Beta-Carotene, Glycemic Control And Dyslipidemia In Type 2 Diabetes Mellitus

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Summary:

Background : *Diabetes mellitus is a stressful condition in which the increased production of free radicals impairs the generation of naturally occurring antioxidants like vitamins and carotenoids .*

Aim :*The present study deals with the changes in serum ß-carotene in type 2 diabetes mellitus, as modulated by glycemic control and oxidative stress .*

Subjects & methods : *Multiple biochemical parameters were obtained from plasma of **57 patients** with type 2 diabetes mellitus (25 males and 32 females) , on oral hypoglycemic with a disease duration of 1- 15 years and **37 healthy normal subject s** of matching age and sex to serve as controls .*

The biochemical parameters measured in the present study included the glycated Hb (HbA1c), serum lipids (total cholesterol TC, triglycerides TG , high and low density lipoprotein cholesterols , HDL-C & LDL-C) , lipid peroxides and serum ß-carotene.

results *revealed a marked reduction of ß- carotene in the diabetics in a pattern proportional to that of the glycemic control ,dyslipidemia and oxidative stress .*

Possible causes , mechanisms and suggestions underlining these changes are discussed.

Key words : *ß –carotene , diabetes mellitus , oxidative stress ,Oxidized Lipoproteins .*

Introduction :

Type 2 diabetes mellitus(DM) is a disease of carbohydrate metabolism characterized by a number of metabolic abnormalities , including impaired pancreatic beta cell functions and insulin resistance in the skeletal muscles ,adipose tissue and liver [1,2]. Mortality from cardiovascular disease is increased by a factor of 2-3 in persons with DM as compared with the general population [3]

Genetic and environmental factors contribute to the pathogenesis of type 2 DM while life style factors act as triggers for the disease for persons at high risk because of inherited susceptibility [4]. The composition of human diet has changed considerably during the past few decades. These recent changes are thought to be contributing greatly to the increasing incidence of type 2 DM.[5].

Hyperglycemia is a widely known cause of enhanced plasma free radical concentration and development of oxidative stress [7,8]. Impaired generation of naturally occurring antioxidants in diabetes can also be expected to result in increased oxidative cell damage[9]. Vitamins (A,E & carotenoids) are considered the most prominent dietary antioxidants [10] and have the highest protective action in corporation with the scavenging enzymes in reducing the toxicity of free radicals [11].These antioxidant vitamins may also inhibit the oxidation of low density lipoprotein (LDL) particularly the atherogenic forms that can cause vascular damage [12,13,14].

Beta-carotene can not be synthesized by the human cells but it is provided by the diet[15]. Some workers concluded that the intake of vegetables and fruits rich in carotenoids may be a protective factor against hypoglycemia while others suggested that the administration of beta-carotene suppresses the elevation of lipid peroxidation and reduces the symptoms of diabetes mellitus in streptozotocin – induced diabetic rats [16]

The present study deals with the relation of serum beta –carotene with the degree of glycemic control ,dyslipidemia and lipid peroxidation in type 2 DM.

Subjects and methods:

A- patients:

Twenty five male and 32 female diabetic patients on oral hypoglycemic agents were included in the study, age range was 35 -65 years, none of them was on insulin or having a previous history of renal , liver or absorption disorders.

B- Control group

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A total of 37 (15 male and 22 female) healthy subjects with age range of 31 – 62 years were included in the study.

C- Blood samples :

About 12 mL of venous blood were collected from each patient and normal subject after a 12 hour fast. Two mL of the whole blood were transferred to an EDTA containing tubes for measurement of glycated hemoglobin HbA1c ,the rest of the blood was centrifuged and serum separated was used for measurement of total malon-dialdehyde (MDA), total cholesterol (Tc) ,high and low density lipoprotein cholesterols (HDL-c & LDL-c), triglycerides (TG) ,oxidized HDL and Beta-carotene.

