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Serum Ascorbic Acid Level In Type 2 Diabetes Mellitus

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Abstract:
Introduction: *Ascorbic acid (Vitamin C) is an important scavenger of oxygen derived free radicals, structurally similar to glucose, which can replace it in many chemical reactions and thus is effective for prevention of nonenzymatic glycosylation of protein and helps in the regeneration of α-tocopherol and prevents LDL oxidation thereby reducing cardiovascular risk.*
Aims and Objectives: *To estimate Haemoglobin A1c (HbA1c), Triglycerides (TG) and Vitamin C levels in type 2 diabetes mellitus patients and compare it with that of healthy individuals.*
Materials and methods: *A cross sectional study done in the department of Biochemistry in collaboration with Department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur from September 2011 to August 2013 included eighty confirmed cases of type 2 diabetes mellitus (T2DM) patients and forty age and sex matched healthy controls. Vitamin C and Triglyceride levels were estimated colorimetrically while HbA1c was measured by Fast Ion Exchange Resin Separation Method.*
Results: *The highest level of Vitamin C was that of control group (1.00±0.37, p <0.001) followed by cases without complication group (0.99±0.41, p<0.001) and cases with complication group (0.67±0.27, p<0.001). Values of HbA1c and TG were found to be highest for diabetes with complications. Post Hoc test for better understanding of multiple comparison showed difference between control and diabetes with complications and was significant.*
Conclusion: *The decreased level of vitamin c found in the present study could be used for screening and diagnosis of diabetes mellitus. However, this observation requires further study.*
Keywords: *Haemoglobin A1c, Triglyceride, Vitamin C.*

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I. Introduction

Diabetes mellitus is one of the most common metabolic disorder. Due to altered insulin secretion or action, there is altered metabolism of carbohydrates and lipids. Hyperglycemia and lipid abnormalities are features of Diabetes mellitus (DM). Hyperglycemia is a recognized pathologic factor of long term complications in diabetes mellitus. It does not only generate reactive oxygen species, but also alters antioxidant mechanism, creating a state of oxidative stress [1]. The hemoglobin A1c (HbA1c) assay provides a reliable measure of chronic glycemia and correlates well with the risk of long-term diabetes complications, so that it is currently considered the test of choice for monitoring and chronic management of diabetes mellitus [2]. It is believed that one of the mechanisms responsible for secondary complications of diabetes involves non-enzymatic glycosylation of proteins by glucose auto-oxidation [3]. Non-enzymatic glycosylation is a spontaneous chemical reaction between glucose and the amino groups of protein resulting in the formation of reversible schiff's bases and Amadon products. These products have been reported to generate free radicals, causing oxidative stress and tissue damage [4].Ascorbic acid (Vitamin C) is known for its beneficial effects on serum lipids and glycated haemoglobin (HbA1c). Vitamin C is an important antioxidant in human [5], capable of scavenging oxygen-derived free radicals [6]. Several investigators have implicated the role of free radical mediated pathology in diabetes mellitus [7,8]. Previous study has shown decrease in basal vitamin C level in type 2 DM [9] and is

structurally similar to glucose replacing it in many chemical reactions proving effective for prevention of nonenzymatic glycosylation of proteins [10].

The main quantitative abnormalities of lipids in diabetes are increased triglyceride (TG) levels, related to an augmented hepatic production of VLDL and a reduction of both VLDL and IDL catabolism, and decreased HDL-Cholesterol levels due to an accelerated HDL catabolism. The main qualitative abnormalities include large VLDL particles (VLDL1), relatively rich in TG, small dense LDL particles, increase in TG content of LDL and HDL, glycation of apolipoproteins and increased susceptibility of LDL to oxidation. Moreover, although plasma LDL-cholesterol level is usually normal in type 2 diabetic patients, LDL particles show significant kinetic abnormalities, such as reduced turn-over, which is potentially harmful [11]. LDL particles are small and dense in type 2 diabetes and are susceptible to oxidation. α-tocopherol is a lipid soluble antioxidant and protects LDL particles from oxidative attack. Vitamin C is required for regeneration of α-tocopherol and may thus prevent LDL oxidation [12]. The study was conducted to estimate serum Glycated haemoglobin (HbA1c), Triglyceride (TG) and Ascorbic acid (Vitamin C) levels in type 2 diabetes mellitus patients with and without micro-vascular complication and to compare with that of healthy individuals. And also, to identify the inter-relationship among these components with the help of correlation matrix in healthy controls, type 2 diabetes mellitus with and without microvascular complications.

