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Original Article

Biochemistry Section

Assessment of Vitamin A and Vitamin E Levels in Patients with Controlled and Uncontrolled Type 2 Diabetes Mellitus: A Case-control Study

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ABSTRACT

Introduction: The prevalence of diabetes in India according to the International Diabetes Federation (IDF), Diabetes Atlas 2015 is reported to be 8.7%. Diabetes mellitus is a metabolic disorder, which results from body's insensitivity to insulin and affects humankind at an alarming pose. Glycated Haemoglobin (HbA1c) is an important biomarker in assessing glucose level serologically. If HbA1c level is <7% the diabetes is said to be in controlled conditions. Vitamin A and E plays pivotal role as antioxidants in order to control oxidative stress which is an important contributing factor in diabetes mellitus by neutralising free radicals generated.

Aim: To assess the antioxidants vitamin A and vitamin E levels in controlled and uncontrolled Type 2 Diabetes Mellitus (T2DM) patients and also to correlate the vitamin A and E levels with HbA1c in controlled and uncontrolled T2DM patients.

Materials and Methods: The present case-control study was conducted for 12 months from January 2019 to December 2019 in the Department of Biochemistry, Jawaharlal Nehru Medical College Belgaum, Karnataka, India. The blood samples were collected from KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum, Karnataka, India. A total of 110 subjects were divided into two group's controlled Group 1 (55) and uncontrolled Group 2 diabetes (55) on the basis of HbA1c levels. Vitamin A and E levels were assessed by Enzyme Linked Immunosorbent Assay (ELISA) method. HbA1c was estimated by using Bio-Rad D-10 HbA1c program. The data was assessed using Chi-square test, Independent t-test, and Karl-Pearson corelation test.

Results: There were a total of 29 males and 26 females in controlled T2DM group and a total of 34 males and 21 females in uncontrolled T2DM group. The mean ages in controlled and uncontrolled T2DM subjects were 57.11±8.82 and 54.22±7.93 years respectively. The HbA1c (%), vitamin E and vitamin A in controlled T2DM subjects were 6.01±0.56, 1.01±0.43 mg/dL and 21.66±7.94 µg% respectively. The HbA1c (%), vitamin E and vitamin A in uncontrolled T2DM subjects were 9.31±0.25, 0.58±0.29 mg/dL and 14.66±5.36 µg% respectively. Correlation of vitamin A and E with HbA1c was found to be non significant statistically.

Conclusion: Vitamin A and E levels were comparatively higher in controlled diabetes patients in comparison to uncontrolled T2DM patients.

Keywords: Antioxidant vitamins, Hyperglycaemia, Metabolic disorder, Oxidative stress

INTRODUCTION

India is a storehouse of 69.1 million diabetic people [1]. Diabetes can be defined as the metabolic disorder, which results in body's insensitivity to insulin affecting multiple metabolic and cytological systems [2]. HbA1c indicates three months prior blood glucose level. The diabetic is said to be in control if HbA1c is <7% [3]. If not resolved hyperglycaemia can amount to several complications in the end. These complications include neuropathy (i.e. nerve damage, for e.g. diabetic foot disorders which may require amputation later, nephropathy (kidney failure), retinopathy (i.e. retinal blood vessels getting damaged which may lead to blindness) as well as cardiovascular disorder such as heart attack and stroke [4,5].

Vitamin A is one of most vital and countable micronutrient used by organisms. In human body, it cannot be synthesised metabolically and thus should be obtained through supplementary or dietary measures. Vitamin A along with vitamin E acts as antioxidant with many other minerals and compounds. Antioxidants are not usually accounted in aetiology of T2DM but may help to improve the disorder-associated complications. Origin of free radicals due to stress by oxidative can cause damage to the blood vessels and organs [6]. Studies done by Tsutsumi C et al., [7], Lobo GP et al., [8], Kato M et al., [9], Chertow BS et al., [10], Souza FI et al., [11], Musso G et al., [12], and Chaves GV et al., [13] suggest that retinoid may be contributing factor to hepatic lipid metabolism, synthesis of fat and metabolism of β-cell of pancreas. Even though there are not enough studies to identify precise mechanism by which vitamin A affects metabolic pathways in diabetic patients. Vitamin A reserve should be maintained anyway in subjects of diabetes mellitus and other carbohydrates, lipids and protein related diseases and disorders [14].

