Original Article

Correlation Between Abnormal Liver Enzymes and Serum Adiponectin Level in Obese Type 2 Diabetic Patients.

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Abstract:

Background: Adiponectin seems to be secreted as a protective response to increased levels of pathologic adipocytokines and hypoadiponectinaemia is implicated in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), insulin resistance and the metabolic syndrome (MetS). Patients with obesity and co-morbid type 2 diabetes mellitus (T2DM) are at increased risk for NAFLD and cirrhosis compared to obese nondiabetic patients. **Aim:** This work was done to correlate between serum adiponectin level and abnormal liver enzymes (ALT and AST) in obese type 2 diabetic patients. **Patients:** Thirty obese type 2 diabetic patients (as patients group) and ten non diabetic obese subjects (as a control group) were included. Thorough Clinical examination was done, serum ALT, AST, fasting and 2 hours postprandial blood glucose, C-reactive protein , uric acid , HbA1c, s. triglycerides, s. cholesterol, s. HDL-cholesterol and s. adiponectin were measured. **Results:** Serum adiponectin was significantly lower in type 2 diabetic obese patients than non diabetic obese control subjects. Within the patient group serum adiponectin was significantly higher in female than male among obese type 2 diabetic patients, serum adiponectin correlated negatively with Alanine transaminase (ALT), Aspartat transaminase, age, systolic BP, diastolic BP, body mass index(BMI), W/H ratio, fasting blood glucose ,2 hours post prandial blood glucose, C-reactive protein , HbA1c, Uric acid, s. triglycerides and s. cholesterol in type 2 diabetic obese patients. But it correlated positively with HDL-cholesterol. **Conclusion:** Hypoadiponectimia is associated with NAFLD in type2 diabetic obese Egyptian patients especially among obese males.

Keywords: adiponectin, BMI, insulin resistance, MetS and NAFLD

Introduction:

Metabolic syndrome (MetS) refers to a condition associated with insulin resistance in which three of five signs must be present, including abdominal obesity, impaired fasting glucose, elevated blood pressure, hypertriglyceridemia and low HDL cholesterol**,** in addition to the classic five signs mentioned above, the metabolic syndrome is associated with a number of other conditions, including microalbuminuria, fatty liver, endothelial dysfunction, hyperuricaemia and systemic inflammation.[1]

Adiponectin is a hormone secreted by Adipocytes. It is found in relatively high circulating levels in plasma but is decreased in patients with NAFLD and in clinical states associated with insulin resistance such as MetS and type 2 diabetes mellitus**.** It regulates energy homeostasis and glucose and lipid metabolism.[2]

A low serum level of adiponectin has been found to be an independent risk factor for the development of hypertension in both cross sectional and prospective studies. Serum levels of adiponectin are significantly lower in individuals with essential hypertension than in normotensive healthy individuals, even after adjustment for confounding factors.[3]

Plasma adiponectin levels have been reported to be reduced in obese humans, particularly those with visceral obesity, and to correlate inversely with insulin resistance. Prospective and longitudinal studies have shown that lower adiponectin levels are associated with a higher incidence of diabetes.[4]

In our work serum adiponectin concentration was estimated in thirty obese type 2 diabetic patients and ten matched age and gender obese non diabetic as a control group from outpatient clinic.

Patients and Methods:

1-Patients:

This cross sectional study was conducted on 40 subjects from the outpatient clinic of Kasr El Aini Hospital. Faculty of Medicine. Cairo University. A written informed consent was obtained from all eligible patients. The research protocol was approved by the local university review committee.

These patients were categorized into:

Group 1: 30 obese type 2 diabetic patients

Group 2: 10 matched age and gender obese non diabetic subjects (as a control group)

All the patients included in the study were subjected to full history and thorough clinical examination. Parameters were obtained as age, gender, blood pressure (BP) in mmHg, weight (WT) in kg, height (HT) in cm, body mass index (BMI) in Kg/m^2, (WC) in cm, hip circumference (HC) in cm, waist / Hip ratio (W/H)

2- Investigations:

The patients were subjected to the following investigations:

1- Serum Alanine transaminase (ALT)

2- Serum Aspartat transaminase (AST)

3- Fasting blood glucose, 2 hours postprandial blood glucose

4- Serum uric acid (UA)

5- C-reactive protein (CRP)

6- S. Triglycerides, S. Total cholesterol, HDL-cholesterol (HDL).

7- Hemoglobin A1c (HbA1c)

8- Total serum adiponectin will be measured using Enzyme Linked Immune Sorbant Assay (ELISA) technique

NB: Fasting adiponectin in obese non diabetic subjects (as a control group) {4400-4700ng/ml in female} and {3700-3900ng/ml in male}.

