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Role of Adiponectin in Development and Progression of Diabetic Complications

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Abstract
This study aimed to elucidate the role of adiponectin in evolution of microvascular complication of type 2 diabetes mellitus, in particular retinopathy and nephropathy.
In addition, we attempted to find a correlation between the serum levels of adiponectin and different parameters such as duration of diabetes, age, sex, glycemic state, insulin resistance, lipid profile and albumin-creatinine ratio in type 2 diabetic patients with retinopathy and nephropathy in order to understand more about these risk factors .
This study involved sixty diabetic patients with type 2 diabetes mellitus with or without micro vascular complications involving either retinal or renal vasculatures or both. These patients were recruited from the outpatient clinic (A written consent was obtained from each case included in the study according to the Ethical Committee Approval of the Research Institute of Ophthalmology (RIO), Giza, Egypt. In addition to fifteen healthy subjects were involved and served as reference group.
In addition to serum levels of adiponectin, routine diabetic diagnostic markers such as blood glucose levels, glycosylated Hb, insulin resistance, lipid profile, and kidney function tests were performed to all individuals included in the study.
Full ophthalmological assessment including Fundus Fluorescein Angiography and clinical examinations (blood pressure, search for lower limb oedema, pallor, renal mass, etc.) have done for each subject.
Data of this study revealed that the levels of serum adiponectin were decreased in patients with diabetic retinopathy while they were increased in patients with either diabetic nephropathy alone and to a lesser extent, in diabetic patients with nephropathy and retinopathy.
Serum adiponectin levels were found to be correlated with some parameters of lipid profile in patients with mixed conditions, a notion that highlight the role of adiponectin in the pathogenesis of microvascular complications of diabetes. Moreover, these results suggest that increased total serum adiponectin may predict coexistence of vascular endothelial dysfunction in diabetic nephropathy patients. Therefore, we recommended that adiponectin can be used as a prognostic marker for diabetic complications which will initiate a new era for the management of diabetes.

Introduction

Adiponectin, which is mainly produced in white adipose tissue (WAT), characteristically differs from most adipokines as it is negatively correlated with obesity.

Adiponectin, a hormone, exerts multiple biological effects throughout the body mediated by the specific receptors AdipoR1, AdipoR2, and T-cadherin. [1,2] Adiponectin has been reported to have a broad spectrum of effects, including antiatherogenic, anti-inflammatory, and insulin-sensitizing properties [3]. Adiponectin may decrease T2DM risk via a number of mechanisms including hepatic fatty acid oxidation, enhanced peripheral glucose uptake, and stimulated insulin secretion [4].

Diabetic retinopathy (DR) is a common micro vascular complication in patients with diabetes and may have a sudden and debilitating impact on visual acuity, eventually leading to blindness [5]. Diabetic retinopathy progresses from mild nonproliferative abnormalities (characterized by increased vascular permeability) to moderate and severe nonproliferative diabetic retinopathy (NPDR) (characterized by vascular closure) to proliferative diabetic retinopathy (PDR) (characterized by the growth of new blood vessels on the retina and posterior surface of the vitreous. Macular oedema, characterized by retinal thickening from leaking blood vessels can develop at all stages of retinopathy. New blood vessels of PDR and contraction of all accompanying fibrous tissue can distort the retina and lead to tractional retinal detachment, producing severe and irreversible vision loss. [6]

Yilmaz et al. reported that adiponectin plasma concentrations are lower in patients with diabetic retinopathy than those without it and are involved in the development of diabetes and diabetic retinopathy. [7]

Diabetic nephropathy (DN) is one of the most common micro vascular complications of diabetes [8], defined as rise in urinary albumin excretion rate, often associated with an increase in blood pressure but without evidence of other causes of renal disease. [9,10] Specifically, it represents a major cause of morbidity and mortality in diabetic subjects [8]. In both type 1 and type 2 diabetic patients, it is found that adiponectin is inversely associated with renal function [11–13].

Subjects and Methods

This study included sixty adult diabetic patients type 2 of both sexes (from 40-60) who were under hypoglycemic agents, no exogenous insulin intake.

The patients included in this study were classified into four groups:

Group 2: fifteen diabetic patients without renal or retinal involvement.

Group 3: fifteen diabetic patients with retinopathy, no renal involvement.

Group 4: fifteen diabetic patients with nephropathy, no retinal involvement.

Group 5: fifteen diabetic patients with retinopathy and nephropathy.

Commercially available kits purchased from (Spectrum) were used in this study.

Serum markers of these groups were compared to control group (Group 1) which consisted of fifteen normal, non diabetic individuals.