By inhibiting lipid-per-oxidation in muscles vitamin E plays one of the most pivotal roles as a potent soluble antioxidant. It decrease HbA1c level inadequate glycaemic control or low serum level of vitamin E [15]. Patients with diabetes do not have vitamin E deficiency in general. In fact, with respect to incidence of diabetes mellitus, plasma and platelet content of vitamin E increases [16,17]. Vitamin E have been associated with decrease rate of cardiovascular disease risk, diabetes complications, certain cancers and cataract disease of the eye hence, it can be concluded that vitamin E is a liposoluble antioxidant which helps in scavenging peroxides radicals, produced during lipid peroxidation of the lipid membrane of cells [18-20].

Few studies [4,21,22] showed high level of Vitamin A and Vitamin E in Diabetes mellitus patients, while [23-25] showed low level of Vitamin A and Vitamin E in diabetes mellitus. Studies by Firoozrai M et al., [26] and Merzouk S et al., [5] showed no significant difference in level of antioxidant vitamins in diabetes mellitus patients compared to controls. Therefore, the present study was undertaken to assess the antioxidants vitamin A and vitamin E levels in controlled and uncontrolled T2DM patients and to study

the correlation of vitamin A and E levels with HbA1c in controlled and uncontrolled T2DM patients.

MATERIALS AND METHODS

The present case-control study was conducted for 12 months from January 2019 to December 2019 in the Department of Biochemistry, Jawaharlal Nehru Medical College Belgaum, Karnataka, India. The blood samples collected from KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum. Ethical clearance obtained from Institutional Ethical Committee (Ethical clearance approval number MDC/DOME/44).

Inclusion criteria: Fifty five patients with T2DM having HbA1c <7%, with the age group 35-70 years were included as cases in the study. Fifty five patients with T2DM having HbA1c >7%, with the age group 35-70 years were included as controls in the study.

Exclusion criteria: Patients with hyperlipidaemia and systemic disease such as kidney disease, neuropathy, cardiovascular disease, liver disease, heart disease were excluded from the study.

Sample size calculation: Sample size was 110 calculated according to formula

$$n=\frac{Z^2_{\alpha/2}\times SD^2}{(P.\ \%\ SD)^2}$$

At 95% confidential Interval, Z2 $\alpha/2=1.96$

A 20% error is estimated, i.e. 80% power taken from previous studies [27,28] and 10% attribution.

Study Procedure

The HbA1c estimation in whole blood was done by using a Bio-Rad D-10 (HbA1c program) using High Performance Liquid Chromatography (HPLC) method based on the principle of ion exchange. The samples were injected into the analytical cartridge after dilution. The D-10 increase ionic strength in cartridge by delivery of buffer. With an absorbance of 415 nm, the separated haemoglobin was passed along the flow cell of the filter photometer [29].

Vitamin A and Vitamin E estimation was done by ELISA method. 50 µL of sample were added per well. Blank well was set without any solution. 50 µL of Horseradish Peroxidase (HRP) conjugate was added to each well (not to blank well), then 50 µL antibody was added to each well. Mixed well and then incubated for 1 hour at 37°C. Each well was aspirated and then washed; the process was repeated two times for a total of three washes. Then each well was washed with wash buffer (200 µL) .After last wash, any remaining wash buffer was removed by aspirating. Then the plate was inverted and blotted against clean paper towels. 50 µL of substrate A and 50 µL of substrate B were added to each well, mixed well and incubated for 15 minutes at 37°C. 50 µL of stop solution was added to each well, and then plate was gently tapped to ensure thorough mixing. Optical density was determined of each well within 10 minutes, using a microplate reader set to 450 nm [30].

Study population divided into two groups depending on value of HbA1C,

- **Group 1:** 55 T2DM patients in controlled group (having HbA1c <6.5-7%).
- **Group 2:** 55 T2DM patients in uncontrolled group (having HbA1c >7%) [31-33].

STATISTICAL ANALYSIS

Data analysis was done by using Statistical Package for the Social Sciences (SPSS) Software version 16.0. Data was analysed using independent t-test within the groups and Karl Pearson Correlation Coefficient in between the groups. The p-value <0.05 was considered statistically significant.