All blood samples were drawn after 12 hours an overnight fast. Plasma samples were kept at - 70°C for subsequent assays.

Estimation of Serum adiponectin:

Adiponectin was assayed using ELISA technique AviBion Human Adiponectin (Acrp30) ELISA Kit Orgenium Laboratories Viikinkaari 6 FIN-00790 Helsinki FINLAND. Datacollection Anthropometric data including age, sex, height, weight and WC were collected. Height was measured with subject standing without shoes by standard stadiometer and the nearest one centimeter. Weight was measured with subjects wearing light clothing, the nearest 0.5kilogram was recorded. WC was measured at the end of a normal expiration, midway between the inferior margin of the ribs and the superior boarder of the iliac crest in a horizontal plane. Body mass index (BMI; kg/m2) was collected according to the Quetelet equation (weight in kilograms divided by height in square meter)

Statistical Analysis:

Data were statistically described in terms of range, mean ± standard deviation (± SD), frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using student t test for independent samples. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. Correlation between various variables was done using Pearson moment correlation equation for linear relation. A probability value (p value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 16 for Microsoft Windows.

Results:

As shown in table 5 above, there was strong negative correlation between adiponectin and (Age, Systolic BP, Diastolic BP, BMI, Waist circumference and W/H ratio). There was a moderate negative correlation between adiponectin and (Height and Hip circumference). There was no significant correlation between adiponectin and weight.

As shown in table 6, there was strong negative correlation between adiponectin and (ALT, AST, Fasting blood glucose, 2 hours post

prandial blood glucose C-reactive protein, Triglycerides and HbA1c). There was moderate negative correlation between adiponectin and (Uric acid and Cholesterol). There was moderate positive correlation between adiponectin and HDL-cholesterol.

Table 1: Descriptive statistics of clinical data of patients included in the study.

Variable	Minimum	Maximum	Mean±SD
Age(years)	38	58	46.40 ±5.92
Systolic BP(mmHg)	110	190	144 ±23.13
Diastolic BP(mmHg)	70	110	91.33 ±11.67
Weight (Kg)	69	105	87.10 ±9.02
Height (cm)	145	175	160.60±9.08
BMI (kg/m^2)	31	38.7	33.59 ±2.27
Waist circumference(cm)	109	142	121.50±10.53
Hip circumference(cm)	104	115	109.17±3.15
W/H ratio	1.03	1.28	1.12 ±0.07

Table 2: Descriptive statistics of clinical data of control group included in the study

Variable	Minimum	Maximum	Mean±SD
Age(years)	40	55	47 ±5.46
Systolic BP(mmHg)	110	130	117±6.75
Diastolic BP(mmHg)	70	90	79±5.68
Weight (Kg)	70	101	86.1±8.87
Height (cm)	150	180	165.9±9.05
BMI(kg/m^2)	31	31.5	31.29±0.16
Waist circumference(cm)	96	121	111.8±8
Hip circumference(cm)	106	128	115.1±5.84
W/H ratio	0.90	1.05	0.97±0.06

Table 3: Comparison between descriptive statistics of clinical data of patients and control group included in the study.

Variable	Patients (30) Mean±SD	Control(10) Mean±SD	P-value	Significance
Age(years)	46.40 ±5.92	47 ±5.46	0.779	N Sig
Systolic BP(mmHg)	144 ±23.13	117±6.75	0.001	Sig
Diastolic BP(mmHg)	91.33 ±11.67	79±5.68	0.003	Sig
Weight (Kg)	87.10 ±9.02	86.1±8.87	0.762	N Sig
Height (cm)	160.60±9.08	165.9±9.05	0.118	N Sig
BMI(kg/m^2)	33.59 ±2.27	31.29±0.16	0.000	Sig
Waist circumference(cm)	121.50±10.53	111.8±8	0.011	Sig
Hip circumference(cm)	109.17±3.15	115.1±5.84	0.000	Sig
W/H ratio	1.12 ±0.07	0.97±0.06	0.000	Sig

p-value is considered significant if < 0.05
Sig: significant
N Sig: non significant

Table 4: Comparison between descriptive statistics of investigations of patients and control group included in the study.

