Adiponectin, Insulin and Interlukin-8 in Type-2 Diabetic Obese Patients in Kerbala Province: Iraq

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Abstract

Background: The association between obesity and type-2 diabetes mellitus may be partly mediated by altered secretion of adipokines by adipose tissue. Adiponectin is an adipokines with anti-inflammatory and insulin sensitizing properties; it is secretion is down regulated in obesity. The correlation of plasma adiponectin with the risk of type-2 diabetes mellitus and hyperlipidemia is unclear.
Objective: To study the association between plasma adiponectin level with insulin and IL-8, in type-2 diabetic obese patients in Karbala province: Iraq.
Material and method: A total number of 110 sample subjects with different age, and different gender were classified according to diabetes mellitus and obesity. Fasting serum adiponectin, insulin and IL-8 were measured by ELISA method.
Results: It was obtained that adiponectin level was lowered significantly in diabetic group, obese group, and diabetic obese group as compared with control group. Serum adiponectin was correlated negatively with serum insulin and with body fat percent (BF %), while there was no relation with IL-8 level.
Conclusion: According to the presented data, adiponectin as hormone plays an important role in prevention of obesity and insulin resistance, and increase insulin sensitivity.

الخلاصة

الخلفية:من المعروف أن السمنة وداء السكر نوع 2 متلازمان في اغلب الاحيان, حيث ان السمنة وزيادة الخلايا الدهنية السبب الاساسي لمقاومة الانسجة للانسولين. اضافة الى ان الخلايا الدهنية تعمل كغدة صماء مسؤولة عن افراز العديد من الهرمونات التي لها دور كبير في تحول الشخص المصاب بالسمنة الى مريض بداء السكر, من بين هذه الهرمونات هرمون الاديبونيكتين المكتشف حديثا, حيث يتميز هذا الهرمون عن بقية الهرمونات التي تفرزها الخلايا الدهنية بان نسبتها تقل كلما ازدادت نسبة السمنة, وكذلك تشير بعض الدراسات السابقة ان نسبة الاديبونكتين تقل في المرضى المصابين بداء السكر نوع 2, اضافة الى خاصية مضاد الالتهابات وعلاقتها باضطرابات نسبة الدهون بالدم. بالرغم من ذلك فان المعلومات المتوفرة حول العلاقة بين السمنة وهرمون الاديبونكتين وداء السكر نوع 2 في المرضى لاتزال قليلة في العراق.
الأهداف: لدراسة العلاقة بين هرمون الاديبونكتين, الانسولين, انترلوكين-8 في المرضى الذين يعانون من السمنة وداء السكري نوع 2 في العراق.
الأشخاص و طرق العمل:تم اختيار أشخاص الدراسة من المرضى المصابين بداء السكر نوع 2 في العيادة الاستشارية لداء السكر في مستشفى الحسين التعليمي في مدينة كربلاء خلال الفترة الواقعة بين شهر تشرين الثاني من عام 2010 إلى شهر نيسان من عام 2011.العدد الكامل للاشخاص الذين تم اختيارهم لهذه الدراسة 110 شخصا, وتم تقسيمهم الى ثلاث فئات وذلك حسب الاصابة بداء السكر نوع 2 مع او بدون السمنة :-

1. الفئة الأولى تتكون من مجموعتين:
أ. مجموعة المرضى (أشخاص مصابون بداء السكري نوع 2, العدد = 67).
ب. المجموعة الضابطة (أشخاص غير مصابين بداء السكري, العدد = 43).
2. الفئة الثانية تتكون من مجموعتين:
أ. المجموعة الأولى (أشخاص مصابون بالسمنة , العدد =65).
ب. المجموعة الثانية (أشخاص غير مصابين بالسمنة , العدد = 45).
3. الفئة الثالثة تتكون من مجموعتين:
أ. المجموعة الأولى (أشخاص مصابون بالسمنة وداء السكري, العدد = 42).
ب. المجموعة الثانية (أشخاص غير مصابين بالسمنة او بداء السكري, العدد = 20).

لكل من هذه المجاميع تم قياس نسبة هرمون الاديبونكتين للشخص الصائم, هرمون الانسولين, الانترلوكين-8 في مصل الدم.احصائيا تم استعمال اختبار (t) لتقييم الاختلاف في قيمة المتوسط للفحوصات المختارة بين المجاميع, واستعمل عامل الارتباط (r) لفحص الارتباط بين الواسمات الحيوية المختلفة.

النتائج:- في الفئة الاولى اظهرت النتائج انخفاض معنوي لهرمون الاديبونكتين(P= 0.001) في مجموعة المرضى مقارنة مع المجموعة الضابطة, مع ارتفاع غير معنوي (p> 0.05) للانسولين, انترلوكين-8.