Full ophthalmological assessment including Fundus Fluorescein Angiography and clinical examinations (blood pressure, search for lower limb oedema, pallor, renal mass, etc.) were performed to all the study subjects.

Fasting blood samples were collected from all the subjects and sera were separated and used for analysis of biochemical parameters including fasting blood glucose, glycosylated Hb, fasting insulin levels, calculated insulin resistance, lipid profile (total cholesterol, triglyceride, HDL and LDL), kidney function(urea and creatinine) and finally, adiponectin levels (Orgenium Laboratories).

Urine samples were also collected at the same time and used to determine albumin/creatinine ratio.

Statistical Analysis: All results were expressed as the mean SD. Statistical analysis was performed with *Statistical Package for the Social Science for Windows* (SPSS, version 16.0).

Results

Fasting blood glucose levels (FBS) in groups 2, 3, 4 and 5 showed a significant elevation when compared to that of the control group. In the meantime, patients of groups 3, 4 and 5 (diabetic patients with renal and / or retinal involvements) exhibited significant increase in FBS levels when compared to that of the diabetic patients (Group2).

There was a statistically high significant increase in HBA1c in diabetic groups with and without complications (group 2,3,4 and 5) in comparison with its percentage in the control group and also on comparing its percentage in group (1) and groups (2 and 3), HBA1c was increased in groups 4, 5 and 3 when compared to its percentage in group (2) and this increase was statistically highly significant ($P < 0.0001$).

Insulin resistance, measured as HOMA-IRfasting insulin (micro IU /L)*fasting glucose (mg/dl)/405), in all diabetic patients showed a significant increase compared to normal adults. Diabetic patients with complications (G3,4 and 5) show more insulin resistance than patients without complications.

Table (2) demonstrated the lipid profile pattern in all groups: There were significant elevations in serum cholesterol, triglycerides and LDL while HDL showed significant reduction compared to normal control. Diabetic patients with complications had significantly higher levels of serum cholesterol, triglycerides and LDL while they had significantly lower HDL level than diabetic patients without complications.

In this study, there was a significant increase in the levels of serum urea in diabetic patients (Groups 3, 4, 5) in comparison with its corresponding levels in the control group ($P < 0.0001$). Non-significant increase in the level of serum urea in group (2) when compared to its corresponding levels in the control group. There was non-significant difference between the levels of serum creatinine in groups (2&3) and its corresponding level in the control group. Significant increase in the levels of serum creatinine in group 4 and 5 was present when compared with its corresponding level in groups (2&3) and the control group. The differences of Albumin/creatinine ratio were significant between all diabetic patients and control group. In diabetic patients with complications (G3, 4 and 5) these differences were significantly increased than group 2.

Results of adiponectin in diabetic patients with nephropathy and with mixed condition(G 4,G5) were significantly increased while its levels were significantly decreased in diabetic patients without complications and patients with retinopathy(G2, G3) when compared to the normal levels of control subjects.

Table (1): Fasting serum glucose, Glycosylated hemoglobin, Fasting serum insulin and HOMA-Insulin Resistance of participants in the different studied groups

Parameters Groups	Glycemic Status			
	Fasting Serum Glucose (mg/dl)	Glycosylated Hb (%)	Fasting Serum Insulin (microIU/ML)	Insulin Resistance Mass/unit
Group 1	86.5+5. 1	4.2+0.17	15.1+0.6	3.18+0.28
Group 2	117.7+20.1	7.5+0.49	26.9+1.4	7.77+1.44
Group3	253.7+70.8	10.8+1.34	33.7+25.6	16.91+5.13
Group 4	254.7+62.4	9.9+1.16	10.3+3.4	6.53+2.91
Group 5	180+30.9	9.5+2.61	13.3+5.3	5.79+2.10

Table (2): Lipid profile of participants in the different studied groups

Parameters Groups	Lipid Profile			
	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group 1	115.3+14.3	66.8+12.9	61.8+3.8	40.2+16.7
Group 2	176.1+40.3	96.7+31.3	53.1+3.4	103.4+41.8
Group 3	237.1+51.7	226.3+73.3	41.4+6.5	142.1+31.7
Group4	263.7+55.5	224.1+59.1	43.9+7.4	155.8+37.9
Group5	239.1+68.9	265.9+52.5	41.5+5.6	125.9+28.8

