Serum Level of Adiponectin as a Risk Marker for Cardiovascular and Type 2 Diabetes Mellitus Patients

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Abstract

Adiponectin has anti-atherogenic and anti-inflammatory properties. its' study as a risk marker for Cardiovascular and type -2- diabetes mellitus patients. This study was done in the period from 1/7/2011 to 1/11/2012 in Al-Hussein hospital in Karballa government. The study groups were divided into four groups ,one is control ,the others were patients (type -2- diabetic , type - 2- diabetic with cardiovascular and Cardiovascular diseases) . The results were showed , there were lowering in fasting serum adiponectin hormone concentration for (three study groups) with respect to than normal subjects , significant different ($p< 0.001$).

When use adiponectin measurement as a marker to limitation the kind of pathogenic case and from Least Significant different (L..S.D) a statistical system were observed there were highly significant different between DMII group compared to control group while there were medium significant different with (DMII- CVD and CVD) groups . The results indicated that there were no significant different between (DMII-CVD and CVD) groups. while there were a significant difference between(DMII -CVD and CVD) groups and control group less than previous group DMII .Therefore concentration of adiponectin were considered as predictor of incident of diabetes mellitus more than cardiovascular diseases.

دراسة مستوى ألأديبونكتين في مصل الدم كعلامة خطر لمرضى ألوعاء ألقلبي والسكر نوع ٢

الخلاصة

ألأديبونكتين هو هرمون مضاد لمرض تصلب الشرايين والالتهابات .وتمت دراسته كأحد العلامات الدالة على مرضى القلب و السكر نوع –٢– . أجريت هذه الدراسة على ثلاث مجاميع من المرضى، المجموعة الأولى مرضى سكر نوع –٢– والمجموعة الثانية مرضى سكر نوع –٢– بالإضافة إلى ا مراض الوعاء القلبي والمجموعة الثالثة مرضى ا مراض الوعاء القلبي فقط بالإضافة إلى مجموعة السيطرة ؛عدد الأشخاص لكل مجموعة (50) شخصا .أجريت الدراسة في مستشفى الحسيني التعليمي في محافظة كربلاء وشخصت ألعينات من قبل طبيبين أحدهما اختصاص باطنية و سكر ,والأخر اختصاص قلبية .

لقد تمت مقارنة النتائج لكل مجموعة من المرضى مع نتائج مجموعة السيطرة وأوضحت ألنتائج إن تركيز الهرمون ألأديبونكتين كان واطئ نسبيا في المجاميع المرضى الثلاثة مقارنة بمجموعة السيطرة وان التأثير كان معنويا (ارتباط عكسي)، وعند استخدام تركيز هذا ألهرمون كأحد العلامات الدالة لتحديد نوع الحالة المرضية ومن استخدام النظام الإحصائي لتحديد أقل قيمة معنوية تبين إن هذا الهرمون هو عالي ألمعنوية بالنسبة لمجموعة سكر نوع –٢– ومتوسط ألمعنوية بالنسبة لمجموعتي (سكر نوع –٢– وقلب ، قلب فقط) مقارنة بمجموعة السيطرة ($p< 0.001$) ،ولا يوجد اختلاف معنوي بين مجموعتي (سكر نوع –٢–وقلب ، قلب فقط) . لذا يعتبر تركيز هرمون ألأديبونكتين كمنبئ لمرض السكر–٢– أكثر من ا مراض الوعاء القلبي .

Introduction

Type 2 diabetes mellitus (DMII) is not one disease – but rather is heterogeneous group of syndromes characterized by an elevation of fasting blood glucose caused by a relative or absolute deficiency in insulin

.Diabetes mellitus is the leading cause of adult blindness and amputation and a major cause of renal failure, heart attack , strokes ,most cases of diabetes mellitus can be separated into two groups type 1 ,type 2 [1]. One of the very important parameters to follow up DMII is HbA1c as a monitor.

