Vitamin C in Plasma Chronic Kidney Disease: Comparison between Diabetic and Non-Diabetic Patients

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Abstract

Objective: The aim of the study is to evaluate the relationship between renal functions and plasma vitamin C concentration in non-diabetic and diabetic patients with CKD.

Background: A decreased plasma level of vitamin C has been reported to be associated with an increased risk of cardiovascular morbidity and mortality. Here, we sought to determine the vitamin C status of patients with chronic kidney disease and the pathophysiological role of vitamin C in these patients.

Materials and methods: Fifty patients were included in the study and classified into two groups. Group1, chronic kidney disease patients with diabetes, and group2, chronic kidney disease patients without diabetes and evaluated the relationship between renal function and plasma vitamin C concentration, as well as the effect of diabetes on this relationship.

Results: Statistical comparison revealed a significant statistical increase in the mean value of vitamin C in the group II compared to group1 (P = < 0.001). Also, there was a significant positive correlation between Vitamin C level and both weight and e-GFR, in the patients of the study. While, there was a significant negative correlation between Vitamin C level and each of serum creatinine, blood urea, A/C ratio and glycosylated hemoglobin in the patients of the study.

Conclusion: Renal dysfunction was associated with a decreased level of plasma vitamin C in patients with CKD. Diabetic patients showed a lower level of vitamin C at any given eGFR compared with non-diabetic patients.

Keywords: Vitamin C, estimated glomerular filtration rate, blood urea, serum creatinine, HB A 1C, Albumine creatinine ratio in urine.

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Introduction

CKD is a worldwide public health problem that affects millions of people from all racial and ethnic groups. Diabetes mellitus is the leading cause of CKD, and the rapidly increasing prevalence of diabetes worldwide virtually assures that the proportion of CKD attributable to diabetes will continue to rise [1].

Diabetic nephropathy is the leading cause of end-stage renal disease in the United States. The progression of kidney disease in patients with diabetes can take many years, and interventions such as glycemic control, blood pressure control, and inhibition of the renin-angiotensin-aldosterone system have been shown to slow this progression. Despite the implementation of these strategies, the number of patients with diabetes that ultimately develop end-stage renal disease remains high [2].

A decreased plasma level of vitamin C has been reported to be associated with an increased risk of cardiovascular morbidity and mortality. There is need to determine the vitamin C status of patients with chronic kidney disease and the pathophysiological role of vitamin C in these patients [3].

Decreased vitamin C may cause endothelial dysfunction via an increase in oxidative stress in non-diabetic chronic kidney disease patients [3].

Experimental lines of evidence have been accumulated that vitamin C has beneficial effects on endothelial function and formation of atherosclerotic lesions [4].

Materials and methods

This study was carried out on 50 patients selected from the inpatient department and outpatient clinics of the Internal Medicine Department, Nephrology Division in Menoufiya University Hospital. The selected participants provided consent for participation in the study before they were subjected to examinations and investigations, and the study was approved by the Ethics Committee of Menoufiya University Hospital. The study was carried out from October 2013 to December 2015.

The selected participants were classified into two groups. Group1, chronic kidney disease patients with diabetes.Group2, chronic kidney disease patients without diabetes. All patients met the following criteria: estimated glomerular filtration rate (eGFR) <60 mL/min/ 1.73 m2, no previous history of myocardial infarction, heart failure or stroke within 6 months; and were not taking vitamin C.

All patients were subjected to the following:

I. *Assessment of history*: Assessment of history was performed for all participants in the study group, with a special focus on the age and sex
of the patients, duration of diabetes mellitus, and the presence or absence of specific diabetic complications and treatment.

II. *Complete clinical examination:* All participants were subjected to a complete clinical examination including measurement of weight, Signs of diabetic complications including: Signs of diabetic nephropathy (e.g. edema usually around the eyes in the mornings later, may be generalized edema) and Signs of diabetic neuropathy: sensory loss to light touch, vibration, and temperature. Abnormalities in more than one test of peripheral sensation are > 87% sensitive in

detecting the presence of neuropathy. Patients who have lost 10-g monofilament sensation are at considerably elevated risk for developing foot ulceration [5].