في الفئة الثانية اظهرت النتائج انخفاض معنوي لهرمون الاديبونكتين (P< 0.05) في المجموعة الاولى مقارنة مع المجموعة الثانية, وارتفاع معنوي للانسولين (p< 0.05) في المجموعة الاولى مقارنة مع المجموعة الثانية, مع ارتفاع غير معنوي (p> 0.05) للانترلوكين-8 في المجموعة الاولى مقارنة مع المجموعة الثانية, علما ان هناك ازدياد معنوي واضح (p= 0.001) بالنسبة لكل من منسب كتلة الجسم (BMI) ونسبة الشحوم في الجسم (BF %) بين المجموعة الاولى والمجموعة الثانية.

في الفئة الثالثة اظهرت النتائج انخفاض معنوي لهرمون الاديبونكتين(P< 0.01) في المجموعة الاولى مقارنة مع المجموعة الثانية, وازدياد معنوي للانسولين (p< 0.05) في المجموعة الاولى مقارنة مع المجموعة الثانية, مع ارتفاع غير معنوي (p> 0.05) للانترلوكين-8 في المجموعة الاولى مقارنة مع المجموعة الثانية, كذلك ازدياد معنوي واضح (p< 0.001) بالنسبة لكل من منسب كتلة الجسم (BMI) ونسبة الشحوم في الجسم (BF %) بين المجموعة الاولى والمجموعة الثانية.

وبينت النتائج وجود علاقة معنوية سالبة بين مستوى هرمون الاديبونكتين وهرمون الانسولين [r=-0.304, p< 0.05] . وكذلك وجود علاقة معنوية سالبة لهرمون الاديبونكتين مع منسب كتلة الجسم ,r=-0.314] (BMI) [p=0.01, اضافة الى وجود علاقة معنوية موجبة لهرمون الانسولين مع منسب كتلة الجسم ,r=0.313].(BMI) p=0.017]

الاستنتاج:-

1. هناك انخفاض واضح في مستوى هرمون الاديبونكتين في المرضى الذين يعانون من السمنة وداء السكر نوع2 مقارنة بالاشخاص الاصحاء.

2. ارتفاع مستوى هرمون الانسولين في المرضى الذين يعانون من السمنة مقارنة بالاشخص الاصحاء.

Introduction

Adiponectin is a 244 – amino acid collagen like protein that is exclusively secreted by adipocytes and acts as a hormone with anti-inflammatory and insulin sensitizing properties by several mechanisms through which adiponectin may decrease the risk of type-2 diabetes mellitus, including suppression of hepatic gluconeogenesis, stimulation of fatty acid oxidation in the liver, stimulation of fatty acid oxidation and glucose uptake in skeletal muscle, and stimulation of insulin secretion [(1)]. These effects may be partly mediated by stimulatory effects of adiponectin on signaling pathways for 5'-adenosine monophosphate activated protein kinase (5'-AMPK), and peroxisome proliferator-activated receptor gamma (PPAR-γ) [(2)]. Adiponectin secretion, in contrast to secretion of other adipokines, is paradoxically decreased in obesity. These may be attributable to inhibition of adiponectin gene transcription by inflammatory and angiogenic factors secreted by hypertrophic adipocytes [(3)]. A number of studies have shown that obesity, insulin resistance and atherosclerosis are accompanied by decreased adiponectin levels and that adiponectin replacement under experimental settings is able to

diminish both insulin resistance and atherosclerosis [4].

Insulin resistance is defined where a normal or elevated insulin level produces an attenuated biological response; classically this refers to impaired sensitivity to insulin mediated glucose disposal. Compensatory hyperinsulinemia occurs when pancreatic β cell secretion increases to maintain normal blood glucose levels in the setting of peripheral insulin resistance in muscle and adipose tissue[5].

Insulin resistance is widely recognized as a fundamental defect seen in obesity and type-2 diabetes. The development of type-2 diabetes is strongly associated with overweight and obesity in both genders and all ethnic groups. Over 90% of diabetics are overweight or obese. Weight gain and insulin resistance usually precede the onset of diabetes [6].

The aim of the presented work is to study the association between insulin resistance, with plasma adiponectin and IL-8 levels, in type-2 diabetic obese patients in Karbala province of Iraq.

Materials and Methods

Samples were recruited at the Al-Husain Teaching Hospital / Karbala, during Nov. 2010 - April, 2011. A total number of 110 subjects with age ranged between (22 - 75) years and different gender were stratified according to obesity and/or diabetes mellitus into three categories:

1. In the first category, study subjects were divided according to diabetes mellitus into two groups: (Diabetic, n =67 and Non-diabetic n =43).
2. In the second category, study subjects were divided according to obesity into two groups: (Obese with body mass index ≥ 30 kg/m^2, n =65 and non-obese with normal body mass index < 25 kg/m^2, n =45).
3. According to both obesity and diabetes mellitus, study subjects in the third category were divided into two groups: (diabetic obese patients with body mass index ≥ 30 kg/m^2 , n =42 and controls non-diabetic non-obese with body mass index < 25 kg/m^2 , n =20).

