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RESEARCH ARTICLE

Estimation of Adiponectin Hormone and Antioxidants Levels in Type 2 Diabetic Patients in Dhiqar Province

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Abstract

The present study was aimed to investigate the changes associated with type II diabetes in the level of adiponectin and antioxidants: (Catalase, Glutathione, Superoxide dismutase and Vitamin C). Seventy Samples (taken from male patients) were collected in cooperation with the Specialized Center for Diabetes and Endocrinology in the Directorate of Health in Thi-Qar Governorate. The numbers of healthy samples were 30. The results showed a significant decrease ($P \leq 0.05$) in the level of adiponectin and antioxidants (catalase, glutathione and vitamin C) in diabetic patients, while it was observed that there was a significant increase in the level of antioxidant superoxide dismutase in diabetic compared with the control group. Moreover, the effect of both the smoking and age on the level of adiponectin and antioxidants was studied. The results showed a significant decrease in the level of adiponectin and catalase in smoking diabetic patients compared to non-smokers. On the other hand, the study showed that there was a significant increase in the level of superoxide dismutase in smoking patients compared to non-smokers. As for the effect of age, the results showed a significant increase in superoxide dismutase with aging in patients with diabetes, while there was a significant decrease in the levels of both glutathione and vitamin C with aging.

Keywords: *Diabetes mellitus, Adiponectin, Antioxidants.*

Introduction

Diabetes mellitus develops as a result of many metabolic disorders, including disorders that occur in the adipose tissue, which is one of the leading causes of diabetes, especially type II, which in turn is caused by a condition called insulin resistance [1]. On the other hand, adipose tissue is an endocrine organ that produces a large number of biologically active hormones or cytokines, where recent research indicates that the adipose tissue has the ability to produce a number of hormones, which play a large role in the prevention of the body of many metabolic disorders, including the case of insulin resistance, Some of these hormones are (adiponectin, leptin, resistin, and interleukins) [2].

Adiponecin was first described more than two decades ago by Scherer *et al.* 1995, this hormone is abundantly produced by adipose tissues, the adiponectin gene is located on chromosome 3q27 [3] in a region recently mapped as a susceptibility locus for type II diabetes and adiposity [4]. Contrary to expectations, despite its production in adipose tissue, Adiponectin was found to be decreased in obesity [5]. In humans, adiponectin is encoded by Adipo Q gene [6]. Adiponectin is an adipocytokine hormone that has many positive effects on metabolism, including improving insulin function and reducing atherosclerotic processes [7].

Adiponectin acts as a hormone with insulin-sensitive and anti-inflammatory properties [8], as well as having a significant relationship with cardiovascular risk factor in type II diabetic patients [9]. Apart from its main activities, adiponectin has shown to modify eating and energy consumption during fasting (increase food intake and low energy consumption) by influencing the central nervous system [10].

Adiponectin has an effect on the energy and metabolism of glucose and fat through phosphorylation and the activation of adenosine mono-phosphate-activate protein kinase (AMPK) [11].In addition, diabetes may develop as a result of the increase in oxidative stress, which is a temporary or chronic condition in the body.

The increase in the production of active Reactive Oxygen Species (ROS) is a major manifestation of type II diabetes [12, 13]. The increase in ROS production may weaken cellular metabolic processes and may destroy the components of the cell [14]. Therefore, in healthy cells, there are antioxidant defense mechanisms that remove the active molecules from the body and maintain a low degree of oxidative stress, this is characterized by many substances that have the ability to offset the oxidative stress of the active varieties of the body, including catalase and superoxide dismutase enzymes, as well as glutathione peroxidase, as well as non-enzymatic substances such as glutathione [15, 13]. Glutathione is an important antioxidant in the body, which in turn regulates the oxidative environment or reverses oxidative damage [16].

It is a non-protein thiol component. It has been found that the presence of the free thiol group in the glutathione provides a major protection against oxidation, as the thiol group removes free radicals and the thiol group is oxidized by a two-component compound GSSG, that is why glutathione contains the two forms, which are GSH and the oxidative form GSSG in most human cells where the percentage of GSSG / GSH is 1/10 [17].

Superoxide dismutase is an enzyme classified as metallo protein that stimulates the conversion of the superoxide anion $O_2^{\bullet -}$ to hydrogen peroxide H_2O_2 [18]. Thus, the superoxide dismutase is an important antioxidant that reduces stress oxidation and preservation of the cellular membrane from damage caused by the presence of superoxide radical [19].