Table (3): kidney functions of participants in the different studied groups

Parameters Groups	Kidney Functions		
	Urea (mg/dl)	Creatinine (mg/dl)	Albumin/Creatinine Ratio
Group 1	20.3+3.5	0.83+0.12	6.8+5.4
Group 2	23.2+6.1	0.87+0.16	7.7+6.3
Group 3	28.3+7.7	0.79+0.13	8.8+3.8
Group4	95.9+7.9	3.42+0.70	112.9+56.7
Group5	67.9+11.6	1.94+0.30	45.7+24.3

Table (4): Adiponectin concentrations of participants in the different studied groups

Parameters Groups	Adiponectin (ng/ml)
Group 1	13.3+1.27
Group 2	6.1+1.38
Group 3	2.8+0.97
Group4	68.6+18.53
Group5	31.6+9.06

Discussion

Adiponectin is an adipokine that is specifically and abundantly expressed in adipose tissue and directly sensitizes the body to insulin. Hypoadiponectinemia, caused by interactions of genetic factors in the adiponectin gene, insulin resistance, type 2 diabetes, obesity, coronary disease and hypertension [14].

Serum total adiponectin not only has been shown to be related to diabetes mellitus (type2) without complications [15] but also to diabetic patients with complications as those with diabetic retinopathy [16] and diabetic nephropathy [17].

This study tries to determine the role of total serum adiponectin in diabetic patients (type 2) and the correlation of it with the progression of the disease and its complications mainly diabetic retinopathy and diabetic nephropathy.

The results presented in this study (table4) showed a significant difference (decrease) in total serum adiponectin values in diabetic patients (Group 2) compared to healthy controls. Total serum adiponectin level was lower in patients with PDR (Group 3) and the difference between its level in diabetic patients (Group 2) and the healthy controls (Group 1) was significant.

These findings were consistent with those of Sameha et al. [18] who have showed lower levels of total serum adiponectin (TSA) in diabetic patients without complications & that the decrease in serum concentration of adiponectin was associated with the severity of diabetic retinopathy. Also, these findings were in agreement with the results of Skiko et al. [19] who have showed that adiponectin suppresses pathological micro vessel formation in retina of mice which was similar to that observed in the human ischemic retinopathy as PDR. The results of this study were against the results of Matsuda et al. [20] who have showed that adiponectin level was not associated with the presence of diabetic retinopathy.

In the current study, a statistical significance increase in TSA was shown in diabetic patients with nephropathy G4. These results agreed with the results of Julie et al. [21] and these of Ran J.et al. [22] who have concluded a higher serum adiponectin concentration which was associated with reduced renal function in type 2 diabetics as the kidney was involved in the degradation and/or elimination of adiponectin, although it was unlikely to be the sole mechanism.

Yilmaz et al. reported that vascular endothelial dysfunction existed in untreated early diabetic nephropathy patients with severe proteinuria and decreased plasma adiponectin concentration, which was contradictory to our results. [23]

In this work, in Group 5 that included diabetic patients with retinopathy and nephropathy, adiponectin level showed significant increase than its corresponding levels in Group 2 and Group 3 but its level was significantly decreased than the corresponding level in Group 4. These results could be explained by the balance between the increased level of total serum adiponectin in diabetic nephropathy and its decreased level in diabetic retinopathy.

Inflammation could be a common antecedent for diabetes. Hyperglycemia and insulin resistance could also promote inflammation, and may be factor linking diabetes to the development of atherosclerosis. Elevated glucose levels could promote inflammation by increased oxidative stress. [22,23,24] Another possibility is that inflammatory response is a result of vascular complications following diabetes.

The acute-phase response refers to a non specific and complex reaction of the individual that occurs shortly after a tissue injury. The origin of this reaction can be attributable to infectious, immunologic, neoplastic, traumatic or other causes and its purpose is to restore homeostasis and to remove the cause of its disturbance. This response, among other systemic effects, includes the change in serum concentrations of the so-called acute phase proteins (APP). [25]

There is increasing evidence that acute-phase response is closely involved in the pathogenesis of type 2 diabetes and associated complications such as dyslipidemia and atherosclerosis. [26] Elevated circulating

inflammatory markers such as C-reactive protein(CRP) and interleukin-6 predict the development of type 2 diabetes. [26]

CRP causes expression of endothelial adhesion molecules [27] and Chemoattractants[28] and mediates LDL uptake by macrophages.[29] Bound CRP activates complement, colocalizes with it in human hearts during acute myocardial infarction[32], and increase infarct size after experimental coronary artery ligation.[33] Cytokines such as IL-6 and TNF-α have many pro-atherosclerotic actions, including promoting leukocyte recruitment to the endothelium by inducing adhesion molecule and Chemoattractants synthesis and increasing capillary permeability. [34] Such cytokines may be produced by the endothelium, smooth muscle cells, and macrophages at the site of atherosclerosis and contribute to a systemic acute-phase response, and/or cytokinemia and augmented acute-phase reactants inherent to type 2 diabetes may promote arterial disease [28].