II. Materials and methods

A cross sectional study was carried out in the Department of Biochemistry in collaboration with Department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur from September 2011 to August 2013. The study group comprised of eighty randomly selected cases of confirmed T2DM patients diagnosed for more than one year among patients coming from different areas of Manipur and attending diabetic clinic and/or admitted in the Medicine ward, irrespective of sex, religion and socio-economic status. Cases were divided into two groups: (i) Forty confirmed type 2 diabetes mellitus patients without complication who were under treatment with insulin or oral hypoglycemic agents and/or diabetic diet. (ii) Forty confirmed T2DM cases with microvascular complications like nephropathy, retinopathy or neuropathy etc under treatment. Forty age and sex matched apparently healthy individuals were selected as controls.All cases and controls were aged 18 years and above. Each individual enrolled in study underwent a detailed history, clinical examination and laboratory examination designed for the study. Type 2 DM patients with and without complication were diagnosed on the basis of history, physical examination, biochemical investigations and according to revised criteria for diagnosing DM issued by consensus panel expert from the National Diabetes Group and World Health Organization.

Patients suffering from carcinoma, any chronic systemic disease, smokers, alcoholics, pregnant lady, lactating mothers, with history of acute infections and thyroid dysfunction were excluded from the study. Study was approved by the Institutional Ethics Committee. Informed consent was obtained from all individuals and nature of the study was explained to them.Five ml of blood was collected from each individual after 12 hours overnight fasting. Four ml was collected in sterile plain vial for estimation of ascorbic acid (vitamin C) and triglyceride (TG). One ml was collected in EDTA vial for estimation of glycosylated hemoglobin (HbA1c). It was processed within 1 hour of collection.Serum HbA1c was estimated using commercially available HbA1c kit according to Fast Ion Exchange Resin Separation Method described by Goldstein DE, Little RA and Widdmayer HM [13]. Quantitative estimation of serum triglycerides was done by enzymatic colorimetric test for TG with Lipid Clearing Factors (LCF) as described by Bucolo G and Harold D [14]. Ascorbic acid was estimated by colorimetric method using commercially available kit. Statistical analysis was performed using SPSS version 16. Data were expressed in Mean ± SD. Statistical tests like $\chi 2$-test, independent t-test, ANOVA (F-test) and correlation coefficient 'r' were applied whenever found suitable and necessary. The P-value less than 0.05 was considered significant.

III. Results

Table 1: Group-wise distribution of HbA1c

HbA1c (%)	Types of group					
	Control (no.)	(%)	Case with complication	%	Case without complication	%
< 3.5	5	12.5	-	-	-	-
3.6 – 4.5	9	22.5	-	-	3	7.5
4.6 – 5.5	14	35	2	5	17	42.5
5.6 – 6.5	12	30	1	2.5	19	47.5
6.6 – 7.5	-	-	22	55	1	2.5
7.6 – 8.5	-	-	4	10	-	-
8.6 – 9.5	-	-	1	2.5	-	-
10.6 – 11.5	-	-	4	10	-	-
11.6 – 12.5	-	-	6	15	-	-
Total	40	100	40	100	40	100

Table 1 shows distribution of HbA1c according to groups. None of the controls have HbA1c level above 6.5%, majority (35%) of the controls have HbA1c level of 4.6 – 5.5 %, whereas majority (47.5%) of diabetics without complications have HbA1c level in the range of 5.6 – 6.5 %, and majority (55%) of diabetics with complications have HbA1c level in the range of 6.6 – 7.5%.

