

 <p>ISSN NO. 2320-5407</p>	<p>Journal Homepage: -www.journalijar.com</p> <p>INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)</p> <p>Article DOI:10.21474/IJAR01/13276 DOI URL: http://dx.doi.org/10.21474/IJAR01/13276</p>	
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RESEARCH ARTICLE

A COMPARATIVE STUDY OF SERUM ASCORBATE BETWEEN NEWLY DIAGNOSED TYPE 2 DIABETICS AND LONG STANDING TYPE 2 DIABETICS ON TREATMENT

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Manuscript Info

Manuscript History

Received: 15 June 2021
Final Accepted: 19 July 2021
Published: August 2021

Key words:-

Vitamin C, Diabetes Mellitus, Glycated Hemoglobin and Vitamin C

Abstract

Introduction: diabetes mellitus is a metabolic disorder prevalent worldwide. Oxidative stress has been the cause of complications which arises gradually due to prolonged hyperglycemia. Vitamin C being a potent antioxidant is well consumed in diabetes mellitus.

Methods: Serum samples of diabetes mellitus type 2 patients from Jorhat Medical College of Assam were tested to estimate ascorbate levels using spectrophotometer. Whole blood samples were tested to estimate glycated hemoglobin levels in d-10 biorad hplc machine. A total of 58 patients and equal numbers of age and sex matched apparently healthy controls were included.

Results: significantly lower levels of ascorbate were recorded in diabetic cases compared to controls. Newly diagnosed cases had lesser serum ascorbate levels compared to patients who were on treatment with oral hypoglycemic drugs and insulin.

Conclusion: the study was done to look for any alteration in serum ascorbic acid levels in diabetes mellitus patients. Patients newly diagnosed had lesser vitamin C levels compared to patients already on medications. It is found that vitamin C is significantly lower in diabetic patients with or without medications compared to healthy controls.

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Introduction:-

According to various studies done worldwide it is evident that there is generation of free radicals in diabetes mellitus and hence damages caused by them eventually leads to its complications. Free radicals arise due to high plasma glucose autooxidation, non enzymatic glycation of proteins and polyol pathway. Antioxidants play a very important role in controlling free radicals and reduce oxidative stress. Ascorbate levels in plasma are reported to be depleted in diabetes mellitus patients [1,11,21]. Vitamin C is a potent antioxidant in humans and protects tissues from free radical damage [2,3]. Ascorbate is proven to be the most effective aqueous-phase physiological antioxidant in human blood plasma and protects against diseases and degenerative processes caused by oxidant stress [4]. Reduced levels of ascorbic acid in diabetic patients may easily aggravate oxidative stress and complications [5]. Quenching of antioxidants is due to increase in generation of free radicals in diabetes mellitus [6]. Vitamin C is an aldose reductase inhibitor and therefore keeps a check on excess sorbitol formation due to prolonged hyperglycemia [7]. Vitamin C is required in biosynthesis of nitric oxide, an important and potent vasodilator [8] and fat metabolism.

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Materials And Methods:-

Patient selection: The present study comprised of 58 numbers of diagnosed cases of diabetes mellitus and equal number of age and sex matched apparently healthy controls. The cases were divided into three groups. From and above 30 years to 40 years patients were newly diagnosed and did not take any oral hypoglycemic agents or insulin. Above 40 years to 50 years patients were diagnosed with type 2 diabetes mellitus within 6 months to 8 months from the date of specimen collection and were on oral medications only. Some of the patients were not on regular medications as they skipped drugs sometimes in this age group. Above 50 years to 60 years the patients suffered from type 2 diabetes mellitus for more than 1 year and were on both oral and insulin therapy.

Patient selection were done by purposive sampling technique and physical examination. Appropriate inclusion and exclusion criteria was followed to enroll the patients in this study. Exclusion criteria: hemoglobinopathies, acute blood loss, anemia, acute kidney injury, chronic renal failure, chronic alcoholic, physical stress, smoking, COPD, patients receiving steroids, high risk obesity, pregnant woman, pancreatitis, cancer patients and tuberculosis patients.

Sample collection and estimation: Blood samples for serum vitamin C and HbA1C were collected by following aseptic and antiseptic measures. Samples for vitamin C were centrifuged and serum separated, and protected from light and stored at -20 degree celcius. Samples for HbA1c were estimated on the same day of sample collection. Vitamin C was estimated in spectrophotometer (Genesys-UV-Vis nanodrops) - OD value measured at 536nm. HbA1c was estimated in D-10 HPLC Biorad machine.

Statistics:

Analysis of data was done using Microsoft Excel. P value was calculated with the help of unpaired student t test. P value for correlation was calculated by using sossicstatistics.com.

Results:-

Table 1:- Comparison of parameters between total number of cases and controls (unpaired student's t test).