RESULTS

The blood samples of 110 T2DM subjects were collected as per the inclusion and exclusion criteria's in which the group 1 subjects (29 males and 26 female) (n=55) and group 2 subjects (34 males and 21 female) (n=55) were obtained based on HbA1c level. Gender distribution among group 1 and group 2 was statistically non significant (p-value=0.3353) [Table/Fig-1]. The mean ages in group 1 and group 2 T2DM subjects were 57.11±8.82 years and 54.22±7.93 years respectively.

Gender	Group 1 (%)	Group 2 (%)	Total (%)	p-value
Male	29 (52.7)	34 (61.8)	63 (57.3)	0.3353
Female	26 (47.3)	21 (38.2)	47 (42.7)	
Total	55 (100)	55 (100)	110 (100)	

[Table/Fig-1]: Distribution of male and female in Group 1 (controlled) T2DM and Group 2 (uncontrolled) T2DM.
Independent t-test

The subjects were categorised into four age groups [Table/Fig-2]. In a group of 35-40 yrs there were minimum number of subjects and in groups 51-60 years and 61-70 years there were maximum number of subjects. Age distribution among group 1 and group 2 was stastisticaly significant (p-value=0.046) [Table/Fig-2].

Age groups (years)	Group 1 (%)	Group 2 (%)	Total (%)	p-value
35-40	3 (5.5)	1 (1.8)	4 (3.6)	0.046
41-50	8 (14.5)	20 (36.4)	28 (25.5)	
51-60	22 (40)	20 (36.4)	42 (38.2)	
61-70	22 (40)	14 (25.4)	36 (32.7)	
Total	55 (100)	55 (100)	110 (100)	

[Table/Fig-2]: Comparison of age between Group 1 (controlled) T2DM and Group 2 (uncontrolled) T2DM.
Independent t-test

The comparison of HbA1c (%), vitamin E and vitamin A in group 1 and group 2 was statistically significant [Table/Fig-3].

In Group 1 (controlled) type 2 diabetes mellitus subjects, the correlation between HbA1c (%) and vitamin E (mg/dL) was found to be r-value -0.0539 [Table/Fig-4] and it was not statistically significant (p value-0.6961). Correlation between HbA1c, and vitamin A (µg %) was found to be r-value -0.0812 which was not statistically significant (p value-0.5557) [Table/Fig-5].

Variables	Group 1 (Controlled T2DM)	Group 2 (Uncontrolled T2DM)	t-value	p-value
HbA1c (%)	6.01±0.56	9.31±0.25	12.76	<0.0001*
Vitamin E (mg/dL)	1.01±0.43	0.58±0.29	6.17	<0.0001*
Vitamin A (µg%)	21.66±7.94	14.66±5.36	5.42	<0.0001*

[Table/Fig-3]: The Glycated haemoglobin HbA1c (%), vitamin A (µg %) and vitamin E (mg/dL) in Group 1 (controlled) and Group 2 (uncontrolled) T2DM.
Independent t-test

Groups	Parameters	N	r-value	p-value
Group 1	HbA1c and vitamin E	55	-0.0539	0.6961
Group 2	HbA1c and vitamin E	55	-0.0635	0.6451

[Table/Fig-4]: Correlation between Glycated haemoglobin HbA1c (%) and vitamin E (mg/dL) in Group 1 (controlled) T2DM and Group 2 (uncontrolled) T2DM.
(Karl Pearson's correlation coefficient method; p<0.05 was considered statically significant)

Groups	Parameters	N	r-value	p-value
Group 1	HbA1c and vitamin A	55	-0.0812	0.5557
Group 2	HbA1c and vitamin A	55	-0.0084	0.9513

[Table/Fig-5]: Correlation between Glycated haemoglobin HbA1c (%) and vitamin A (µg %) in in Group 1 (controlled) T2DM and Group 2 (uncontrolled) T2DM.
(Karl Pearson's correlation coefficient method; p<0.05 was considered statically significant)

In Group 2 (uncontrolled) type 2 diabetes mellitus subjects, the correlation between HbA1c (%) and vitamin E (mg/dL) was found to

be r-value -0.0635 [Table/Fig-4] and it was not statistically significant (p value- 0.6451). Correlation between HbA1c and vitamin A (µg %) was found to be r-value -0.0084 and it was not statistically significant (p value- 0.9513) [Table/Fig-5].