Cardio Vascular disease [CVD] a group of medical problems that effect the heart and surrounding blood vessel . CVD can take many forms such as high blood pressure , coronary artery disease, valvular heart disease or rheumatic heart disease . There are many factors affecting at CVD, DMII such as cholesterol ,triglycerides ,and lipoproteins (HDL-C VLDL-Tg , LDL-C) [2].

Adiponectin, also known as adipocytes complement-related protein of 30kDa (ACRP30), is a secreted serum protein expressed exclusively in differentiated adipocytes. Studies indicated that decreased plasma adiponectin concentration is associated with obesity, insulin resistance [3] essential hypertension [4], inflammation and atherosclerosis [5], and acute myocardial infarction [6].

On the other hand, increased adiponectin level leads to nephritic syndrome [7,8].

Obesity is a major risk factor for insulin resistance , type 2 diabetes with cardiovascular diseases ,orthopedic problems ,and many other chronic diseases.

The incidence of obesity has dramatically increased and has become epidemic in the western world [9]. The etiology multifactor , with genetic ,environmental [10].Obesity is the final consequence of a chronic positive energy balance ,regulated by a complex network between endocrine tissues and the central nervous system [11,12].

Objective

To study the importance of adiponectin and some biochemical variables in serum as a markers for cardiovascular and type -2- diabetes mellitus disease and the relationship between adiponectin and other factors such as obesity ,hypercholesterimia and Insulin resistance .

Subjects

1-Aquestionnaire which include (name ,.age, length ,weight)

2-Determination the body mass index (BMI) weight(kg)/ (length in meter)^2 .

3 - Determination the serum level of adiponectin (ADN).

4-Determine the serum level of fasting glucose, glycated hemoglobin HbA1c %, lipid profile [total cholesterol (Tc), triglyceride (Tg)], lipoprotein (high density lipoprotein(HDL), low density lipoprotein(LDL), very low density lipoprotein) and insulin hormone.

5-Evaluation of the insulin resistance.

6-.Determination of the relationship between adiponectin and insulin resistance, obesity in three study groups(type-2- diabetic(DMII), type -2- diabetic with cardiovascular (DMII-CVD) and cardiovascular disease (CVD) groups .

Methods

This study was done in the period from 1/7/2011 to 1/11/2012 in Al-Hussein hospital in Karballa government ,department of biochemistry . The study groups were divided into four groups ,one is control ,the others were patients as follow:

Control group included 50 apparently healthy subjects (25 males and 25 females). The ages of the apparently healthy individuals were range from 35-up to 65 years. They were collected from my family, medical

staff and relatives who were free from signs and symptoms of any chronic diseases like diabetes, hypertension and others.

Patients groups:- This part include three groups, each one have 50 subjects , the ages were range from 35-up to 65 years, as shown below:

group one.: DMII patients only (30 females ,20 males).

group two : DMII with CVD patients (24 females , 26 males).

group three : CVD patients (23 females, 27 males).

Patients suffered from kidney failure or/and liver diseases were not included and excluded from the current study . All samples were diagnosed by two drs. One of them diabetic specialist and another Cardiologist . .

1-Collection of samples

Venous fasting blood samples (5- 6 ml) were collected from the patients

and healthy volunteers after an over night fasting in Al-Hussein hospital in Karballa government. The samples were put in tubes containing no anticoagulant. Disposable syringes and needles were used for blood collection . After allowing the blood to clot at 37°C for about 15 min, blood samples were centrifuged for 15 min . The sera were separated in a disposable tubes and stored at(-18 °C) .

2- Assay Procedure of Adiponectin [3]

It was assayed according to the procedure of the manufactures of its assay kit ,Assay Max company.

3-Fasting serum sugar (FSG) [13], Glycolated hemoglobin (HbA1C)[14] Triglycerides (Tg.) [15], total cholesterols (T-C.) [16], High density lipoprotein cholesterol (HDL-C) [17] and Insulin hormone were assayed according to the procedure of the manufactures of its' kits [18].