Table 2: Group-wise distribution of Triglycerides

TG (mg%)	Types of group					
	Control (no.)	(%)	Case with complication	%	Case without complication	%
< 120	1	2.5	-	-	-	-
121 – 130	27	67.5	-	-	-	-
131 – 140	12	30	7	17.5	12	30
141 – 150	-	-	21	52.5	17	42.5
151 – 160	-	-	12	30	11	27.5
Total	40	100	40	100	40	100

The levels of TG were divided into five groups as shown in Table 2. It is seen that majority (67.5%) of the control group have TG level in the range of 121-130 mg%, whereas majority of diabetes groups viz., diabetes with complications (52.5%) and diabetes without complications (42.5%) have TG level in the range of 141 – 150 mg%.

Table 3: Group wise distribution of Vitamin C

Vitamin C in mg%	Types of group					
	controls	%	Cases with complications	%	Cases without complications	%
≤0.50	0	-	0	-	0	-
0.51-1.00	14	35	34	85	18	45
1.01-1.50	6	15	6	15	22	55
≥ 1.50	20	50	0	-	0	-
Total	40	100	40	100	40	100

Vitamin C level is also clubbed into four categories as shown in the above table. It is observed that none of the groups have vitamin C level below 0.50mg%. It is also evident that majority of the individuals in the control group (50%) have vitamin C level in the range of ≥ 1.50 mg%. Majority of the diabetes without complications (55%) have vitamin C level in the range of 1.01–1.50 mg%, whereas in the diabetes with complications, majority (85%) have vitamin C level in the range of 0.51–1.00 mg%. The variations observed here could not be tested for statistical significance as most of the cell frequencies are either nil or very few.

Table 4: Group-wise mean± SD values of Vitamin C, Glycosylated Hb (HbA1c) and Triglycerides (TG)

	Glycosylated Hb	Triglyceride	Vit C
	Mean ± SD	Mean ± SD	Mean ± SD
Control	5.04 ± 0.88	131.85 ± 7.09	1.00 ± 0.37
Cases with complication	8.11 ± 2.12	159.62 ± 8.69	0.67 ± 0.27
Cases without complication	5.52 ± 0.52	154.10 ± 6.72	0.99 ± 0.41
P-value	< 0.001	< 0.001	< 0.001

Table 4 shows distribution of mean and standard deviation of HbA1c, TG and Vitamin C among the three study groups. Values of HbA1c and TG were found to be highest for diabetes with complications followed by diabetes without complication. These values were lowest for controls. Whereas, for vitamin C, control group has highest level followed by diabetes without complication and lowest level in diabetes with complications. The variation of mean for HbA1c, TG and vitamin C was found to be highly significant ($p < 0.001$).

Table 5: Pair-wise (group) comparision of mean of Vitamin C, Glycosylated Hb (HbA1c) and Triglycerides (TG)

Variable	(I)	(II)	Mean difference* (I-II)	p-value
HbA1c	Control	Case with complication	-3.07000	0.001
		Case without complication	-0.47250	0.373
	Case with complication	Case without complication	2.59750	0.001
Triglyceride	Control	Case with complication	-21.77500	0.001
		Case without complication	-22.25000	0.001
	Case with complication	Case without complication	-0.47500	1.000
Vit C	Control	Case with complication	0.33475	0.001
		Case without complication	0.01400	1.000
	Case with complication	Case without complication	-0.32075	0.001

In order to evaluate better understanding of multiple comparison Post Hoc test was done and the findings are shown in Table 5. It is observed that the paired combinations for HbA1c is found to be statistically significant between control and diabetes with complications whereas it is not significant between control and diabetes without complications. In case of TG the paired combinations between control and the diabetes is found to be significant, however it is found to be insignificant between the two groups of diabetes i.e. diabetes with complications and diabetes without complications. For vitamin C this difference was found to be statistically significant between control and diabetes with complications whereas insignificant between control and diabetes without complications.