D- Methods :

Serum total MDA was measured by the thiobarbiturate method [7,18] while serum lipids (Tc ,LDL-c ,HDL-c and TG) were measured by enzymatic methods using kits from bioMeriux , France.

Serum oxidized HDL was measured by precipitation of all lipoproteins ,except HDL-c which was measured by phospho-tungstic acid – Mgcl2 reagent The supernatant was used for estimation of oxidized HDL by the same method used for the measurement of total MDA.

Serum Beta-carotene was measured by the method of Pesce & Kaplan (1987)[19]

Results :

Table (1) shows the distribution of the 57 diabetic patients according to the level of Haemoglobin A1c (HbA1c) into three groups ,depending on The American Diabetes Association (ADA) HbA1c guidelines [20]

1- Total lipid peroxides:

Serum MDA was significantly increased in all groups of patients when compared to the controls . There was ,also, a significant variation between diabetic groups when compared with each other (ANOVA-P value was 10^{-3}), as shown in Table (*2)*

2- Oxidized lipoproteins :

All diabetic groups had a significant reduction of Oxidized HDL% and an elevation in oxidized non –HDL% when compared to the controls (P<0.05).There was a significant variation among diabetic groups when compared with each other (ANOVA-P = 10^{-3}) , table (*2*)

3- Serum lipid profile :

All patients showed an increase in all lipid parameters measured in diabetics except the HDL-C which showed a significant reduction (table *3*).

4- Serum β-Carotene :

Table *(4)* shows no significant reduction in serum β-carotene concentration in diabetic group I (HbA1c <7%) (p=0.12) , while group II & III (HbA1c 7-8% & HbA1c >8%) showed a significant reduction in the serum β-carotene level (P= 10^{-4} and 10^{-6} respectively) when compared with the normal control group. By comparing the three groups with each other a significant difference is noticed (P = or < 0. 05).

Table *(5)* illustrates a good percentage of β-carotene level in group I (91.6% above 30 Ug/dL), and as there is a deterioration in the glycemic control there is a reduction in serum β-carotene level (83.4% &37% above 30 Ug/dL in groups II & III respectively.

5- Correlation between serum Beta-carotene and glycemic control :

There was a significant negative correlation between Serum β-Carotene concentration and HbA1c level in the controls (r = - 0.33, p<0.05) and all the diabetic groups (r =- 0.47, p<0.05) , fig.(*1 & 2*).

6- Correlation between serum beta-carotene and oxidative stress:

Figure *(3)* illustrates a negative correlation between serum β--carotene level and serum MDA among the diabetic Groups (r = - 0.49, p <0.05) , which was not be found in the normal control subjects .

Table (1) Distribution of type 2 diabetic patients according to the level of HbA1c

HbA1c level	No. of cases	Percent %
<7 %	12	21
7-8 %	18	31.5
>8 %	27	47.3
TOTAL	*57*	*100*

Table (2) :Lipid peroxidation and it's fractions (mean ± sd) in different diabetic groups and control group

Groups	Serum MDA Umol/L	t-test P-value*	Oxid. HDL %	t-test P-value*	Oxid. non-HDL %	t-test P-value*
HbA1c <7%	0.65 ± 0.08	0.03	65.3 ± 7.9	0.05	34.7 ± 7.79	0.05
HbA1c 7-8%	0.74 ± 0.2	0.01	62.5 ± 5.95	H.S	37.5 ± 5.95	H.S
HbA1c >8%	0.87 ± 0.26	H.S	59.5 ± 12.06	H.S	40.5 ± 12.12	H.S
ANOVA P-value	~	H.S	~	H.S	~	H.S
Controls	0.51 ± 0.12	~	72 ± 15.03	~	28 ± 15.03	~

- **Student t-test was done between each diabetic group and control.**
- **P value is considered significant at 0.05 or less.**
- **H.S (Highly Significant) when P value = or <10 $^{-3}$.**

Table (3): Serum lipid profile (mean ± sd) in diabetic and control groups expressed in mg/dl