4-Very low density lipoprotein triglycerceride (VLDL-Tg) concentration was calculated by divided triglycerceride concentration by five VLDL = Tg./5 [19].

5-Low density lipoprotein cholesterol was calculated from the formula :- (LDL.C) = TC –(HDL+TG/5) [20].

6-insulin resistance in type 2 diabetic patients was estimated by Homeostasis model assessment (HOMA-IR): [21,22]

HOMA = [glucose in (mmole/l) * insulin in (microIU/mL)] / 22.5.

Calculations

1-The absorbance for each set of references standard ,control and sample were measured at wave Length 450 nm using Bio-ELISA reader Elx 800 .

2-A standard curve was constructed by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper.

3-The corresponding concentration of adiponectin (ng/ml) from the standard curve was determined.

4-The adiponectin concentration in each samples of patients and control sera were calculated by measuring Its conc. With Bio-ELISA reader Elx 800 at wave Length 450 nm , then multiplied by the dilution factor of (500) to obtain Adiponectin results in µg/ml.

Standard curve of adiponectin determination

The standard curve of adiponectin determination was shown in figure -1- and the adiponectin level in each sample was determined.

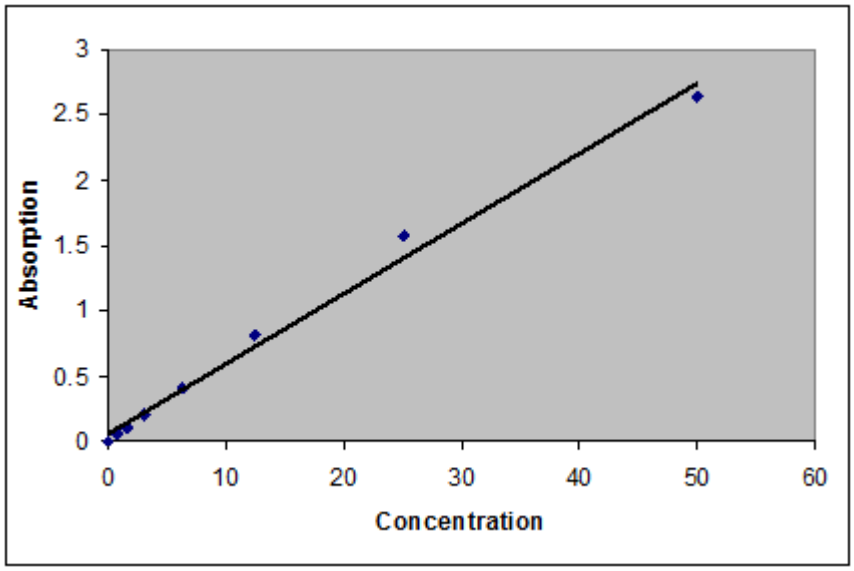

Figure 1 The standard curve for determination of human adiponectin concentration (ng/ml)

Statistical analysis

Student t-test was applied to assess the significance and linear regression analysis (bivariants correlation analysis) was done to determine the relationships between adiponectin and other risk variables and Least Significant Different (L.S.D) a statistical system to limitation significant.

Results and Discussion

Table1 clinical and biochemical variables of study subjects, type-2-diabetic mellitis .