Variable	Patients(30) Mean±SD	Control(10) Mean±SD	P-value	Significance
ALT(u/l)	46.73±14.99	21 ±3.27	0.000	Sig
AST(u/l)	41.43±15.35	18.7±3.23	0.000	Sig
Fasting blood glucose (mg/dl)	134±35.91	80.3±4.64	0.000	Sig
Post prandial blood glucose (mg/dl)	194.80±48.66	107.5±12.51	0.000	Sig
Uric acid (mg/dl)	5.28±1.47	3.95±0.55	0.080	N Sig
C-reactive protein(mg/dl)	18.3±15.22	7.8±7.1	0.060	N Sig
Triglycerides(mg/dl)	168.63±39.14	110.30±7.42	0.000	Sig
Cholesterol (mg/dl)	213.57 ±43	188.2±40.23	0.109	N Sig
HDL-cholesterol(mg/dl)	41.2±8.62	45.2±6.6	0.189	N Sig
HbA1c %	6.29±0.57	5.96±0.34	0.090	N Sig
Adiponectin(ng/ml)	2693.3±863	4390±338.13	0.000	Sig

p-value is considered significant if < 0.05 Sig: Significant N Sig: Non significant

Table 5: Correlation between Adiponectin and clinical data of patients group included in the study

Variable	adiponectin in the patients		
	r	p-value	Significance
Age(years)	-0.810	0.000	Sig
Systolic BP(mmHg)	-0.814	0.000	Sig
Diastolic BP(mmHg)	-0.773	0.000	Sig
Weight (kg)	-0.293	0.116	N Sig
Height (cm)	-0.688	0.000	Sig
BMI(kg/m^2)	-0.862	0.000	Sig
Waist circumference(cm)	-0.854	0.000	Sig
Hip circumference(cm)	-0.688	0.000	Sig
W/H ratio	-0.832	0.000	Sig

p-value is considered significant if < 0.05 Sig: significant N Sig: non significant
r: is considered weak if < 0.25 , mild if ≥ 0.25- <0.5 , moderate if ≥ 0.5- <0.75 and strong if ≥ 0.75 .

Table 6: Correlation between Adiponectin and other investigations of patients group included in the study

Variable	adiponectin in the patients		
	r	p-value	Significance
ALT(u/l)	-0.943	0.000	Sig
AST(u/l)	-0.808	0.000	Sig
Fasting blood glucose (mg/dl)	-0.921	0.000	Sig
Post prandial blood glucose (mg/dl)	-0.877	0.000	Sig
Uric acid (mg/dl)	-0.699	0.000	Sig
C-reactive protein(mg/dl)	-0.806	0.000	Sig
Triglycerides (mg/dl)	-0.911	0.000	Sig
Cholesterol (mg/dl)	-0.537	0.002	Sig
HDL-cholesterol(mg/dl)	0.635	0.000	Sig
HbA1c %	-0.945	0.000	Sig

p-value is considered significant if < 0.05 Sig significant
r : is considered weak if < 0.25 , mild if ≥ 0.25- <0.5 , moderate if ≥ 0.5- <0.75 and strong if ≥ 0.75

Discussion:

It has been reported that the prevalence of obesity in adults is very high in Egypt, particularly among women, and that the prevalence of diabetes and hypertension parallels that of obesity. The overall prevalence of central obesity among Egyptian adults, according to the 2 indicators; WC and W/H ratio was 24.1% and 28.7% respectively. These figures are relatively high if the association of central obesity with morbidity and mortality is taken into consideration (Abolfotouh M A, et al.,2008).[5]

Adiponectin is a recently described adipokine that has been recognized as a key regulator of insulin sensitivity and tissue inflammation. It is produced by adipose tissue (white and brown) and circulates in the blood at very high concentrations. It has direct actions in liver, skeletal muscle and the vasculature, with prominent roles to improve hepatic insulin sensitivity, increase fuel oxidation [via up-regulation of adenosine monophosphate activated protein kinase (AMPK) activity] and decrease vascular inflammation (Whitehead J. P. , et al,.2006).[6]

Adiponectin seems to be secreted as a protective response to increased levels of pathologic adipocytokines as it possesses antidiabetic, antiatherogenic and anti-inflammatory properties (Kadowaki T et al., 2006).[7]

Hypoadiponectinemia has also been demonstrated to be independently associated with the metabolic syndrome indeed. Reduced plasma adiponectin levels are also commonly observed in a variety of states frequently associated with insulin resistance, such as cardiovascular disease and hypertension (Adamczak, M., et al. 2003).[8]

In our work serum adiponectin concentration was estimated in thirty obese type 2 diabetic patients with (24) females and (6) males (80% and 20% respectively) 70 % of them had fatty liver by abdominal U/S and ten (8 females and 2 males) matched age obese non diabetic as a control group.