Laboratory investigations included the following:

- ***Glycosylated Hemoglobin (HbA1c):*** HbA1c levels depend on the blood glucose concentration. That is, the higher the glucose concentration in blood, the higher the level of HbA1c. Levels of HbA1c are not influenced by daily fluctuations in the blood glucose concentration but reflect the average glucose levels over the prior six to eight weeks.
- ***Kidney function tests:*** (blood urea nitrogen, serum creatinine).
- ***Estimated glomerular filtration rate (eGFR):*** GFR was estimated by Cockroft-Gault equation (ml/min):

eGFR = [140 - age (years)] × weight (kg)/[72 × serum creatinine (mg/dl)] × 0.85 (if a woman)

- ***Albumin-creatinine(A/C)ratio:***

Morning 10 ml midstream urine samples were collected and were centrifuged at 3000 rpm for 10 min before analysis. Microalbuminuria levels were measured using an autoanalyzer (Beckman Coulter Synchron CX 9;
Beckman Coulter Inc.). Urine creatinine levels were measured in the first morning urine using a spectrophotometer (Clinical Chemistry Analyzer 7)
and the ratio was calculated as the urinary albumin/ creatinine ratio.

Micro-albuminuria ranges between 30-300 mg/gm creatinine.

- ***Plasma Vitamin C level:***

plasma vitamin C is found as ascorbic acid as well as its oxidized form, dehydro-ascorbate. Both forms are biologically active. In our vitamin C assay, an oxidation is induced prior to analysis so that both forms are measured. A dose response curve of the absorbance unit (optical density, OD at 492 nm) vs. concentration is generated, using the values obtained from the standard. The concentration of the patient sample is determined directly from the linear standard curve.

Plasma sample was not diluted and used directly for the sample preparation. 2 ml blood were taken in a tube with EDITA and centrifuged at 3000 rpm for 5 min. 200 µl of the supernatant (plasma) was added to 200 µl of meta-phosphoric acid (MPA) and centrifuged for 30 min at 4 oC at 10000 rpm and the supernatant then stored at -20 oC. Samples were kept in a cool and dark place.

Normal range: 4-15 mg/L

Statistical Analysis

Data were collected, tabulated, statistically analyzed by computer using SPSS version 10.

Two types of statistics were done:

① **Descriptive:** No., %, Mean, SD and Range.

② **Analytical:** This included:

1. **Correlation (r):** The correlation analysis is used to know the relation between variables. It aims at expressing the changes that occur in one variable which follow the changes in other variables.
2. **Chi-Squared (χ^2):** It is used to compare between two or more qualitative variables contingency table or r x c complex 2 x 2 in table.
3. **P value:**
    - P <0.05 indicates significant difference.

- P <0.01 indicates high significant difference.

4. T-test

Results

There was no significant difference between the two groups studied as regards age, sex, and weight (P = 0.843, 0.247, 0.117 respectively).

Plasma Vitamin C in group I ranged between 0.6-13.0 mg/l with a mean value of 5.013±3.825, while in group II it ranged between 1.4-21.5 mg/l with a mean of 10.656±6.433; there was a significant statistical increase in the mean value of vitamin C in the group II compared to group1 (P < 0.001) (Table 1 and Fig. 1).

Table 1: Vitamin C value in both studied groups

Vit C (mg/l)	Group I (diaetics)	Group II (non-diabetics)	t test	p value
Range	0.6-13	1.4-21.5	-3.770	<0.001*
Mean±SD	5.01±3.82	10.65±6.43		

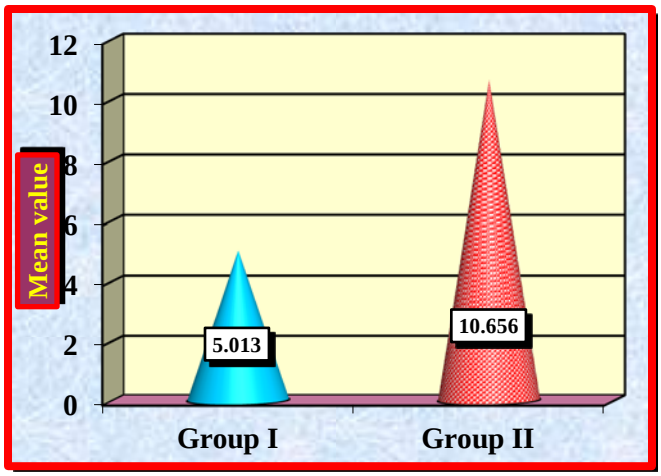

Fig. 1: Vitamin C value in both studied groups.