Vitamin C, an antioxidant, is found in food in the form of ascorbic acid (AA), its oxidizing form is known as dehydro ascorbic acid (DHAA), which has vitamin C activity, the reason for the inability of the body's cells to produce ascorbic acid is due to the absence of active enzyme L- gulonolocton oxidase, both ascorbic acid and dehydro ascorbic acid are the physiologically active forms of vitamin C [20].Catalase is an enzyme found in almost all organisms (such as bacteria, plants and animals), which stimulates the degradation of hydrogen peroxide into water and oxygen [21].

It is an important enzyme in protecting the cell from oxidative damage by reactive oxygen species, one molecule of catalase can convert approximately 6 million molecules of H_2O_2 into water and oxygen per minute [22, 23].Therefore, because of the important role played by adipose tissue hormones and antioxidant in all the functional activities of the body and the consequent and metabolic damage in the disturbance of secretions in the body, the current study tries to investigate the relationship between the concentrations of adiponectin secreted from the adipose tissue and some antioxidants with patients with the Type II diabetes.

Materials and Methods

The Control Group

In the present study, 30 Serums were used. These were taken from healthy men after confirming that they did not have diabetes. These men range between 30 to 65 years. The samples were collected on the basis of three age groups of 10 samples per age group.

The Patients' Group

A total of (70) serum samples were collected from people with type II diabetes. The samples were divided according to age groups as follows:

- 22 serum samples of people with diabetes whose age range between 30-39 years.
- 24 serum samples of people with diabetes whose age range between 40 and 49 years.
- 24 serum samples of people with diabetes whose age range between 50-65 years.

The samples were collected form cases diagnosed by specialized doctors in cooperation with the Specialized Centre for Diabetes and Endocrinology in Thi Qar Health Directorate.

Preparation of Serum

Blood samples were taken in the morning between 8.30 and 11.30 hours when patients were examined in the Specialized Centre for Diabetes and Endocrinology by specialist doctor who sent them to the laboratory unit of the Center. At this time, the patient must be fasting for 8-12 hours; the laboratory worker withdraws about 4 to 5 ml of the blood from the Antecubital vein. The blood is placed in a special tube (gel tube) and left for

5-10 minutes after which the sample centrifugation is performed at 3500 (r.p.m) for 15 minutes (to get the serum), after which the serum was divided and placed in an eppendorf tube to avoid remelting, the samples were kept at -20 ° C until the tests were performed.

Determination of the Concentration of Adiponectin Hormone and Antioxidants in the Serm

The method of Enzyme-Linked Immunoassay (ELISA) (type sandwich) technique was adopted in the study to estimate the concentration of adiponectin and antioxidants throughout using the ELISA Reader (type Biotek-USA) and the Kits equipped by Elabascince Corporation (USA).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 23 was used in the statistical analysis of data, where both T-test and ANOVA were employed to compare the mean of control group samples with patient's samples at the ($P \leq 0.05$) probability level.

Results

The results of the present study showed a significant decrease at the probability level ($P \leq 0.05$) in adiponectin hormone in patients with diabetes (17.75 ± 6.31 pg / mL) compared to its level in the healthy people (25.64 ±5.53 pg / mL) as shown in Table (1). The results also showed a significant decrease at ($P \leq 0.05$) in glutathione in patients with diabetes (30.38 ±6.42 µg / mL) compared to its level in the healthy people (37.0 ±8.68 µg / mL).

Moreover, the results showed a significant increase at ($P \leq 0.05$) in SOD in patients with diabetes (1319.16 ±235.70 pg / mL) compared to its level in the healthy people (828.57 ±181.34 pg / mL). As can be observed in table (1), there was a significant decrease at ($P \leq 0.05$) in the catalase in patients with diabetes (120.50 ±25.19 pg / mL) compared to its level in the healthy people (157.85 ±39.96 pg / mL).

The results have also showed a significant decrease in vitamin C in patients with diabetes (30.16 ±3.95 µg / mL) compared to its level in the healthy people (35.28 ±4.24 µg / mL) as in table (1).

Body Mass Index (BMI)

The results of the present study showed a significant increase at the probability level ($P \leq 0.05$) in the BMI in patients with diabetes type II (28.91 ± 14.41 Kg / H2) compared to its level in the healthy people (25.87 ±2.72 Kg / H2) as can be seen in Table (1).