In our study, C-reactive protein was positive in seven patients of Group 2, 10 patients of Groups 3, 4 patients in Group 4 and 12 in Group 5.The highest level of TSA was in Group 4 which has the least number of diabetic patients with positive CRP. This result may support the fact that adiponectin have anti-inflammatory activity.

For the two studied multiple biochemical pathways have been proposed to explain pathogenesis of diabetic retinopathy, all starting initially from hyperglycemia. These mainly include increased polyol pathway, increased advanced glycation end- products (AGEs) formation, activation of protein kinase C (PKC) and increased hexosamine pathway flux. [35]AGEs are heterogeneous groups of macromolecules that are normally formed non-enzymatically by the interaction of reducing sugars with free amino groups of proteins, lipids and nucleic acids, but their formation increases under high glucose ambiance [36-38]. Engagement of AGEs to their receptors (RAGE) has been shown to play a critical role in diabetic complications. [39,40]

Among the most investigated AGEs is glycated hemoglobin (HbA1c) which is the product of the slow and largely irreversible reaction that occurs through non-enzymatic glycation of hemoglobin. [41] Chronic hyperglycemia, as measured by HbA1c, is an established risk factor for diabetes associated microvascular diseases. [42] A reduction of 1% in HbA1c is associated with a 37% decrease in microvascular endpoints. [43] In other studies, fasting plasma glucose concentration and HbA1c, each predict elevated albuminuria, defined as an albumin / creatinine ratio (ACR) ≥ 30 mg/g, after adjusting for age, sex and duration of diabetes.[44] The risk of hyperglycemia (HbA1c) amplifies the risk of microalbuminuriaconferred by increased systolic blood pressure. [45] In support, Nikzamir, A., et al. found that more severe albuminuria was significantly associated with higher levels of HbA1c among diabetic group with (normo-, micro- and microalbuminuria). In this regard, the study of El-Wakf, A. M., et al. (40) showed that, serum fasting blood sugar (FBS) was significantly increased in all diabetic subjects when compared to control. At the same time, all diabetic subjects showed significantly increased levels of HbA1c when compared to control subjects. For the two studied variables [(FBS) and (HbA1c)], statistically significant increases were detected on comparing each of diabetic groups with micro- and macro-albuminuria relative to normo-diabetic patients. Thus, indicating the importance of both glucose and HbA1c levels as predictors for developing nephropathy status (micro- and macro- albuminuria) among diabetic patients.[40]

Sato, K. K., et al. [47] found that combined measurement of FBS and HbA1c is effective for prediction of type 2 diabetes. Also, The International Expert Committee (2009). [48] reported on the role of HbA1c assay in the diagnosis of diabetes as there was a recommendation that an HbA1c value of 6.0 to < 6.5% is associated with diabetes risk and its complications.

In table(2),results of lipid profile showed that there was a significant increase of total serum cholesterol in groups of diabetic patients (groups 2,3,4&5) in comparison with its values in the control group (P <0.0001). There was a significant decrease in HDL-cholesterol levels in groups 2, 3, 4 and 5when compared to its

corresponding levels in the control group (P < 0.0001).There was a significant increase in LDL-cholesterol levels in groups 2,3,4 &5 in comparison with its levels in the control group (P <0.0001) but this increase was insignificant on comparing LDL-cholesterol levels between groups (2) and Group 5.

Adiponectin has potent effects on carbohydrate and lipid metabolism in skeletal muscle. Numerous studies have shown that treatment with the globular domain of adiponectin improves fatty acid utilization, both in isolated muscle as well as in cultured skeletal muscle cells [49, 50, and 51]. The action of adiponectin in muscle is mediated by adiponectin receptors, AdipoR1 and AdipoR2. In human skeletal muscle, AdipoR1 is expressed at the highest level, with lower levels of AdipoR2 [52]. The binding of globular and full-length adiponectin to adiponectin receptors increased PPARa activity and stimulated glucose uptake and fatty acid oxidation in myocytes [52].