BMI was measured according to World Health Organization (WHO) procedure [7]. Body fat percentage can be estimated from a person's BMI by the following formula [8]:

Body Fat% = (1.2 x BMI) + (0.23 x age) − 5.4 − (10.8 x gender)

Where gender is 0 if female and 1 if male.

Seven milliliters of fasting venous blood was aspirated using disposable syringes and needles. The blood was allowed to clot in plain tubes for 30-45 minutes at room temperature and serum was recovered by centrifugation at 2000 xg for 10 minutes and transferred into plain plastic tubes and kept frozen at -18^oC until the time of assay.
All hormones were determined by ELISA method. Available kits for adiponectin, insulin, and IL-8, were supplied from DRG Company / Germany.
In ELISA: the patient's hormone is bound to an enzyme-labeled antibody and the unbound enzyme-linked antibody is removed from the system, in order to detect antigen-antibody enzyme catalyzes a reaction involving the substrate. From which a colored production is formed as a result of that reaction, the intensity of the color, measured by a spectrophometer, is directly proportional to the amount of hormone present in the patient's serum[9].
Statistical analysis was used to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by Student T-Test. The probability $P< 0.05$ = significant, $P> 0.05$ = non-significant. Correlation analysis was used to test the linear relationship between parameters. ANOVA test was used to show the

differences between variables of differentiated groups.

Results and Discussion

Estimation of hormones (adiponectin, insulin) and IL-8 in sera of 67 type-2 diabetic patients and 43 non diabetic subjects as control group was performed.
Adiponectin is a hormone of adipocyte origin that is involved in the homeostatic control of circulating glucose and lipid levels, so the level of adiponectin was reduced in type-2 diabetic patients in association with insulin resistance. These data demonstrate a significant reduction in adiponectin level in type-2 diabetic as compared with control ($p<0.001$) as shown in **table-1**. Insulin level was not significantly elevated ($p> 0.05$) as indicated in the same table. Previous study performed by Weyer *et.al*. in (2001) demonstrated a hypo-adiponectinemia in obesity and type-2 diabetes mellitus and a close association with insulin resistance and hyperinsulinemia [(10)]. Interleukin-8 level was not significant elevation in diabetic patients as compared with control group.

Table 1. Mean ± SD and P values of serum adiponectin, insulin, IL-8, BMI, BF%, and age, in diabetic and non-diabetic subjects

Parameters	Diabetic group N = 67	Non-Diabetic group N = 43	*P* value
Adiponectin (µg/ml)	3.79 ± 3.16	7.06 ± 4.09	0.001
Insulin (µI.U./ml)	21.36 ± 19.96	18.80 ± 21.25	0.606
IL-8 (ng/ml)	38.69 ± 17.96	36.87 ± 17.40	0.663
BMI (kg/m^2)	31.79 ± 6.31	28.93 ± 8.24	0.083
BF%	39.80 ± 9.94	31.55 ± 13.86	0.010
Age (year)	43 ± 9	40 ± 9	0.258

Estimation of hormones (adiponectin, insulin) and IL-8 in sera of 65 obese subjects and 45 non obese subjects as control group was performed.
Adiponectin is exclusively secreted from adipose tissue into the bloodstream and in contrast to other adepokines; its level is inversely correlated with obesity and body fat percentage in adults [(11)]. A number of previous clinical studies showed a decrease in adiponectin levels in obese humans relative to lean subjects, and found a negative correlation between body mass index and plasma adiponectin levels in men and women [(12)]. Our result demonstrate significant reduction of adiponectin level in obese group as compared with control ($p< 0.05$), whereas, insulin level was significantly elevated in obese group as compared with control group ($p< 0.05$) as shown in **(table-2)**.

The presented data indicated that the mean value of body mass index (BMI) was significantly higher in obese group as compared with that found in control group ($p < 0.001$), while the mean value of body fat (BF%) percent was significantly higher in obese group as compared with control group ($P< 0.001$) as shown in **(table-2)**.

The elevation of serum insulin in obese group show the association between obesity and type-2 diabetes mellitus which may be explained by the fact that the initial increases in fat mass are mostly due to adipocyte hypertrophy [(13)]. Because large adipocytes are less insulin sensitive than small adipocytes [(14)], the expanded fat depot loses most of its ability to take up glucose in response to insulin because the enlarged lipid droplet pushes cell organelles, such as mitochondria, against the cell surface, the role of mitochondria may be important especially in visceral fat.