Table 1: The Concentration of Adiponectin and Antioxidants in the Type II Diabetic Group Compared to the Control Group

Parameters	mean ± Standard Deviation	
	Control Group	Patients' Group
Adiponectin (pg/mL)	25.64 ± 5.53	17.75 ± 6.31*
Glutathione (µg/mL)	37.0 ± 8.68	30.38 ± 6.42 *
Superoxide dismutase(pg/mL)	828.57 ± 181.34	1319.16 ± 235.70 *
Catalase (pg/mL)	157.85 ± 39.96	120.50 ± 25.19 *
Vitamin C (µg/mL)	35.28 ± 4.24	30.16 ± 3.95 *
BMI (Kg/H2)	25.87 ± 2.72	28.91 ± 4.41*

* Significant difference at ($P \leq 0.05$) compared to the control group

Effect of Age

The effect of age on the level of adiponectin and antioxidants was studied in patients with type II diabetes. The results proved that with aging, there was a significant increase in SOD in diabetic patients, while there was a significant decrease in levels of both

vitamin C and glutathione. On the other hand, there were no significant differences in both adiponectin and catalase among the different age groups of diabetic patients as in Table (2).

Table 2: the Concentration of Adiponectin and Antioxidants in Type II Diabetic Patients according to Age Group

Parameters	Age Group			P-value
	(30-39)	(40-49)	(50-65)	
Adiponectin (pg/mL)	19.40 ± 5.08	16.28 ± 4.27	15.95 ± 5.51	P = 0.151
Vitamin C (µg/mL)	32.72 ± 8.10 [a]	31.66 ± 6.01 [b]	26.47 ± 5.43 [c]	P = 0.021
Catalase (pg/mL)	126.36 ± 46.03	126.95 ± 71.19	124 ± 31.92	P = 0.216
Glutathione (µg/mL)	32.18 ± 8.15 [a]	25.19 ± 5.67	19.19 ± 2.82 [b]	P = 0.001
Superoxide dismutase (pg/mL)	1184.09 ± 427.44 [a]	1395.23 ± 344.20	1414.28 ± 444.16 [b]	P = 0.000

A, b, c the different letters indicate significant differences (P≤ 0.05) between groups
Values represent: mean ± standard deviation

The relationship between the level of adiponectin and antioxidants was also studied in different age groups in comparison to the corresponding age groups of the healthy people. The results Table (3) showed that there was a significant decrease in the level of adiponectin, vitamin C and glutathione, and on the other hand, a significant increase in superoxide dismutase in the serum of patients with type II diabetes in the first group (30-39 years) when compared to the control group within category of age itself. There was also a significant decrease in the level of adiponectin, catalase, glutathione and a significant increase in superoxide dismutase in the serum of type II diabetics within the second age group (40-49 years) when compared to the control group within the same age group as in Table (4). In the third age group (50-65 years) (Table 5), there was a significant increase in the superoxide dismutase and a significant decrease in adiponectin, vitamin C, catalase and glutathione when compared to the control group within the same age group.

Table 3: Comparison of Type II Diabetic Patients with the Control Group within the First Age Group (30-39 years)

Parameters	mean±Standard Deviation	
	Control Group	Patients' Group
Adiponectin (pg/mL)	25.30±6.73	19.40±7.08 **
Vitamin C (µg/mL)	34.60±6.05	31.72±5.10 *
Catalase (pg/mL)	138±37.13	126.36±26.03
Glutathione (µg/mL)	36.30±9.71	32.18±8.15 *
Superoxide dismutase (pg/mL)	793±117.07	1184.09±227.44 **

* P< 0.05 , ** P< 0.01 , *** P< 0.001

Table 4: Comparison of Type II Diabetic Patients with the Control Group within the Second Age Group (40-49 years)

Parameters	mean±Standard Deviation	
	Control Group	Patients' Group
Adiponectin (pg/mL)	24.20±4.40	16.28±3.27 ***
Vitamin C (µg/mL)	33.40±4.11	32.66±6.01
Catalase (pg/mL)	175±35.22	130.95±41.19*

Glutathione (µg/mL)	35.20±7	30.19±9.67*
Superoxide dismutase(pg/mL)	988±153.35	1514.28±244.16***

* P< 0.05, ** P< 0.01, *** P< 0.001

Table 5: Comparison of Type II Diabetic Patients with the Control Group within the Third Age Group (50-65 years)