Parameters	Healthy normal subjects n=50	Type 2 diabetic subjects n=50	P-Value
BMI(Kgs/m²)	23.979±0.659	31.8±4.23	< 0.001
Fasting glucose (mmol/L)	4.994±0.650	9.24±3.00	< 0.001
HbA1c (%)mg/dl	5.112±0.286	10.96±2.7	< 0.001
S.Cholesterol(mmol/L)	4.066±0.701	4.83±1.53	< 0.002
S.Triglyceride (mmol/L)	1.510±0.468	2 .35±0.84	< 0.001
High density lipoproteins	1.130±0.156	0.86±0.13	< 0.001
Low density lipoproteins	2.680±0.819	3.45±0.12	< 0.001
Very low density lipoproteins	0.272±0.105	0.45±0.16	< 0.001
Insulin(µIU/ml)	6.557±1.742	18.7±15.8	< 0.001
Insulin resistance (HOMA,IR)	1.458±0.381	7.55±5.96	< 0.001
Adiponectin(µg/ml)	17.857 ±3.664	11±2.8	< 0.001
Systolic BP(mm Hg)	118.4±4.4	129.6±10.7	< 0.001
Diastolic BP(mm Hg)	78.6±3.3	89.8±11.4	< 0.001

Table 2 clinical and biochemical variables of study subjects, diabetic Mellitis with cardiovascular diseases .

Parameters	Healthy normal subjects n=50	DMII with CVD subjects n=50	P-Value
BMI(Kgs/m²)	23.979±0.659	31.16±5.60	< 0.001
Fasting glucose(mmol/l)	4.994±0.650	11.81±4.43	< 0.001
HbA1c %(mg/dl)	5.112±0.286	12.39 ±2.40	< 0.001
S.Cholesterol (mmol/L)	4.066±0.701	4.83 ±1.52	< 0.002
S.Triglyceride (mmol/L)	1.510±0.468	2.40 ±1.42	< 0.001
High density lipoproteins (mmol/L)	1.130±0.156	0 .87±0.12	< 0.001
Low density lipoproteins	2.680±0.819	3.53 ±1.54	< 0.001
Very low density lipoproteins	0.272±0.105	0.45±0.27	< 0.001
Insulin(µIU/ml)	6.557±1.742	24.90±28.7	< 0.001
Insulin resistance (HOMA-IR)	1.458±0.381	12.89±18.03	< 0.001
Adiponectin(µg/ml)	17.85 ±3.664	12.48±6.87	< 0.001
Systolic BP(mm Hg)	118.4±4.4	137.1±13.6	< 0.001
Diastolic BP(mm Hg)	78.6±3.3	93.6±12.5	< 0.001

Table 3 clinical and biochemical variables of study subjects, Cardiovascular diseases

Parameters	healthy normal subjects n=50	CVD subjects n=50	P-Value
BMI(Kgs/m²)	23.979±0.659	31.65±5.7	<0.001
(Fasting glucose mmol/L)	4.994±0.650	5.37±0.69	< 0.001
HbA1c (%)mg/dl	5.112±0.286	6.73±1.36	<0.001
(S.Cholesterol mmol/L)	4.066±0.701	5.05±1.10	< 0.002
(S.Triglyceride mmol/L)	1.510±0.468	2.39 ±1.29	< 0.001
High density lipoproteins	1.130±0.156	0.87±0.37	< 0.001
Low density lipoproteins	2.680±0.819	3.82±1.12	< 0.001
Very low density lipoproteins	0.272±0.105	0.45±0.25	< 0.001
Insulin(µIU/ml)	6.557±1.742	93.4±14.3	< 0.001
Insulin resistance (HOMA.IR)	1.458±0.381	4.93±5.43	< 0.001
Adiponectin(µg/ml)	17.857 ±3.664	11.5±6.75	< 0.001
Systolic BP(mm Hg)	118.4±4.4	136.4±15	< 0.001
Diastolic BP(mm Hg)	78.6±3.3	93.4±14.3	< 0.001

Table 4 Bivariats correlation analysis of Adiponectin with high risk variables in DMII patients**.**

Parameters	r	P-Value
BMI(kgs/m²)	-0.830**	< 0.01
Fasting glucose(mmol	- 0.278	0.051
HbA1c (%) mg/dl	- 0.327*	0.020
S.Cholesterol(mmol/l)	- 0.224	0.119
S.Triglyceride (mmol/L)	- 0.358	0.011
High density lipoproteins (mmol/L)	0.189	0.190
Low density lipoproteins	- 0.173	0.229
Very low density lipoproteins	- 0.343*	0.015
Insulin (µIU/ml)	- 0.603**	< 0.01
Insulin resistance(HOMA.IR)	- 0.669**	< 0.01
Systolic BP(mm Hg)	- 0.180	0.211
Diastolic BP(mm Hg)	- 0.181	0.209