Parameters	Diabetic patients	Controls	p value
Glycated hemoglobin (%)	10.88 ± 2.82	5.01 ± 0.53	p<0.0001
Ascorbate (µg/ml)	9.13 ± 4.08	25.91 ± 2.31	p<0.0001

The levels of biochemical parameters are expressed as mean + sd.

Table 2:- Agewise comparison of parameters between cases and controls with and without treatment (unpaired student's t test).

SERUM ASCORBATE (µg/ml)				GLYCATED HEMOGLOBIN (%)		
AGE (years)	CASES(µg/ml)	CONTROLS(µg/ml)	p VALUE	CASES(%)	CONTROLS(%)	p VALUE
Not on Oral Hypoglycaemics and insulin , 30 to 40 years.				Not on Oral Hypoglycaemics and insulin, 30 to 40 years.		
30 – 40 years	7.19±1.31 (µg/ml)	28.61 ± 0.71 (µg/ml)	p<0.0001	11.38 ± 3.02 (%)	4.73 ±0.25 (%)	P<0.0001
Only on Oral Hypoglycaemics and some with irregularity, 40 to 50 years.				Only on Oral hypoglycaemics and some with irregHlarity, 40 to 50 years.		
40 – 50 years	9.22 ± 3.58 (µg/ml)	26.89 ± 1.54 (µg/ml)	p<0.0001	11.90 ±3.05 (%)	5.05 ± 0.60 (%)	p<0.0001
On Insulin therapy and Oral Hypoglycaemics, 50 to 60 years.				On Insulin therapy and Oral Hypoglycaemics, 50 to 60 years.		
50 – 60 years	9.69 ± 5.0 (µg/ml)	23.96 ± 1.72 (µg/ml)	p<0.0001	9.61 ± 1.96 (%)	5.06 ± 0.52 (%)	p<0.0001

The levels of biochemical parameters are expressed as mean+sd.

Discussion:-

In this present study patients were allowed to eat their normal diet before giving blood samples. Cases comprised of both newly diagnosed, and long standing diabetes mellitus.

The increase of HbA1C levels in diabetic patients were significantly higher than controls ($p < 0.0001$) and levels of vitamin C levels in diabetic patients were significantly lower than controls ($p < 0.0001$), (table 1).

In all age groups we observed significant decrease of vitamin C levels in diabetic patients compared to apparently healthy controls. In the 30 to 40 age groups newly diagnosed patients were compared to other age groups who received treatment. In the 40 to 50 age group who received only oral hypoglycaemic agents, few patients were irregular with their medications. Cases in 50 to 60 age group were on insulin and oral hypoglycaemic agents, (table 2). However we did not found a significant lower values of vitamin C in newly diagnosed patients compared to patients receiving treatment.

This is in accordance to the scientific findings of the previous studies that vitamin C acts as an antioxidant in diabetes mellitus [9]. The study done by Will JC, got significant lower levels of serum ascorbate in newly diagnosed diabetes patients compared to controls [10]. This findings are similar to the findings recorded by Sargeant LA et al [11]. Ali SK and Chakraborty SK recorded significant lower values of serum ascorbate in diabetic retinopathy compared to controls irrespective of treatment [12]. Another study with similar significant findings were recorded by Sundaram RK et al where they found decrease in vitamin C levels in patients with complications of higher magnitude and duration compared to healthy controls [1]. Gupta M and Chari S recorded significant lower vitamin C in diabetics with ischaemic heart disease compared to diabetics without complications receiving treatment [13]. Study done by Ganesh N. Dakhale et al obtained significant increase in ascorbate levels and significant decrease of HbA1c levels in patients receiving combination of metformin and vitamin C when compared to patients receiving metformin with placebo [14], however Merzouk et al [15] did not get a difference between vitamin C levels in diabetics and controls.

It is evident that vitamin C is well consumed against reactive oxygen species in diabetes mellitus patients. ROS develops due to advanced glycated end products, glucose autooxidation, polyol pathways [16]. Studies suggests that Vitamin C metabolism may be altered in diabetes mellitus [17,18] and hyperglycemia decreases active transport of vitamin C [19]. Vitamin C is similar to glucose in structure and hence it can replace glucose in various chemical reactions and thus is effective in prevention of non-enzymatic glycation of proteins [20].

Conclusion:-

In this present study a significant decrease of vitamin C levels is observed in diabetes mellitus patients compared to their counterpart controls across all age groups in newly diagnosed cases as well as patients on treatment. Vitamin C may play role in controlling complications arisen due to chronic hyperglycemia. As vitamin C is reduced significantly in patients, supplementation with ascorbic acid may benefit the chronically ill patients and may reduce disease burden having that vitamin C is a potent antioxidant available in market at reasonable price. Further studies are required for better understanding the disease progression because there is claimed to have rise of ROS yet to be proven apart from above mentioned pathways. Awareness can be created for better management of this chronic illness and its complications.

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