Parameters	mean±Standard Deviation	
	Control Group	Patients' Group
Adiponectin (pg/mL)	25.10±6.13	15.95±4.51 ***
Vitamin C (µg/mL)	37±8.44	26.47±5.43***
Catalase (pg/mL)	155±33.59	102.38±25.92***
Glutathione (µg/mL)	39.30±9.77	29.19±7.82**
Superoxide dismutase (pg/mL)	715±168.4	1295.2±244.2***

* P< 0.05, ** P< 0.01, *** P< 0.001

Effect of the Smoking

The effect of smoking on the level of adiponectin and antioxidants was studied in patients with type II diabetes. The results Table (6) showed that there was a significant decrease in the level of adiponectin and catalase in smoking diabetic patients compared to non-smokers. There was also a significant increase in the level of superoxide dismutase in smoking diabetic patients compared to non-smokers.

Table 6: The Concentration of Adipocetin and Antioxidants in Type II Diabetes Patients according to smoking

Parameters	mean±Standard Deviation		P-value
	Smokers	Non-smokers	
Adiponectin (pg/mL)	14.58±3.34	19.92±3.97	**P = 0.005**
Vitamin C (µg/mL)	31.44±5.36	30.84±2.23	**P = 0.542**
Catalase (pg/mL)	115±16.62	130.69±30.73	**P = 0.027**
Glutathione (µg/mL)	31.64±4.85	29.72±3.84	**P = 0.098**
Superoxide dismutase (pg/mL)	1348.48±134.54	1274±114.67	**P = 0.001**

Discussion

The most effective factor in decreasing the concentration of adiponectin in patients with type II diabetes is obesity [24], the process of building adiponectin in adipose cells consumes high energy, so its structure is regulated in adipose cells by the cellular energy production system(mitochondria), the adiponectin is largely built in small adipose cells because of the increased mitochondrial activity, In contrast, the process of building adiponectin in Bloated adipose cells is reduced due to impaired function or decreased mitochondrial activity, therefore, when Bloated adipose cells increase in obese people, the levels of adiponectin decrease [25, 27].

The results of some studies indicate that the low expression of adiponectin in adipose tissue may be due to insulin resistance, which has resulted mainly from obesity or increase of the proportion of triglycerides in the blood, as one of the most important reasons for resistance to insulin is increased triglycerides, which in turn lead to disruption of the work of hormone Insulin [28, 29]. There was also evidence that insulin stimulates adiponectin secretion in rodents, so adiponectin levels may reduce in type II diabetic patients due to insulin resistance. Moreover, adiponectin levels may decline in obese individuals due to the accumulation of triglycerides, which in turn affect the genetic expression of adiponectin [30, 31].

On the other hand, it has been found that the increase in the levels of adiponectin may be associated with a decrease in the concentration of triglyceride and glucose; these changes suggest that adiponectin increases the sensitivity of cells to insulin. Adiponectin increases the sensitivity of insulin in part by enhancing phosphorylation

and activating AMPK in skeletal muscles, liver and adipose cells, where the AMPK signal affects many aspects of cellular metabolism including glucose metabolism and adipose acid oxidation [32].

The relationship between adiponectin and insulin sensitivity has been observed through the ability of the hormone to stimulate the use of glucose frequently by cells in the case of high levels in the blood. In addition, the gene expression of adiponectin in rodents has been shown to be strongly influenced by changes in adipose mass and insulin sensitivity [33, 34].

The decrease in the level of glutathione in serum in diabetic patients may be due to the active participation of the glutathione in preventing oxidative stress by direct removal of the free radicals, thus reducing oxidative stress and decreasing serum levels [35].

Furthermore, the level of glutathione decreases in the serum of diabetic patients because of its work on the re-formation of some antioxidants such as vitamin C, as it renews vitamin C, which is oxidized significantly in diabetics, the low levels of glutathione in patients with type II diabetes may be because of the glycation for enzyme that stimulates synthesis of glutathione (enzyme γ - glutamyl cystein synthetase), this process results in the formation of low amounts of GSH in diabetic patients [36, 37].

In addition, under hyperglycemia, up to 30% glucose is channeled to the Polyol pathway, resulting in a significant depletion of NADPH used as an adjuvant to reduce glucose to sorbitol in this path; this pathway depletes NADPH, which causes a significant reduction in the level of glutathione in diabetic patients [38].