Diabetic Groups	<7 % n=12	7-8% n=18	>8 % n=27	ANOVA	Controls n=37
T.Ch	199.5 ± 23.3	213.38 ± 30.3	239.8 ± 52.7	~	171.1 ± 22.3
t-test p-value*	H.S	H.S	H.S	0.02	~
TG	131.6 ± 48.7	158.0 ± 60.5	226.7 ± 95.3	~	95 ± 30.5
t-test p-value*	0.02	H.S	H.S	0.05	~
HDL-C	32.1 ± 6.5	31.1 ± 7.8	30.8 ± 7.7	~	41.4 ± 5.4
t-test p-value*	H.S	H.S	H.S	0.03	~
VLDL-C	26.3 ± 9.7	31.9 ± 12.6	45.5 ± 19.1	~	18.9 ± 6.1
t-test p-value*	0.15	0.04	0.01	0.07	~
LDL-C	143.1 ± 27.7	144.1 ± 27	162.6 ± 47.5	~	111 ± 21.3
t-test p-value*	0.1	H.S	H.S	0.01	~

- **To convert from mg/dl to mmol/L , simply divide:- T.Ch by 38.9 & T.G by 88.5.**
- **Student t-test was done between each diabetic group and control**
- **Significant p value at 0.05 or less.**
- **H.S (Highly Significant) when P value = or <10 $^{-3}$.**

Table (4): β-carotene level (mean ± sd) in diabetic and control groups

Groups	HbA1c <7%	HbA1c 7-8%	HbA1c >8%	ANOVA	Controls
Beta-carotene Ug/dl	43.08 ± 5.9	36.6 ± 9.8	27.6 ± 10.1	~	50.2 ± 5.4
t-test p-value*	0.12	H.S	H.S	0.05	~

- **Student t-test was done between each diabetic and control groups**
- **P value is considered significant at 0.05 or less.**
- **H.S (Highly Significant) when P value = or <10 $^{-3}$.**

Table (5): Percentage of diabetic patients who have β-carotene level above 30 and below 30 Ug/dl in different diabetic groups

Groups	Beta-carotene 30-50 Ug/dl	Beta-carotene <30 Ug/dl
HbA1c <7% N=12	91.6%	8.4%
HbA1c 7-8% N=18	83.4%	16.6%
HbA1c >8% N=27	37%	63%

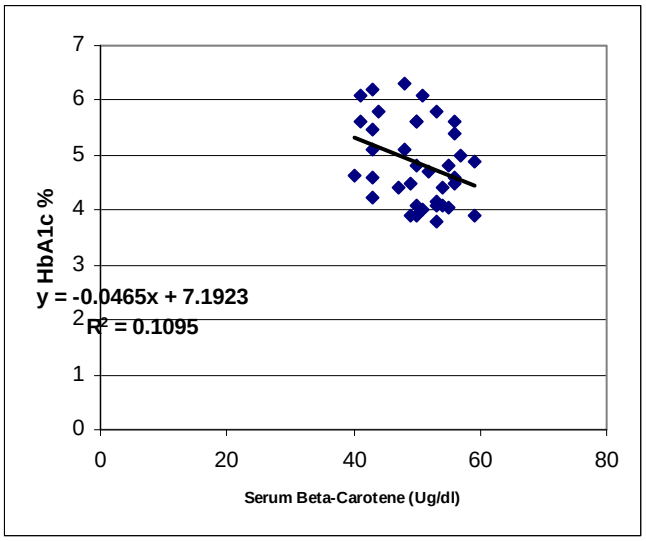

Figure (1): Correlation between serum β-carotene and HbA1c % in the controls, P value < 0.05