Statistical analysis revealed that there was no significant statistical difference between both groups as regards blood urea concentration, serum level of creatinine and rate of e-GFR (P = 0.917, P = 0.663, P = 0.50 respectively).

Urine albumin creatinine ratio of patients of group I ranged between 60-400 µg/mg with a mean value of215.76±118.45 µg/mg, while in group II it ranged between 45-260 µg/mg with a mean of 124.52±72.0 2 µg/mg. Statistical analysis revealed that there was significant statistical difference between both groups as regards A/C ratio (P = 0.002), (Table 2 and Fig. 2).

Table 2: A/C ratio in both studied groups

A/C ratio (µg/mg)	Group I (diaetics)	Group II (non-diabetics)	t test	P value
Range	60-400	45-260	3.291	0.002
Mean±SD	215.76±118.45	124.52±72.02		

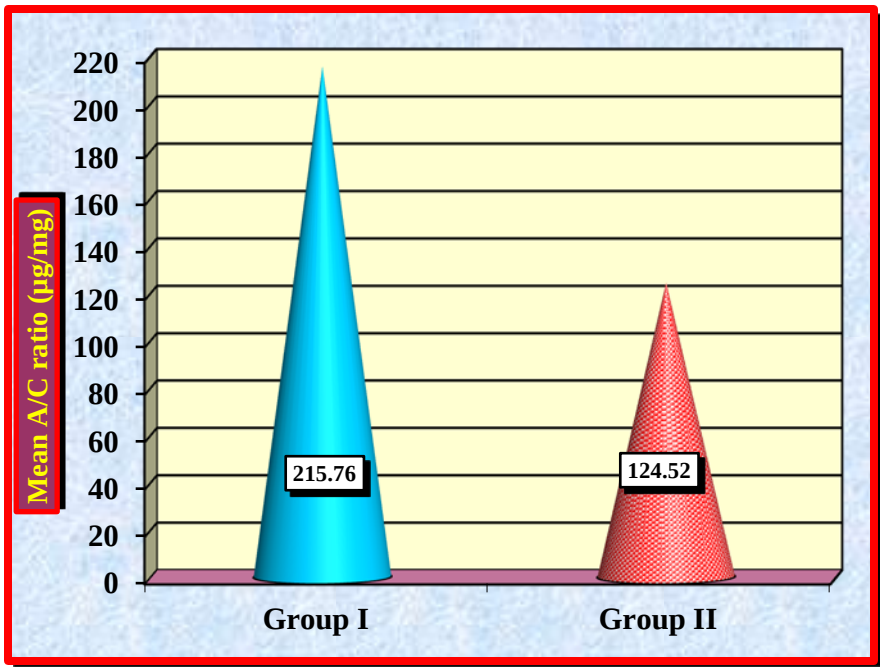

Fig. 2: A/C ratio in both studied groups.

Glycosylated hemoglobin concentration in patients of group I ranged between 7.4-10% with a mean value of 8.27± 0.76%, while in group II it ranged between 4-5.4% with a mean of 4.81± 0.30%. There was significant statistical difference between both groups of the study as regards HbA1c concentration (P = 0.000), (Table 3 and Fig. 3).

Table 3: HbA1c level in both studied groups

HbA1c %	Group I (diaetics)	Group II (non-diabetics)	t test	P value
Range Mean±SD	7.4-10 8.27±0.76	4-5.4 4.81±0.30	21.035	0.000