IV. Discussion

The diabetes complication and control trial (DCCT) established HbA1c as the gold standard of glycemic control. The level of HbA1c value $\leq 6.5\%$ was said to be appropriate for reducing the risk of diabetes complications. In the present study the mean ± SD of HbA1c was 5.04 ± 0.88 in controls, 5.52 ± 0.52 in diabetes without complications and 8.11 ± 2.12 in diabetes with complications and the differences were highly significant statistically. Gabbay KH et al [15] also found that glycosylated hemoglobin concentration was elevated as much as two fold in diabetics and decreased with improvement of glycemic control. Higher glucose concentrations and increased glycosylated hemoglobin might accelerate atherosclerotic process through several plausible mechanisms such as oxidative stress and protein glycation of vessel wallsIn the present study triglyceride levels were increased in diabetic groups, both with complications and without complications, as compared to control subjects. This finding was in agreement with the findings of Ali A et al [16], Taylor R et al [17], Kumar V et al [18] and Madhu SV et al [19].In our study, Vitamin C levels in diabetic patients both with complication and without complications were found to be decreased than controls. Som S et al [20] observed low plasma ascorbic acid level in diabetic patients. A similar finding was observed by Banerjee A [21]. It has been proposed that in diabetic patients, several abnormalities related with absorption develop in the absence of antioxidant vitamins [22]. A substantial body of literature suggests that Vitamin C metabolism is altered in diabetes mellitus [23,24]. The active transport of ascorbic acid also appears to be decreased by hypoglycemia [25] and insulin deficiency [26]. Vitamin C is an important antioxidant in humans [27] capable of scavenging oxygen–derived free radicals [6]. Vitamin C is structurally similar to glucose and can replace it in many chemical reactions, and thus is effective in prevention of non-enzymatic glycosylation of proteins [28].Highly significant test values for HbA1c and TG indicate that HbA1c level as well as TG values were significantly varied over the three groups in the present study. For instance, highest HbA1c and TG levels pertain to diabetes with complications. There were significantly low values of vitamin C. Increased oxidative stress in diabetes could contribute to depletion of antioxidant such as vitamin C [29,30].

Vitamin C has been shown to reduce protein glycosylation and act as scavengers of free radicals. The deficiency of this vitamin has been implicated in the development of late complications of diabetes mellitus, such as cataract, nephropathy and neuropathy.

Absolute or relative deficiency of antioxidant defense may lead to a situation of increased oxidative stress especially in case of obesity, dyslipidemia and insulin resistance. And this may be associated with both cause and consequences of a variety of disorders [31].It was observed that the paired combinations for HbA1c and Vitamin C were found to be statistically significant between controls and diabetes with complications whereas it was not significant between controls and diabetes without complications. And in case of TG, the paired combinations between controls and the diabetes is found to be significant, also it was found to be statistically significant between the two groups of diabetes that is, diabetics with complications and diabetics without complications.Our study supported the hypothesis that hyperglycemia activates cellular and tissue injury by oxidative stress but the compensatory mechanism for defense against the reactive oxygen species (ROS) to normalize oxidative stress is not achieved in the diabetic patients. Moreover this oxidative stress is increased with poor glycemic control. As a result, patient with hyperglycemia and oxidative stress present a high risk for development of diabetic complications and need early intervention in type 2 diabetes mellitus.

