by oxidative stress in the early stage of disease, as oxidative stress also aggravates with the duration of disease[32].

Current study supports the previous published report and demonstrates the potential role of vitamin E against cognitive impairment and oxidative stress in type 2 diabetes mellitus. Cognition impairment induced by T2DM have been previously reported in animal as well as humans[33,34,35]. Some of the previous studies also suggested that use of antioxidants, vitamin E alone[23,36] and combination with vitamin C was associated with a lower risk of cognition impairments, when compared to non users [19,20]. It has also been reported that neuroprotective effect of vitamin E is independent of their antioxidant activity and can modulate apoptosis which intern give beneficial effects[14,37].

It is also well documented that vitamin supplementation like consumption of fruits, vegetables and healthier lifestyle, are likely

associated with higher socioeconomic status[38]. In this study most of the enrolled patients were coming under economically deprived background. Thus, less consumption of vitamins enriched fruits and vegetables, among the patients with T2DM may leads to increase oxidative stress.

The role of oxidative stress in the causation of chronic tissue damage is being increasingly recognized, [10,39] if antioxidant deficiency occurs. The low antioxidant level in type 2diabetes may have resulted from lower intake of antioxidant or increased oxidative stress. So, in this condition the supplementation of antioxidants was found to be beneficial[39].

There were no significant changes with respect to different drug regimen and their combination. Although our results may have some clinical implications, measurement of cognition and antioxidants is not routinely performed in clinical practice, reviewing the intake of food rich in antioxidants particularly citrus fruits and green leafy vegetables and the appropriate antioxidant supplements among patients with the metabolic syndrome may be instructive for the smooth control of diabetes. If their dietary intake of vitamins A, C and E fails to meet the recommended daily allowance, health care professionals should encourage people with the metabolic syndrome to increase their intake of vitamins, preferably through the consumption of healthy food sources rich in these vitamins or otherwise through the use of appropriate vitamin supplements. The future studies may direct towards extended duration of treatment with large number of patients.

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Table 1 DEMOGRAPHIC COMPARISON OF CONTROL AND INTERVENTION GROUP

Variables	Control Group	Intervention group
No. of Patients	36	38
Male/Female	14/22	15/23
Age (years)	56.61±8.17	54.65±8.44 ^{NS}
Smoker	4	4
Alcoholic	5	4
Non Vegetarian	29	31
Duration of disease	5.83±1.99	6.56±1.91 ^{NS}
Body Mass Index (kg/m^2)	25.60 ±3.49	26.52±2.89 ^{NS}

TABLE 2 COMPARISON OF COGNITIVE STATUS BY USING MMSE IN CONTROL AND INTERVENTION GROUP

MMSE Domains	Control group (n=36)		Intervention group (n=38)	
	Baseline	After 3 months	Baseline	After 3 months
Orientation	7.77±0.24	8.72±0.26	7.72±0.38	9.22±0.27***
Registration	3.00±0.00	3.00±0.00	3.00±0.00	3.00±0.00
Attention & calculation	4.11±0.19	4.30±0.18	4.00±0.25	4.61±0.11
Recall	2.77±0.10	2.83±0.09	2.61±0.11	3.00±0.00
Language	7.50±0.20	7.55±0.14	7.50±0.25	7.80±0.12
Copying	0.16±0.09	0.16±0.09	0.27±0.10	0.33±0.11
Total MMSE score	25.3±0.37	26.6±0.54	25.16±0.66	28.00±0.43***

(Data expressed in mean ±SD, *** indicates $p \leq 0.001$)

TABLE 3 COMPARISONS OF BIOCHEMICAL PARAMETERS BETWEEN CONTROL AND INTERVENTION GROUPS AFTER VITAMIN E SUPPLEMENTATION

Parameters	Control group(n=36)		Intervention group(n=38)	
	Baseline	After 3 months	Baseline	After 3 months
TBARs(nmol/ml)	7.25±0.16	7.27±0.20	7.31±0.18	6.35±0.28**
SOD[a](Unit mg /Hb)	2.35±0.09	2.38±0.10	2.36±0.14	2.60±0.05**
CAT[b](Unit mg /Hb)	50.33±4.52	52.05±5.63	48.84±4.03	63.81±3.48**
GSH(mg/dl)	29.72±2.33	30.38±2.74	30.47±2.50	34.92±1.71**
HbA1C (%)	11.71±1.17	10.72±1.26	11.51±1.26	10.35±0.86**

(Data expressed in mean ±SD,** indicates $p \leq 0.05$)

a. One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute.

b. umole of H_2O_2 consumed/minute.

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