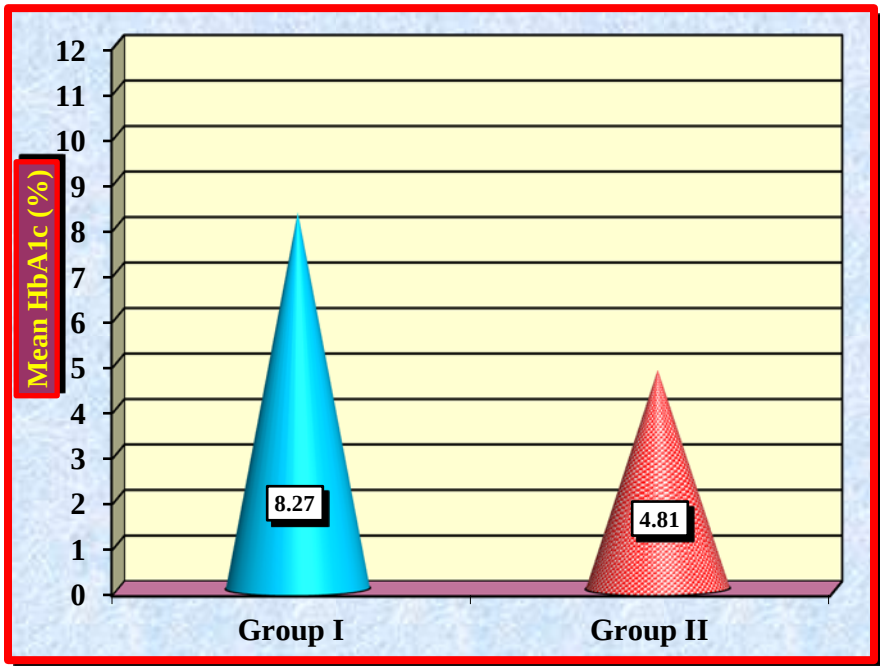

Fig. 3: HbA1c level in both studied groups.

Statistical analysis revealed that there was no significant statistical difference between females and males of group I as regards Weight, Age, Creatinine, Urea and HbA1C, while there was more significant statistical difference as regards A/C ratio, then Vitamin C , then e GFR (Table 4).

Table 4: comparison between females and males in group I as regards vitamin C, Age, weight, serum creatinine, blood urea, e-GFR and HbA1C

Group I (diaetics)	Sex		T-Test	
	Female	Male		
	Mean ± SD	Mean ± SD	t	P-value
Vit C (mg/l)	2.775 ± 1.763	6.066 ± 4.111	-2.154	0.042*
Weight (kg)	69.438 ± 12.281	73.971 ± 9.044	-1.043	0.308
Age (Years)	55.000 ± 14.861	54.588 ± 6.472	0.098	0.923
Creat. (mg/dl)	3.704 ± 1.449	2.771 ± 1.521	1.451	0.160
Urea (mg/dl)	173.125 ± 72.454	110.000 ± 70.534	2.070	0.050
e-GFR (ml/min/1.73m$^{2)}$	23.863 ± 16.822	40.014 ± 17.363	-2.216	0.043*
A/C (µg/mg)	291.00 ± 108.62	180.35 ± 108.27	2.387	0.03*
HbA1C %	8.38 ± 0.82	8.23 ± 0.76	0.44	0.67

Statistical analysis revealed that there was no significant statistical difference between females and males of group II as regards Weight, Age, Creatinine , Urea , HbA1C, Vit C, e-GFR and A/C ratio (Table 5).

Table 5: comparison between females and males in group II as regards to vitamin C, Age, weight, serum creatinine, blood urea, e-GFR and HbA1C

Group II (non-diabetics)	Sex		T-Test	
	Female	Male		
	Mean ± SD	Mean ± SD	t	P-value
Vit C (mg/l)	9.887 ± 7.042	11.367 ± 6.015	-0.567	0.576
Weight (kg)	75.333 ± 7.563	77.731 ± 7.807	-0.779	0.444
Age (Years)	55.417 ± 9.848	52.846 ± 15.545	0.489	0.630
Creat. (mg/dl)	3.542 ± 2.050	3.023 ± 1.405	0.743	0.465
Urea (mg/dl)	133.833 ± 54.045	123.154 ± 51.392	0.506	0.617
e-GFR (ml/min/1.73m$^{2)}$	27.284 ± 13.641	35.623 ± 15.258	-1.436	0.164
A/C (µg/mg)	139.58 ± 75.33	110.62 ± 68.82	1.00	0.330
HbA1C %	4.83 ± 0.26	4.79 ± 0.35	0.33	0.740

Statistical analysis revealed that there was a significant positive correlation between Vitamin C level and both weight and e-GFR, in the patients of the study as shown in the table. Statistical analysis revealed that there was a significant negative correlation between Vitamin C level and each of serum creatinine, blood urea, A/C ratio and glycosylated hemoglobin in the patients of the study as shown in the table. Statistical analysis revealed that there was no significant between Vitamin C level and age (Table 6).