*Correlation is significant at the 0.05 (2-tailed)
**Correlation is significant at the 0.01 (2-tailed)

Table 5 Bivariats correlation analysis of Adiponectin with high risk variables in DMII-CVD patients**.**

Parameters	r	P-Value
BMI(kgs/m²)	- 0.876**	< 0.01
Fasting glucose(mmol/l)	- 0.196	0.172
HbA1c (%) mg/dl	- 0.258*	0.050
S.Cholesterol(mmol/l)	- 0.061	0.672
S.Triglyceride (mmol/L)	-0.008	0.954
High density lipoproteins (mmol/L)	- 0.055	0.707
Low density lipoproteins	-0.064	0.659
Very low density lipoproteins	-0.035	0.811
Insulin (µIU/ml)	-0.432**	< 0.01
Insulin resistance(HOMA.IR)	- 0.478**	< 0.01
Systolic BP(mm Hg)	- 0.251	0.078
Diastolic BP(mm Hg)	-0.137	0.341

*Correlation is significant at the 0.05 (2-tailed)
**Correlation is significant at the 0.01 (2-tailed

Table 6 Bivariats correlation analysis of Adiponectin with high risk variables in CVD patients**.**

Parameters	r	P-Value
(BMI(kgs/m²)	- 0.889**	< 0.01
Fasting glucose(mmol/l)	- 0.214	0.137
HbA1c (%) mg/dl	- 0.567**	< 0.01
S.Cholesterol(mmol/l)	0.046	0.751
S.Triglyceride (mmol/L)	-0.186	- 0.196
High density lipoproteins (mmol/L)	- 0.025	0.580
Low density lipoproteins	0.069	0.632
Very low density lipoproteins	-0.171	0.235
Insulin (µIU/ml)	- 0.653**	< 0.01
Insulin resistance(HOMA.IR)	- 0.463**	< 0.01
Systolic BP(mm Hg)	-0.026	0.858
Diastolic BP(mm Hg)	-0.002	0.966

*Correlation is significant at the 0.05 (2-tailed)
**Correlation is significant at the 0.01 (2-tailed

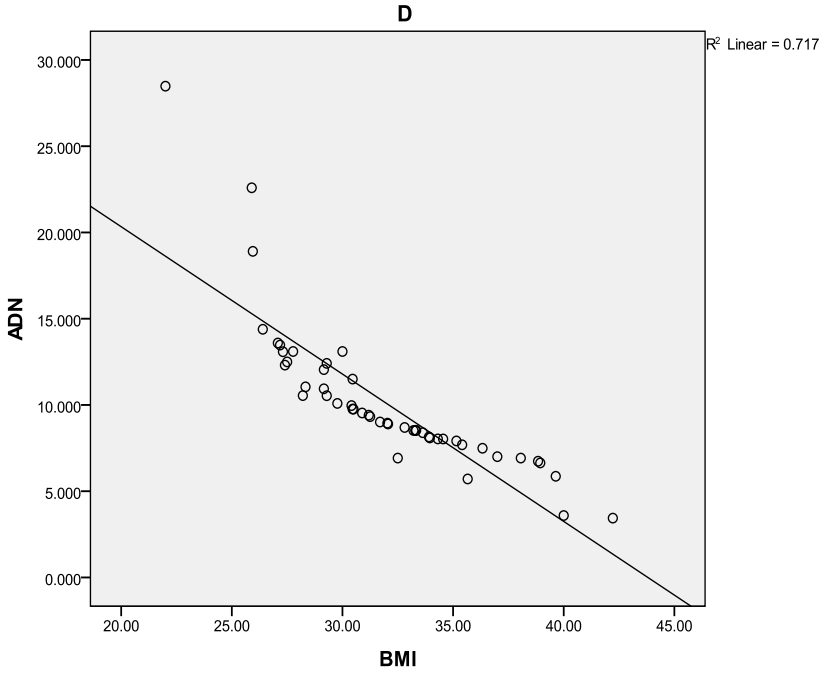

Figure 2 relationship between adiponectin and body mass index in Type -2- Diabetic Mellitis group (DMII), r =- 0.830**