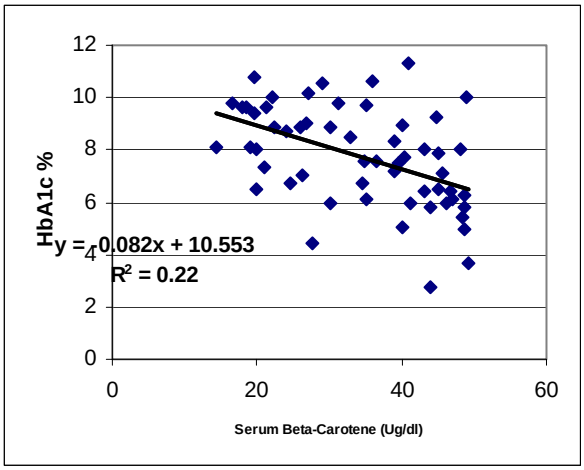

Figure (2): Correlation between serum β-carotene level and HbA1c % in type 2 diabetes mellitus P value < 0.05

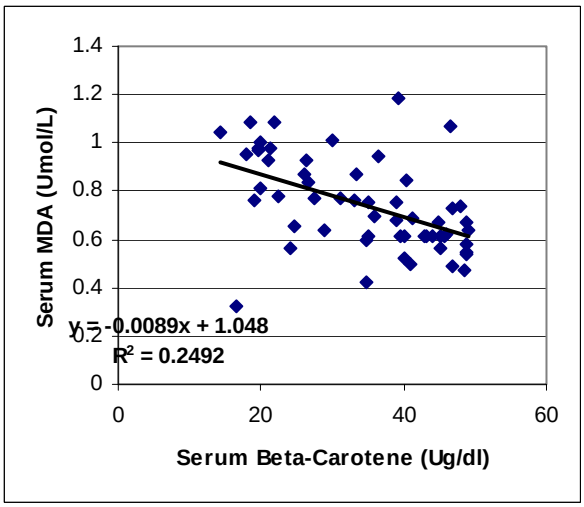

Figure (3): Correlation between serum β-carotene level and serum malondialdehyde in type 2 diabetes mellitus P value < 0.05

Discussion

Excellent diabetic control has implied the maintenance of near normal blood glucose concentration, as reflected by HbA1c **< 7%.** The major cause of death, However, in patients with type 2 diabetes is cardiovascular disease and glucose control may have little impact upon diabetes mortality. In contrast control of other features of the metabolic syndrome, such as hypertension, dyslipidemia and hyper-coagulability appears to be more important in this regard.[23].

Diabetes mellitus and it's complications are important causes of increased rate of oxidative stress and it's consequences , hyperlipidemia and albuminuria [24].

Hyperlipidemia is one of the major risk factor for cardiovascular disease [25 , 26] , and to reduce this increased risk, a multifactorial approach to the management of type 2 diabetes has been advocated [27]. Recommended lifestyle interventions included reduced intake of dietary saturated fat, regular participation in light or moderate exercise, and cessation of smoking [27]. Rise in serum MDA indicates an increased rate of lipid peroxidation which is mostly attributed to hyperglycemia and hyperinsulinemia. [8 , 28]

In this study oxidative stress (which is expressed as total lipid peroxide and oxidized lipid subfractions) had been measured to demonstrate the relation between type 2 diabetes and oxidative stress, and an association of poor glycemic control with oxidative stress in type 2 diabetes was observed , as evident from the higher values of both HbA1c and serum MDA. This is in agreement with *Ali et al.2001.* [29] as shown in table *(2)*.

The oxidation of LDL is a very complex procedure [30 , 31]. The diabetic state alters LDL size and composition [32, 33]. LDL in postprandial state appears to be more susceptible to oxidation than fasting LDL [34]. Oxidation of LDL leads to alteration of the LDL apolipoprotein B (apo B) , recognition site and in the unregulated uptake of the LDL by the macrophages via the scavenger-receptor.[35]

Another important factor in LDL oxidation relates to the ambient HDL concentrations. HDL carries important antioxidant enzymes, paroxanase and platelet activating factor acetylhydrolase, and also it serves to protect LDL from oxidation in other ways. HDL also appears to exchange undamaged phospholipids from oxidized phospholipids in LDL. HDL_2 from diabetic subjects were found to be less protective against an in vitro oxidation of LDL than HDL from non-diabetic control subjects [34].