The increased efficacy of the superoxide dismutase may be resulted from oxidative stress which contributes to an increase in the production of the superoxide anion $O_2^{\bullet -}$ in patients with type II diabetes. This may in turn lead to increase enzyme efficiency to eliminate the generated free radicals [39]. The high SOD in diabetic patients indicates high levels of the superoxide anion$O_2^{\bullet -}$, which is an indication of the high oxidative stress in these patients. Thus, increasing the amount of $O_2^{\bullet -}$ alerts cells to the synthesis of Cu / Zn -SOD in diabetics in order to protect the cell from free radicals damages [40].

The increase in the level of superoxide dismutase with age may be resulted from the increase of oxidative stress with age, which in turn increases the production or generation of $O_2^{\bullet -}$ in patients with diabetes, which leads to increase the level of the enzyme in the body in order to get rid of the generated free radicals [39]. High glucose levels in the serum of diabetic patients also stimulate oxidative stress as a result of free radicalts, thus contributing to the reduction of certain antioxidants, including catalase [41].

Consequently, the decrease in catalase may be caused by hypoglycemia, which in turn causes degradation of Peroxisomes [40]. Alternatively, it could be resulted from the disruption of the catalase through glycation, which is in turn is obtained as a result of high glucose and therefore the increase in blood sugar in patients with type II diabetes. Subsequent reactions of proteins may affect nearby amino acids, thus causing structural and functional changes in the molecules.

In addition, low levels of catalase are an indication of the high concentration of hydrogen peroxide H_2O_2 in diabetic patients, which increases oxidative stress and complications of diabetes [42].The decreased concentration of vitamin C in the serum of diabetics confirms oxidative stress, as vitamin C works to remove free radicals such as H_2O_2, OH, O2- and helps to protect cells and reduces tissue damage [43].

The decrease in the level of vitamin C in patients with type II diabetes may be due to the consumption of vitamin reactive oxygen, which is one of the most important antioxidants because it reacts quickly to free radicals, especially of pyroxyl ($ROO^{\bullet}$) [44], or may consume as a result of its use in the regeneration of vitamin E, which is subsequently consumed by reactive oxygen species [45].

Moreover, the increase of blood sugar in patients with diabetes can promote the loss of renal vitamin C by inhibiting the reabsorption of renal ascorbic acid [46]. On the other hand, the decrease in both vitamin C and glutathione in diabetics with aging may be because of the increase in free

radicals, which leads to loss of balance between free radicals activity and antioxidants. This will result in a decrease in the level of these antigens in the blood [47].

The decrease in the concentration of some antioxidants in type II diabetics in most age groups is due to the increase in free radicals production resulted from oxidative stress, which leads to a loss of balance between levels of antioxidants and reactive oxygen species [48].

The results of the present study also showed a significant decrease in the level of adiponectin and catalase enzyme in smoking patients with diabetes compared to non-smokers, while it was observed that there was a significant increase in the level of superoxide dismutase in smoking diabetics compared to non-smokers, as the decrease in the level of adiponectin in smokers may be because smoking increases the oxidative stress, which reduces the production of adiponectin and its expression throughout inhibiting the function of phosphatidylinositol 3 -Kinase in adipose cells.

Also, the same nicotine stimulates the decomposition of lipids by activating the Nicotinic Cholinergic Receptors in the adipose tissue, or smoking may suppress adiponectin gene expression by up-regulating post-ganglionic sympathetic nerves, In addition, there may be an increased consumption of adiponectin in smokers due to its association with the collagen of infected blood vessels, which leads to decrease its levels [49]. Moreover, smoking is the source of many reactive oxygen species(ROS), including the superoxide anion $O_2^{\bullet -}$ whose main source is smoke, which leads the cell to increase the production of SOD enzyme in order to dismantle it to H_2O_2 before damaging the cell and its contents. Furthermore, continuous smoking leads to the accumulation of H_2O_2 which is resulted from the decomposition of superoxide anion which breaks down into H_2O and O_2 by the catalase enzyme and thus higher levels of hydrogen peroxide leads to decrease the levels of catalase in smokers [50].

Conclusion

There is a significant reduction in the level of adiponectin in patients with type II diabetes and can therefore be used as an important predictor of the disease. The study concluded that fat or obesity is the main cause of the decrease in the level of adiponectin in the blood and, thus, the possibility of causing type II diabetes. It is also clear that the increase in the level of sugar in patients with type II diabetes plays a key role in increasing oxidative stress, which eventually leads to the reduction of some antioxidants, including glutathione, catalase and vitamin C. It is concluded that there is a possibility to use adiponectin as a therapeutic hormone for diabetes type II because of the role it plays in increasing the sensitivity of insulin to sugar and regulating its level in the blood.

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