There is a sexual dimorphism in the circulating levels of adiponectin, indeed, female humans have higher plasma adiponectin levels than males, suggesting that sexual hormones regulate the production of adiponectin, although it is controversial how these hormones, such as estrogen and testosterone, are involved in the regulation of plasma adiponectin level (Xu, A., et al. 2005)[9] Nevertheless, this may partially account for the fact that females are more sensitive to insulin than males.

In our work, there was negative correlation between Adiponectin and (Age, Systolic BP, Diastolic BP, Height, BMI, Hip circumference, Waist circumference and Waist/ Hip (W/H) ratio) in our 30 patients as shown in table 5. This was previously shown by Bilgili,et al.,2008[10] who stated that adiponectin levels were correlated negatively with BMI, waist and hip ratios, systolic and diastolic blood pressures. But they had shown that no significant correlation between adiponectin and age. Low serum level of adiponectin has been found to be an independent risk factor for the development of hypertension in both cross sectional and prospective studies. (Chow W-S et al. 2007)[3]

In our work, there was negative correlation between adiponectin and (ALT, AST) in our 30 patients as shown in table 6. In comparison Hickman, et al .2007[11] had shown that patients with obese T2DM are associated with decreased adiponectin and unexplained increases in liver enzymes and co-morbid type 2 diabetes with increased risk for NAFLD and cirrhosis compared to obese non diabetic patients (Hickman, et al .2007)[11]

Adiponectin has been shown to have a role in hepatic inflammation and steatosis. Hypo-adiponectinaemia is associated with NASH (Targher G, et al., 2004)[12] Adiponectin has been shown to have beneficial anti-inflammatory effects in liver, reducing steatosis, hepatomegaly and inflammation in mouse models of alcoholic and non-alcoholic fatty liver disease, in a study by Xu A. and his colleagues treatment with adiponectin decreased hepatomegaly, steatosis and alanine aminotransferase abnormality (Xu A, et al.,2003)[9]

It has also anti-inflammatory effect since it suppresses the hepatic production and plasma concentrations of TNF-α. In our work there was strong negative correlation between adiponectin and C-reactive protein in our 30 patients as shown in Table 6.

In agreement with our results, Devaraj et al. 2008[13] stated that, there was a significant negative correlation between CRP and adiponectin concentration. MetS seems to be a proinflammatory state characterized by increased concentrations of CRP, the inflammatory response correlates with multiple metabolic markers including obesity (particularly

visceral), dyslipidaemia, hypertension and insulin resistance. (Ouchi N, et al.,2003)[14]

Epidemiological studies reproducibly showed an inverse association between serum adiponectin concentrations and inflammatory markers and manifestations of the metabolic syndrome including CRP, fibrinogen, hypertension and endothelial function (Iwashima Y, et al., 2004)[15] In our work there was negative correlation between adiponectin and (Fasting blood glucose, 2 hours postprandial blood glucose Triglycerides and Cholesterol and uric acid) in patients. But there was positive correlation between adiponectin and HDL-cholesterol in our 30 patients as shown in Table 6.

In agreement with our results, Merja et al., 2006[16] stated that in both the sexes, adiponectin was correlated negatively with measures of body fat, fasting plasma glucose, 2 hours post prandial blood glucose, triglycerides and CRP. A positive correlation was found between adiponectin and fasting plasma insulin and HDL-cholesterol. Chedid R et al., 2010[17] stated that; serum uric acid was inversely correlated with adiponectin. Serum uric acid was positively correlated with BMI, WC, SBP, DBP, FPG, triglycerides, total and LDL-cholesterol, and HOMA index and inversely correlated with adiponectin ($p<0.001$ for all variables, $p<0.05$ for adiponectin). This study has few limitations, the small number of patients included in the study, lack of follow up for these patients, and absence of liver biopsy for staging the NASH and its correlation with adiponectin level

In conclusion serum adiponectin level are significantly lower in type 2 diabetic obese patients than non diabetic obese subjects, serum adiponectin level are significantly higher in female than male subjects in type 2 diabetic obese patients, serum adiponectin level correlate negatively with ALT and AST, age, systolic BP, diastolic BP, BMI and W/H ratio in type 2 diabetic obese patients. Also serum adiponectin level correlates negatively with fasting blood glucose, 2 hours postprandial blood glucose C-reactive protein, HbA1c, Uric acid, triglycerides and Cholesterol in type 2 diabetic obese patients. Finally serum adiponectin level correlate positively with HDL-cholesterol in type 2 diabetic obese patients. Finally, Hypoadeponectinemia is associated with NAFLD in type2 diabetic obese patients especially in obese males.

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