DISCUSSION

The main need of diabetic patients is to attain normal blood glucose. In the present study, authors have compared the levels of HbA1c, vitamin A and E in controlled and uncontrolled T2DM. Previous studies conducted by, Abahusain MA et al., [23]. Firoozrai M et al., [26], Reunanen A et al., [34] and Onyesom I and Agho JE [35] have estimated vitamin A and vitamin E in diabetic groups and compared with normal groups. But in the present study authors divided DM patients like controlled and uncontrolled depending on level of HbA1c. As per the present study findings it was observed that the levels of vitamin E and vitamin A in controlled T2DM were in normal interval when compared with uncontrolled T2DM.

Present study showed that vitamin E levels were significantly lower in uncontrolled diabetes mellitus subjects as compared to the patient of controlled diabetes mellitus with t-value 6.17 and p-value <0.0001. The study done by Odum EP et al., [24], Alamdari MI [36] Ahmad M et al., [37], Veerabhadra GG et al., [38], Sawant J et al., [39], showed that vitamin E level in diabetic patients was found to be less as compared to controlled individuals

In diabetes mellitus, free radicals play a very important role which results in the development of inflammation and oxidative stress. Scavenging action of vitamin E helps to overcome the oxidative stress. Vitamin E interrupts the chain reaction of lipid peroxidation by interacting with lipid peroxy radicals. Thus it is found to protect cells against oxidative damage. Hence they concluded that decrease in vitamin E levels could be the cause of its excess utilisation [40].

In contradicting to present results, few studies by Tavridou A et al., [41], Kimble MS et al., [42], Murrill WA et al., [43] showed that there was no difference in serum vitamin E concentrations between the groups. Present study showed that vitamin A levels were significantly lower in uncontrolled diabetes patients as compared with controlled diabetes mellitus with t value 5.42 and p-value <0.0001. The study done by Aliyu M et al., [2], Erikstrup C et al., [25], Oneysom and Agho JE [35], showed vitamin A level in diabetic patients was less than control group. The reason for low vitamin A level could be due to excess utilisation which results in reducing oxidative stress [44].

In contradicting to present study results few studies done by MA abahusain et al., [23] , Basualdo C G et al., [45] and Peerapatdit T et al., [46] they showed that there was no difference in vitamin A level in diabetic individuals and control group (p<0.001). Previous studies like Abahusain MA et al., [23], Onyesom I and Agho JE [35] and Firoozrai M et al., [26] had correlated HbA1c, vitamin A and vitamin E with each other in diabetic subjects as one group, but in the present study, authors have correlated HbA1c, vitamin A and vitamin E with each other in controlled and uncontrolled T2DM subjects separately. Firstly the correlation in Group 1controlled T2DM subjects was performed for vitamin E and HbA1c it showed insignificant results (r=-0.0539; p=0.6961). Then HbA1c and vitamin A were correlated and the results were insignificant (r=-0.0812; p=0.5557). The same was done in group 2 uncontrolled T2DM subjects and the correlation between HbA1c and vitamin E was insignificant (r=-0.0635; p=0.6451). Then HbA1c and vitamin A were correlated and the results were insignificant (r=-0.0084; p=0.9513). There was a weak and not significant negative correlation observed between two parameters in controlled T2DM and uncontrolled groups. Comparison of finding of present study with contrast studies is shown in [Table/Fig-6] [2,5,23,24,26,35,36,38,46].

In DM there is increased polyol pathways, increased formation of glyacation end product, activation of protein kinase C and increased hexoseamine pathway. All these increases superoxide formation, that leads to increase oxidative stress in DM [4]. Formation of free radicals, causing oxidative stress and tissue damage is mainly due to non enzymatic glycation between glucose and amino group of protein [23,47].

According to the present study authors observed low levels of vitamin A and E in Group 2 (uncontrolled) T2DM subjects than Group 1 (controlled) T2DM which may be due to excess utilisation vitamins A and E, it may be due to increased oxidative stress in uncontrolled T2DM subjects compared to controlled T2DM subjects. The other reason could be due to adequate intake of vitamins and consumption of medicine, which has good control on oxidative stress by controlled T2DM.