According to the present results (table *2*) , there was a significant elevation of the Oxidized LDL% and a reduction of the oxidized HDL % in all diabetic groups with increasing HbA1c level (which is an index of the glycemic control).These results are in accordance with the results obtained from *Sanguinetti ,et al.*[36], *Kontush ,et al.*[37] *and American Diabetes Association position statement* [38] .

Serum lipids were significantly elevated in all diabetic groups except for HDL-c which was significantly reduced in the diabetics as compared to the controls , and as the level of HbA1c increased , there was more pronounced lipid disturbances, as seen in table *(3)* .

It is well documented that nutrition therapy can improve glycemic control [39]. Nutrition therapy can result in a reduction in glycated hemoglobin (HbA1c) of 1.0 to 2.0% and, when used in combination with other components of diabetes care, can further improve clinical and metabolic outcomes .[40 , 41]

An explanation for why fruit and vegetables might be protective against long-term disease . Peto et al (1981) hypothesized that the carotenoid pigments (beta-carotene etc), known to protect the plant against oxidative stress from excessive UV radiation , might exert a similar effect when ingested by humans. They suggested that the high antioxidant content of fruit and vegetables might be a reason for their beneficial effects by protecting the organism against the damage caused by oxidative free radicals in vivo.[42]

A recent European Task Force has reviewed the evidence that the antioxidant content of fruit and vegetables is the reason why increased fruit and vegetable intake has shown a consistent protective effect on the risk of degenerative disease [43].

Antioxidant micronutrients such as ascorbate, alpha-tocopherol, and beta - carotene, levels of which can be favorably manipulated by dietary measures without side effects, could be a safe approach in inhibiting LDL oxidation [44].

Elderly subjects with Type 2 diabetes were reported to have significantly lower levels of plasma antioxidants, compared to matched controls [45].

It has been postulated that carotenoids may form a part of the antioxidative mechanism of cells, acting as antioxidants or modifying the levels of other antioxidants . But conflicting reports regarding the effect of β-carotene on the onset and /or the progression of diabetes mellitus and its complications, some suggest benefit but others show no such benefit .

Ford et al.(1999) in their study showed that all serum carotenoids were found to be inversely associated with the fasting serum insulin levels. Moreover, serum lycopene and β-carotene levels in the diabetic patients were significantly lower as compared to levels in persons with impaired glucose tolerance and was also found to be lower than the levels in subjects with normal glucose tolerance [47].

The absorption of carotenoids, including beta-carotene and vitamin E, has been shown to prevent the oxidation of LDL (or bad) cholesterol [48]. So, as the β-carotene is transported in plasma mainly in the LDL, potentially it could play a protective role in limiting LDL oxidation .[49]

The present results reveal a significant reduction in the mean β-carotene level in plasma of type 2 diabetic patients when compared to the normal controls as shown in table (*5*).

The decrease in β-carotene level may be attributed to its role in scavenging superoxide in cell culture [50]. Deactivation of superoxide can be done by physical and chemical quenching pathways [10] . Fig. (*3*) shows a negative correlation between serum β-carotene level and serum MDA in type 2 diabetic patients, in which the patients who have good glycemic control and low level of the serum MDA show a high β-carotene level ,while those with poor glycemic control and high level of MDA show a low level of β-carotene (*table 5 & fig. 2 & 3*). This can be explained by the fact that β-carotene is considered as one of the antioxidants that can reduce the oxidative stress during any disease , so high β-carotene level may counteract or reduce the stressful condition leading to a reduction in the serum MDA level and vice versa [51].

This may lead to the suggestion that inclusion of Beta-carotene in the diet of diabetics might be of benefit in this respect.

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