The molecular mechanisms underlying adiponectin-dependent increase in muscle fatty acid oxidation include up-regulation of several genes involved in muscle lipid metabolism, such as fatty acid translocase (FAT/CD36); acyl-CoA oxidase (ACO), the rate-limiting enzyme of the b- oxidation pathway in peroxisomes [53]; and mitochondrial uncoupling protein2 (UCP2), accompanied by the induction of PPARa (peroxisomeproliferator-activated receptor alpha) gene expression and increase in PPARa activity [52, 51].

The nuclear receptor PPARa is required for transcription of many genes involved in fatty acid oxidation pathway [54], thus its activation by adiponectin may improve fatty acid utilization in muscle. Additional effect of adiponectin on skeletal muscle is an increased phosphorylation of AMP- activated protein kinase (AMPK) [52, 50]. Moreover, activation of AMPK has been shown to be necessary for adiponectin effects on fatty acid oxidation in skeletal muscle cells [49, 50, and 55]. AMPK activation triggers many metabolic changes that act to restore energy balance in muscle cells, such as increased glucose uptake and metabolism,and increased oxidation of fatty acids [56]. Regulation of fatty acid oxidation pathway by AMPK involves phosphorylation of acetyl- CoA carboxylase (ACC),which leads to the inhibition of ACC activity followed by a decrease in malonyl- CoA levels [56]. Adiponectin-dependent AMPK activation in skeletal musclewas associated with an increase in ACC phosphorylation and a decrease in the concentration of malonyl-CoA [52, 50]. Malonyl-CoA is an allosteric inhibitor of carnitinepalmitoyltransferase 1 (CPT-1), an enzyme responsible for the transport of fatty acids into mitochondria, where fatty acid oxidation occurs [57]. Thus a decrease in malonyl-CoA concentration after adiponectin treatment may be the reason for increased fatty acid oxidation in muscle [50].

Studies done by El-Wakf, A. M., et al. [40] showed the association between the incidence of diabetic complications (retinopathy, neuropathy and ischemic heart disease) and the development of diabetic renal disease starting by nephropathy. [58] The main risk factors for the development and progression of diabetic retinopathy are the duration of diabetes, glycemic state, blood pressure and co-existing nephropathy. [59] In order to explore such associations between diabetic retinopathyand diabetic nephropathy, some parameters like serum creatinine, serum urea and urine albumin/creatinine ratio were measured. The increase in serum creatinine is a well-accepted marker for impaired renal function.[60] Similar findings were also detected in the study done by El-Wakf, A. M., et al. [40] regarding the associationbetween the progression of diabetic renal disease and the increase in the levels of serum creatinine.

The urinary Albumin Creatinine Ratio (ACR) was used as a measure of albumin excretion.[61] Microalbuminuria, defined as an increased urinary albumin excretion rate in the absence of clinically compromised renal function, is a strong risk factor for overt nephropathy in patient with type (1) diabetes. [62, 63] In contrast, in patients with type (2) diabetes, microalbuminuria predicts cardiovascular rather than renal disease. [64-68] It has been suggested that microalbuminuria generally reflects a state of wide-spread endothelial dysfunction and/or vascular damage. [69]

Other workers have presented albuminuria as a powerful predictor of progression of nephropathy in patients with type 2 diabetes [70]. Albuminuria (or elevated urinary albumin excretion) may reflect underlying renal

expression of vascular damage, hypertension, endothelial dysfunction [73-74], and inflammation [72]. Festa, A., et al. [61] showed that chronic inflammation was associated with microalbuminuria in diabetic as well as non-diabetic subjects. This may be explained via elevations of acute-phase proteins and/or inflammatory cytokines that may alter glomerular function.

Table (3) showed that there was a significant increase in the levels of serum urea in diabetic patients (Groups 3, 4, 5) in comparison with its corresponding levels in the control group ($P < 0.0001$). Non-significant increase in the level of serum urea in group (2) when compared to its corresponding levels in the control group.There was a non-significant increase in the levels of urine albumin/creatinine ratio in groups (2&3) when compared to its corresponding level in the control group.Significant increase in the levels of urine albumin/creatinine ratio in group 4 and group 5 when compared to its corresponding level in groups (2&3) and the control group and this increase was statistically significant ($P < 0.0001$).There was non-significant difference between the levels of serum creatinine in groups (2&3) and its corresponding level in the control group. Significant increase in the levels of serum creatinine in group 4 and 5 was present when compared with its corresponding level in groups (2&3) and the control group.

In conclusion, this study shows that adiponectin may play a role in the pathogenesis of diabetic retinopathy. Moreover, these results suggest that increased total serum adiponectin may predict co-existing vascular endothelial dysfunction in diabetic nephropathy patients. We recommend the use of adiponectin in treatment of diabetes as a new era.

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