V. Conclusion

In the present study the mean values of blood HbA1c and TGs were found to be higher in the diabetic patients- either with complications or without complications than controls whereas the mean values of Vitamin C levels were lower in diabetic patients- both with complications and without complications, than controls. The present study showed a highly significant difference in the levels of HbA1c between the diabetics and the controls. Also a significant difference was observed between the two groups of diabetes i.e. diabetes with complications and diabetes without complications.From the present study it may be concluded that altered levels of vitamin C may have a role in the pathogenesis and progression of T2DM. The decreased blood levels of vitamin C as has been found in the present study could be utilized for the screening and diagnosis of diabetes mellitus. However this observation requires further study. Because of the central role of vitamin C in the antioxidant defense system of our body, it is suggested that an adequate supply of these substances in the diet of diabetic patients could be beneficial in the long term management of diabetic patients, and further studies in this field are recommended.

References

[1]. P.A. Mayes. Lipid of physiological significance, in R.K. Murray, D.K. Grammer, P.A. Mayes , VW Rodwel (Ed.), Harpers Biochemistry, 24 (United States: Appleton and Lange, 1996) 147-157.

[2]. [G. Lippi, G. Targher, Glycated hemoglobin (HbA1c): old dogmas, a new perspective? Clinical Chemistry, 48(5), 2010, 609-614.

[3]. M.A. Catherwood, L.A. Powell, P. Anderson, D. McMaster, P.C. Sharpe, and E.R. Trimble, Glucose-induced oxidative stress in mesangial cells, Kidney International, 61, 2002, 599-608.

[4]. A. Cariello, A. Quatraro, and D. Giugliano, New insight on non enzymatic glycosylation may lead to therapeutic approaches for the prevention of diabetic complications, Diabetic Medicine, 9(3), 1992, 297-299.

[5]. S.J. Padayatty, A. Katz, Y. Wang, P. Eck, O. Kwon, J.H. Lee, et al, Vitamin C as an antioxidant: evaluation of its role in disease prevention, Journal of the American College of Nutrition, 22, 2003, 18-35.

[6]. H.H. Ting, F.K. Timimi, K.S. Boles, S.H.J. Creager, P. Gans, and M.A. Creager, Vitamin C improves endothelium dependent vasodilation in patients with non-insulin dependent diabetes mellitus, Journal of Clinical Investigation, 97(1), 1996, 22-28.

[7]. D. H. Alamdari, K. Paletas, T. Pegiou, M. Sarigianni, C. Befani, and G. A. Koliakos, "A novel assay for the evaluation of the prooxidant-antioxidant balance, before and after antioxidant vitamin administration in type II diabetes patients", Clinical Biochemistry, 40(3-4), 2007, 248–254.

[8]. M. Gupta, and S. Chari, "Proxidant and antioxidant status in patients of type II diabetes mellitus with IHD", Indian Journal of Clinical Biochemistry, 21(2), 2006, 118–122.

[9]. G. T. Fadupin, A. U. Akpoghor, and K. A. Okunade, "A comparative study of serum ascorbic acid level in people with and without type 2 diabetes in Ibadan, Nigeria," African Journal of Medicine and Medical Sciences, 36(4), 2007, 335– 339.

[10]. M. A. Ardekani, and A. S. Ardekani, "Effect of vitamin C on blood glucose, serum lipids & serum insulin in type II diabetes patients", Indian Journal of Medical Research, 126(5), 2007, 471–474.

[11]. B. Vergès, New insight into the pathophysiology of lipid abnormalities in type 2 diabetes. Diabetes & Metabolism, 31(5), 2005, 429-439.

[12]. B. A. Mullan, I.S. Young, H. Fee, and D. R. McCance, Ascorbic acid reduces blood pressure and arterial stiffness in type 2 diabetes, Hypertension, 40, 2002, 804-809.

[13]. D.E. Goldein, R. R. Little, and H. M. Widdmayer, Glycosylated hemoglobin estimation in 1990's. A review of assay methods and clinical interpretation, Diabetes Annual, 1994, 193-212.