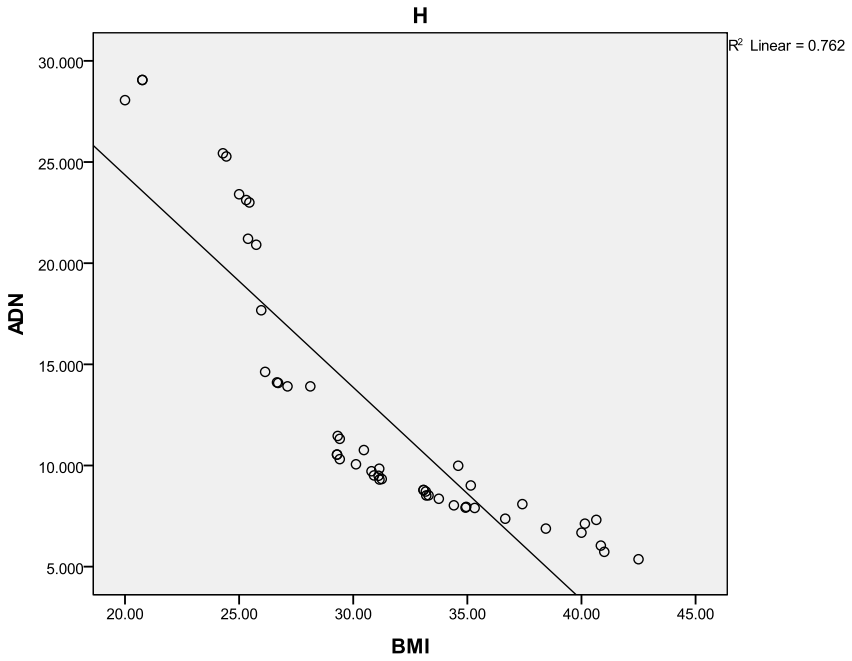

Figure 3 relationship between adiponectin and body mass index in Type -2- Diabetic Mellitis with Cardiovascular diseases group (DMII - CVD) , r = -0.876**