Table 6: Correlation between vitamin C and patients variables of the study

	Vit C	
	R	**P-value**
Weight	0.296	0.037*
Age	-0.101	0.485
Creat.	-0.684	<0.001*
Urea	-0.682	<0.001*
e-GFR	0.648	<0.001*
A/C	-0.725	<0.001*
HbA1C	-0.504	<0.001*

Discussion

Chronic kidney disease (CKD) is an emerging public health problem and one of the most powerful predictors of premature cardiovascular disease [6].

In un-supplemented CKD patients, several deficiencies in various components of antioxidant defense mechanisms have been demonstrated, including reduced plasma vitamin C concentration. Vitamin C is a primary antioxidant that directly neutralizes radical species as well as an essential nutrient required for the formation of collagen and normal immune function [3].

Ascorbic acid is involved in the formation and repair of collagen, in the development of bones and teeth, in amino acid metabolism, in the synthesis of hormones, and in wound healing.It facilitate iron absorption and utilization [7].

Diabetes Mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [5].

Diabetic nephropathy is the leading cause of end-stage renal disease in the United States. The progression of kidney disease in patients with diabetes can take many years, and interventions such as glycemic control, blood pressure control, and inhibition of the renin-angiotensin-aldosterone system have been shown to slow this progression. Despite the implementation of these strategies, the number of patients with diabetes that ultimately develop end-stage renal disease remains high [8].

The aim of this study was to evaluate the relationship between renal functions and plasma vitamin C concentration in non-diabetic and diabetic patients with CKD.

This study was conducted at Menoufeya University Hospital on 50 patients all had CKD, 25 were diabetic (Group I) and 25 were non-diabetic (Group II).

Plasma vitamin C level in patients of our study was 5.013 mg/l in diabetic patients (Group I) and 10.656 mg/l in non-diabetic (Group II) with a significant low level in group 1 compared to group II.

Takahshi et al., 2011 found in their study that the mean vitamin C level was 4.5 μg/mL in diabetic patients group 1 and 6.2μg/mL in non-diabetic group II with no significant difference between the level in both studied groups [3].

Proteinuria is another important factor that causes an increase in urinary loss of vitamin C [9].

We found no statistical significant difference between females and males of group I patients as regards weight, age, creatinine, urea and HbA1C, while there was significant statistical increase in Vit C and eGFR and reduction of A/C ratio in males than females.

There was no significant statistical difference between females and males of group II as regards Weight, Age, Creatinine, Urea , HbA1C, Vit C, e-GFR and A/C ratio.

Lincoln A. et al, 2000 found in their study that there was no significant statistical difference between females and males as regards age, HbA1C. which was in agreement with our study [10].

Our results revealed statistical positive correlation between vitamin C level and both weight and e-GFR in the patients of the study. While there was negative correlation between Vitamin C level and each of serum creatinine, blood urea, A/C ratio and glycosylated hemoglobin in the patients.

Takahashi et al., 2011 reported that there were significant relationships between eGFR and urinary protein excretion in patients of their study, these could explain the relationship between decreased vitamin C concentration and renal dysfunction which was in agreement with our study [3].

Takahashi et al., 2011 reported that there was a negative correlation between vitamin C level and creatinine level, A/G ratio and glycosylated hemoglobin concentration which was in agreement with our study while no relation between vitamin C level and blood urea and this disagreed with our results [3].

Lincoln A. et al, 2000 found in their study that there was a negative correlation between vitamin C level and glycosylated hemoglobin concentration which was in agreement with our study [10].

Diane fennell, 2009 found in his study that A combination of insulin and vitamin C may help stop diabetes-induced blood vessel damage in people with poor blood glucose control, according to new research published in The Journal of Clinical Endocrinology and Metabolism that halting this damage could help prevent a host of complications, such as chronic heart failure, kidney disease and eye disease [11].

Afkhami-Ardekani M. et al., 2006 found in their study that supplementation with 1000 mg/day of vitamin C in addition to the normal diet and treatment schedule may help in improving plasma glucose and lipid profile in patients with type 2 diabetes [12].