Authors name, Ref no. and publication year	Place of study	Study design	Sample size	Parameters assessed	Correlation between parameters
Alamdari MI et al., [36], 2018	Ardabil, Iran	A descriptive and cross-sectional study	186 (97 controlled and uncontrolled 89)	HbA1c ,Vitamin E and lipid profile	No significant difference in vitamin E levels between diabetic controlled patients and uncontrolled patients
Aliyu M et al., [2], 2005	Sokoto, Nigeria	Case control study	300 (150 non insulin dependent diabetes mellitus cases and 150 healthy non diabetic controls)	Vitamins A, E and C	Vitamins A, E and C levels were significantly lower in diabetic patients. They showed the significantly negative correlation between serum glucose and each vitamin A ,E and C in diabetic subjects.
Merzouk S et al., [5], 2003	Dijon, France	Case control study	102 (40 type I, 42 type II diabetic patients and 20 controls)	Vitamins A, E and C, Catalase, Superoxide dismutase, glutathione peroxidase and Glutathione reductase	No significant difference between control and diabetes subjects in relation to vitamin C whereas; there was a significant decrease in the levels of vitamin A and vitamin E in diabetic patients compared to controls.
Abahusain MA et al., [23] ,1999	Riyadh, Saudi Arabia	Case-control study	250 (107 type 2 diabetes mellitus and 143 controls)	Retinol, alpha-carotene, and alpha-tocopherol	No significant difference in both groups in the mean concentrations of retinol, α-carotene and α- tocopherol.
Odum EP et al., [24], 2012	Port Harcourt, Nigeria	Case-control study	105 (55 type 2 diabetes mellitus and 50 healthy individuals)	Glucose, total antioxidant status (TAS), Vitamin E and C	Statistically significant decrease in Total Antioxidant Status (TAS), ascorbic acid and vitamin A in T2DM compared to control . In diabetics cases TAS had a positive correlation with vitamin E but no correlation with vitamin C
Firoozrai M et al., [26], 2006	Tehran, Iran	Case control study	100 (62 type 2 diabetes mellitus cases and 38 controls)	Vitamin A and vitamin E	No significant difference in the level of vitamin A and E in diabetes mellitus compared to health controls
Onyesom I and Agho JE [35], 2011	Nigeria	Case-control study	100 (50 type 2 diabetes mellitus and 50 healthy individuals)	Vitamin A, C and E	They found that statistically significant decrease in the level of Vitamin A, C and E in diabetes mellitus cases compared to controls.
Veerbadragowda GG et al., [38], 2016	Bangalore India	Case-control study	40 (20 controls and 20 Type 2 Diabetes patients)	Glucose, lipid profile, Vitamin E, Malondialdehyde and Ceruloplasmin	Statistically significant decrease in the level of vitamin E in cases than compared to controls.

Peerapatdit T et al., [46], 2006	Bangkok Thailand	Case-control study	75 (9 subjects with poorly controlled type 2 DM (fasting plasma glucose (FPG) >180 mg/dL), 26 subjects with fairly controlled type 2 DM (FPG < or = 180 mg/dL), and 20 subjects with type 2 DM complicated coronary heart disease (CHD). 20 healthy subjects with normal plasma glucose level (FPG <110 mg/dL) as a control group)	MDA, vitamin A, C, E and beta-carotene	Plasma antioxidant vitamin C and E significantly lower only in poorly controlled and CHD complicated type 2 DM, no significant differences in both plasma vitamin A and beta-carotene were noted between any types of DM and age-matched normal healthy group.
Present study, 2022	Belgaum, India	Case- control study	110 (55 controlled and 55 uncontrolled Type 2 Diabetes mellitus)	HbA1c, Vitamin E and Vitamin A	Vitamin A and E levels were significantly lower in uncontrolled diabetes mellitus in comparison to controlled type 2 Diabetes mellitus. The correlation of vitamin A and E with HbA1c was found to be non significant in both controlled and uncontrolled type 2 diabetes mellitus.

[Table/Fig-6]: Comparison of present study with contrast studies [2,5,23,24,26,35,36,38,46].

Limitation(s)

Measurement of other antioxidant levels may yield more meaningful data on the role of the antioxidant system in the clinical course of type 2 DM. Further follow-up studies with vitamin A and E supplementation is needed.

CONCLUSION(S)

Present study concludes that antioxidant vitamins A and E were low in uncontrolled diabetic patients group compared to controlled type 2 diabetes group. Physician should advice uncontrolled diabetic patients to consume adequate vitamin A and E in their diet along with medication which helps them to have appropriate amount of vitamin A and E to control diabetes and prevent complications. Further studies are required to study the beneficial role of antioxidant vitamins supplementation in diabetes mellitus patients.

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