[14]. G. Bucolo, and D. Harold, Quantitative determination of serum Triglyceride by the use of enzyme, Clinical Chemistry, 19(5), 1975, 478-481.

[15]. K. H. Gabbay, K. Hasty, and J. L. Breslow, Glycosylated hemoglobins and long term blood glucose control in diabetes mellitus. Journal of Clinical Endocrinology & Metabolism, 44, 1977, 859-864.

[16]. I. Ali, I. U. Khan, M. K. Baloch, and G. Mustafa, Comparative lipid profile studies in cardiac and diabetic conditions. Pakistan Journal of Pharmaceutical Sciences, 17(1), 2004, 25-30.

[17]. R. Taylor, and L. Agius, The biochemistry of diabetes, Biochemical Journal, 250(3), 1998, 625-640.

[18]. V. Kumar, S.V. Madhu, G. Singh, and J.K. Gambhir, Post-prandial hypertriglyceridemia in patients with type 2 diabetes mellitus with and without macrovascular disease, Journal of the Association of Physicians of India, 58, 2010, 603-607.

[19]. S.V. Madhu, V. Mittal, B.K. Ram, and D.K. Srivastava, Postprandial lipid abnormalities in Type 2 DM, Journal of the Association of Physicians of India, 53, 2005, 1043-1046.

[20]. S. Som, S. Basu, D. Mukherjee, S. Dev, P.P. Choudhury, S. Mukherjee, S.N. Chatterjee, and I.B. Chatterjee, Ascorbic acid metabolism in diabetes mellitus. Metabolism, 30(6), 1981, 572-577.

[21]. A. Bannerjee, Blood DHSS and diabetes mellitus in human beings, Annals of Clinical Biochemistry, 19(2), 1982, 65-70.

[22]. I.N. Zelc, T.J. Mariani, and Folz, Superoxide dismutase multigene family, a comparision of the CuZn-SOD (SOD2), and EC- SOD (SOD3) gene structures, evolution, and expression, Free Radical Biology & Medicine, 33, 2002, 337-349.

[23]. J.J. Cunningham, Altered vitamin C transport in diabetes mellitus, Medical Hypothesis, 26, 1988, 236-265.

[24]. D.K. Yue, S. McLennon, E. Fisher, et al, Ascorbic acid status and polyol pathway in diabetes, Diabetes, 38, 1989, 257-261.
[25]. U. Moser, Uptake of ascorbic acid by leukocyte, Annals of the New York Academy of Sciences, 498, 1987, 200-215.
[26]. A.J. Verlangieri, and J. Sestito. Effect of insulin on ascorbic acid uptake by heart endothelial cells: Possible relationship to retinal atherogenesis, Life Sciences, 29, 1987, 5-9.
[27]. S.J. Padayatty, A. Katz, Y. Wang, P. Eck, O. Kwon, J.H. Lee, et al, Vitamin C as an antioxidant, evaluation of its role in disease prevention, Journal of the American college of Nutrition, 22, 2003, 18-35.
[28]. M. Aftkham, M. Ardekani, A.R. Vahidi, and L. Borjian, Effect of vitamin C supplementation on glycosylated hemoglobin in patients with type 2 diabetes, Shah SAD Univ, 10, 2003, 15-18.
[29]. A.C. Maritim, R.A. Sanders, and Warkins. Diabetes, oxidative stress, and antioxidants: a review, Journal of Biochemical and Molecular Toxicology, 17(1), 2003, 24-38.
[30]. B. Hasanain, and A.D. Mooradian, Antioxidant vitamins and their influences in diabetes mellitus, Current Diabetes Report, 2(5), 2002, 448-456.
[31]. A.S.Urmila, Anti-diabetic activity and mechanism of action of chromium chloride, Experimental and Clinical Endocrinology & Diabetes, 112, 2004, 248-252.

*Thiyam Romola Devi. "Serum Ascorbic Acid Level In Type 2 Diabetes Mellitus." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.12 (2017): 52-57