Harding and Wareham 2008 found in their study that there was a significant inverse association between vitamin C levels in the blood and the risk of getting diabetes.. Also showing

that vitamin C- as well as a high intake of vegetables and fruits- may have protective effects against diabetes [13].

Jayesh k bhatt et al., 2012 found in their study that oral vitamin C supplementation is found to be effective and has potential implications for the prevention of further complications in patients with diabetes mellitus [14].

Daniel HR. 2014 found in his study that combining insulin with vitamin C stops blood vessel damage in type 1 diabetics [15].

Kotb A. et al., 2015 found in their study that oral supplementation of vitamin C as an adjuvant with anti diabetic drugs may be of particularly attractive therapeutic effect in the treatment of type II DM. Studies with a larger sample size and longer follow-up period together with measurement of other related antioxidant levels may be needed to yield more beneficial data on the role of the antioxidant system in the clinical course of type II DM [16].

Conclusion

Renal dysfunction was associated with a decreased level of plasma vitamin C in patients with CKD, and diabetic patients showed a lower level of vitamin C at any given e-GFR compared with non-diabetic patients.

References

1 - De Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. JAMA 2011 ; 305(24): 2532-2539.

2- Noel vanBuren and Robert Toto. Current Update in the Management of Diabetic Nephropathy. Current Diabetes Review 2013; 9: 62-77.

3 -Takahashi N, Morimoto S, Okigaki M, Seo M, Someya K, Morita T et al (2010): Decreased plasma level of vitamin C in chronic kidney disease: comparison between diabetic and non-diabetic patients. Nephrol Dial Transplant 2010; 26: 1252-7.

4 - Aguirre R and May JM. Inflammation in the vascular bed: importance of vitamin C. Pharmacol Ther 2008; 119: 96–103.

5 - ADA (American Diabetes Association). Diagnosis and Classification of Diabetes Mellitus. Diabetes care 2014; 33:62-69.

6 - Williams S, Malatesta K. and Norris K. Vitamin D and chronic kidney disease. Ethn Dis 2009;19: 8-11.

7 - Chen GC, Lu DB, Pang Z and Liu QF. Vitamin C intake, circulating vitamin C and risk of stroke: a meta-analysis of prospective studies. J Am Heart Assoc 2013; 2(6): 329.

8 - Noel vanBuren and Robert Toto. Current Update in the Management of Diabetic Nephropathy . Current Diabetes Review 2013; 9: 62-77.

9 - Hirsch IB, Atchley DH. And Tsai E. Ascorbic acid clearance in diabetic nephropathy. J Diabetes Complications 1998; 12: 259-63.

10 - Lincoln a. sargeant, nicholas j. wareham, sheila bingham, nicholas e. day, phd robert n. luben, Welch et al. vitamin c and hyperglycemia in the european prospective investigation into cancer—norfolk (epic-norfolk) study. Diabetes care 2000; 23: 6.

11 - Fennel 2009: Defeat Diabetes Foundation: Ihnat, Michael. Clay, Diane. University of Oklahoma news release.

12 - Afkhami-Ardekani Mohammad & Ahmad Shojaoddiny-Ardekani . Effect of vitamin C on blood glucose, serum lipids & serum insulin in type 2 diabetes patients 2007. Indian J Med Res; 126: 471-4.

13 - Harding and Wareham. Plasma Vitamin C Level, Fruit and Vegetable Consumption, and the Risk of New-Onset Type 2 Diabetes Mellitus. Arch Intern Med. 2008; 168(14):1493-9.

14 - Jayesh k bhatt, sabin Thomas. And nanjan mj. Effect of oral supplementation of vitamin c on glycemic control and lipid profile in patients with type 2 diabetes mellitus. source. International Journal of Pharmacy & Pharmaceutical Sciences 2012; 4(2): 524.

15 - Daniel Henryk Rasolt. 2011 Complementary and Alternative Medicine, Diabetes ABCs, Diabetes Research Explained and tagged protection, research, type 1, vitamin C by Daniel Henryk Rasolt. Bookmark the permalink.

16 - Ashraf kotb and Khaldun M. Al Azzam. Effect of Vitamin C on Blood Glucose and Glycosylated Hemoglobin in Type II Diabetes Mellitus. BMC 2015; 3: 6-8.