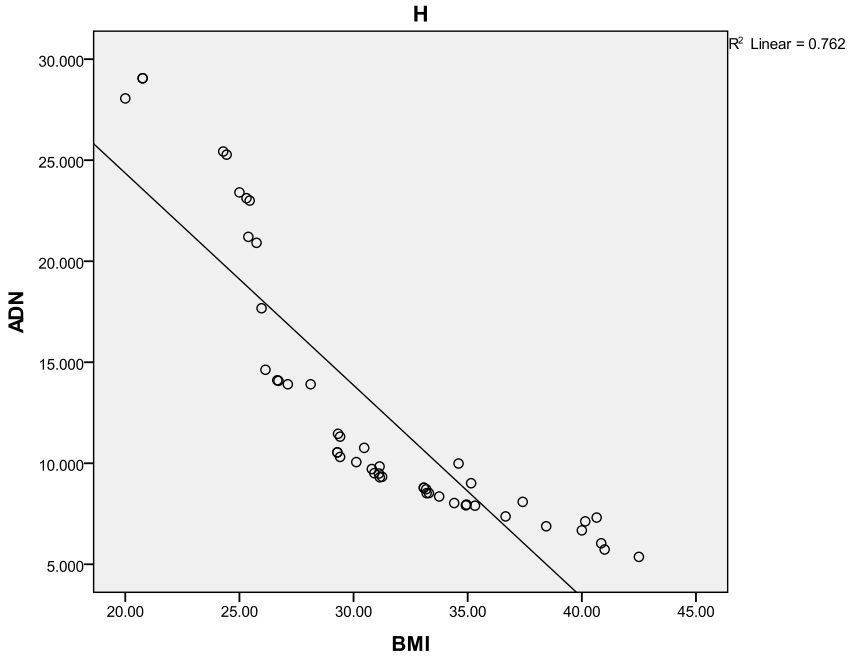

Figure 4 relationship between adiponectin and body mass index in Cardiovascular diseases group (CVD) , r = -0.889**

1- **Evaluation of Adiponectin hormone in (three study groups) Type - 2 - Diabetic, Type - 2- Diabetic with Cardiovascula , Cardiovascular patients and the control group:**

The results showed , lowering in Fasting serum adiponectin hormone concentration for (three study groups) type -2- diabetic , type - 2- diabetic with cardiovascular and cardiovascular disease groups with respect to than normal subjects ,

Significant different (p< 0.001) as shown in tables No.(1, 2, 3).

When use adiponectin measurement as a marker to limitation the kind of pathogenic case and from Least Significant Different (L.S.D) a statistical system were observed there were highly significant difference between DMII group compared to control group while there was a medium significant different for (DMII with CVD and CVD) groups . The results indicates that there were no significant difference between (DMII with CVD and CVD) groups ,while there were a significant different between (DMII with CVD and CVD) groups and control group less than previous group DMII Therefore concentration of adiponectin were considered as predictor to incident by diabetes mellitus more than cardiovascular diseases .

2-Relationship between Adiponectin hormone and biochemical variables in (three study groups):-

The clinical and biochemical variables which was related to adiponectin of the three study groups were shown on the tables No.(1,2,3). Body mass index to patients in three study groups were higher than control subjects, significant p<0.001. They were also had higher systolic and diastolic blood pressure (P<0.001) , HbA1C % (P<0.001) , fasting insulin (P<0.001) and insulin resistance (P<0.001) were also higher in (DMII , DMII with CVD and CVD only) subjects compared to healthy subjects(significant difference). Fasting serum glucose (P<0.001) was higher in subjects with (DMII and DMII with CVD) compared to healthy subjects (P<0.001) (significant different), but Its´ normal in CVD subjects only (P<0.006) compared to healthy subjects (control). Triglycerides (P<0.001), Total Cholesterol (p=0.002) (no significant difference) , LDL- C (P<0.001). VLDL-Tg(P<0.001) all these parameter significantly higher among of the three study groups than control group, HDL-C were lower in three study groups than control group, (significant difference) (P<0.001) , insulin-resistant fat cells undergo greater breakdown of their stored triglycerides and greater release of free fatty acids into the circulation. This was a common abnormality seen in both obese and no obese insulin-resistant subjects and those with type 2 diabetes. Increased fatty acids in the plasma leads to increased fatty acid uptake by the liver; in the fed state and in the presence of adequate glycogen stores, which is the common situation in patients with type -2- diabetes that is reasonably well controlled and certainly the case in the insulin-resistant no diabetic subject, the liver takes those fatty acids and convert them into triglycerides [23]. The presence of increased triglycerides stimulates the assembly and secretion of apolipoprotein (apo) B and very low density lipoprotein. The result is an increased number of VLDL and increased level of triglycerides in the plasma, which leads to the rest of the diabetic dyslipidemic (OLefsky *et al* 1974)

The linear regression analysis was used to examine the relationship of the levels of adiponectin with FSG ,T.C., LDL.C, VLDL. Tg , systolic and diastolic blood pressure of the patients in three study groups, no Significant correlations (negative correlation), tables No.(4, 5,6) were observed for the levels of adiponectin with biochemical variables of patients. These results were in agreement with those obtained by Pischon T *et al* [24]. There were low significant correlations between levels of

HbA1c% and adiponectin levels in DMII group and DMII-CVD group ,while high significant correlations in CVD group (negative correlations), tables No.(4, 5 ,6). There were no significant correlations between levels of Tg. and adiponectin levels in DMII group and low Significant correlations in DMII with CVD group and CVD group (negative correlation) (Tables 4, 5, 6). positively correlation between HDL-C levels and adiponectin levels in DMII group and negative correlations in DMII with CVD group and CVD group ,tables No. (4 ,5 ,6) . In this study and from linear regression analysis , there were high Significant correlations (negative correlations) between levels of insulin, insulin resistance (HOMA-IR) and BMI levels , tables No.(4 ,5 ,6) and figures No. (2, 3 ,4) . These results were in agreement with Yang *et al.* [25] . which found significant relation between the increase of adiponectin concentrations and the reduction of BMI and low adiponectin levels with elevation in lipids (Dyslipidemia) , It is well established that increased availability and utilization of FFA play a critical role in the development of IR [22]. The released FFA, according to the lipid supply hypothesis (Randle hypothesis) act as the predominant substrate in intermediary metabolism. Increased NADH/NAD^+ and acetyl-CoA /CoA ratios could be the reason for decreased glucose uptake which means IR [26]. FFA impairs insulin signaling in insulin responsive tissues, especially in muscle ,liver and adipose tissue. There were many researchers claimed that Circulating adiponectin concentrations are reduced in patients with DMII and are associated with the metabolic syndrome, (Havel PJ 2004).Reduced levels of adiponectin were implicated in the development of insulin resistance in human subjects of obesity [27,28] .

HbA1C levels were independent determinant of circulating adiponectin. Therefore, there was a scientific rationale that serum adiponectin was a target for future research in the treatment and prevention of diabetes. We suggest that increase in adiponectin levels by combination of lifestyle modifications and medications. Circulating levels of adiponectin, an adipose-derived protein proposed to have anti-inflammatory properties are reduced in individuals who are obese with cardiovascular disease or DMII. Analysis [12],[29],[30] of the Health Professionals Follow-up Study showed that low levels of adiponectin seem to be an independent risk factor for the development of Cardiovascular diseases(Pischon T *et al.* 2004) Recently, it was shown that adiponectin independently protects against DMII [31]. Furthermore, it has been postulated that adiponectin modulates the interaction between abdominal obesity and insulin resistance, and weight reduction increases serum levels of adiponectin [32]. Decreased expression of adiponectin correlates with insulin resistance in mouse models. Reduced levels of adiponectin were implicated in the development of insulin resistance in human subjects of obesity [27,28]. This study confirmed the observed strong inverse associations between low adiponectin levels and some of the well-known risk factors for cardiovascular diseases, such as low of HDL-cholesterol levels, hypertriglyceridemia, and high insulin resistance levels [33,34]. Additionally, which has been recently considered as a predictor for development of type -2- diabetes and cardiovascular diseases [35]. These findings clearly support the point of view that adiponectin may have an active role in the pathogenesis of cardiovascular diseases. Thus,

(plasma ,serum),adiponectin integrates with the cardiovascular risk factors and was a potential target for research in reducing morbidity and mortality of atherosclerotic disease. Future research should focus on clinical endpoints such as prevention of diabetes and reduction of cardiovascular morbidity and mortality following adiponectin increase, as well as the ability of adiponectin values to predict these outcome.

Conclusions

The results were obtained from this study indicate that decrease in the levels of adiponectin, which were associated with markers of type 2 diabetes mellitus and elevated of risk factors of cardiovascular diseases , could be significantly increased after weight loss in morbidly obese patients. This increase was more pronounced in healthy subjects compared to those with type -2- diabetes and Type -2- diabetes with cardiovascular diseases, because insulin resistance in those patient is high .we notice adiponectin decrease when insulin resistance increase.

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