In addition, endocrine and paracrine hormonal signals may also play an important role in decreasing oxidative capacity in enlarged adipocytes [15].

Table-2. Mean ± SD and P values of serum adiponectin, insulin, IL-8, BMI, BF%, and age, in obese and non-obese subjects

Parameters	Obese group N = 65	Non-obese group N = 45	*P* value
Adiponectin (µg/ml)	4.19 ± 3.74	6.06 ± 3.43	0.029
Insulin (µI.U./ml)	22.89 ± 20.99	14.95 ± 17.32	0.047
IL-8 (ng/ml)	40.81 ± 18.13	35.59 ± 16.50	0.240
BMI (kg/m^2)	34.81 ± 3.71	21.30 ± 2.30	< 0.001
BF%	42.99 ± 7.69	23.51 ± 7.91	< 0.001
Age (year)	42 ± 8	42 ± 12	0.829

There was no significant differences in IL-8 level between obese and control group as shown in **(table-2)**.

Estimation of hormones (adiponectin, insulin) and IL-8 in sera of 42 diabetic obese patients and 20 non-diabetic non-obese subjects as control group was performed.

The mean value of serum adiponectin level was lowered significantly in diabetic obese group, while the mean value of serum insulin level was elevated significantly in patients group as compared with control group, and there were no significant differences in IL-8 level between diabetic obese and control group as shown in **(table-3)**.

The mean value of body mass index was significantly higher in diabetic obese group as compared with control group, while the mean value of body fat percent was significantly higher in patients group as compared with control group as shown in (table-3). The risk of diabetes increases by 9% for each kg gained in self-reported weight and generally starts to increase at a BMI of 22 and is 40 times higher at a BMI over 35 [16-17].

Insulin resistance is widely recognized as a fundamental defect seen in obesity and type-2 diabetes. The development of type-2 diabetes is strongly associated with overweight and obesity in both genders and all ethnic groups. Over 90% of diabetics are overweight or obese [18]. Weight gain and insulin resistance usually precede the onset of diabetes [19]. Current theories indicate that type-2 diabetes develops when pancreatic beta cell output can no longer satisfy the demands imposed by increased insulin resistance [20]. The current results demonstrate that diabetes mellitus when accompanied with obesity then the patient more liable to develop hyperlipidemia, and these results in consistence with previous reports.

Table-3. Mean ± SD and P values of serum adiponectin, insulin, IL-8, BMI, BF%, and age, in diabetic obese and non-diabetic non-obese subjects.

Parameterss	Diabeticobese group N = 42	Non-Diabetic non-obese group N = 20	p value
Adiponectin (µg/ml)	3.47 ± 3.27	7.97 ± 3.70	0.004
Insulin (µI.U./ml)	23.09 ± 19.31	13.62 ± 8.10	0.017
IL-8 (ng/ml)	39.09 ± 18.96	38.26 ± 20.47	0.871
BMI (kg/m^2)	34.83 ± 3.36	20.18 ± 1.98	< 0.001
BF%	43.92 ± 6.37	18.89 ± 6.34	< 0.001
Age (year)	42 ± 7	38 ± 10	0.238

Adiponectin, Insulin, IL-8 and Lipid profile in various subgroups of type-2 diabetic obese and control group.

To evaluate serum adiponectin, insulin and IL-8 in various subgroups of type-2 diabetic obese patients, subjects were categorized into three groups according to diabetes mellitus and obesity. Group B consist of 42 patients (diabetic obese), group C contained 25 patients (diabetic non-obese), and group D involved 23 subjects (non-diabetic obese), these three groups where compared with control group A which include 20 subjects (non-diabetic non-obese), the statistical analysis of the obtained data was carried out using the ANOVA analysis. The results of adiponectin, insulin, IL-8 levels are shown in **(table-4)**.

Significant decreases of serum adiponectin levels were obtained, while insulin levels were increased significantly in diabetic obese group as compared with the control group. Serum IL-8 levels did not show significant variation.

Table 4. ANOVA analysis of serum hormones (adiponectin, insulin) and IL-8 level in various subgroups of type-2 diabetic obese and control

Parameters	(A and B)*	(A and C)*	(A and D)*
Adiponectin	0.001	0.026	NS
Insulin	0.017	NS	NS
IL-8	NS	NS	NS

***A : Control group N = 20 ; *B : Diabetic obese group N = 42**
***C : Diabetic non-obese group N = 25 ; *D: Non- diabetic obese group N = 23**
NS: Not significant.

On the other hand significant decreases of adiponectin levels were obtained in diabetic non-obese ($p < 0.05$). Serum insulin and IL-8 levels did not show significant variation between diabetic non-obese and control group.

Serum level of adiponectin, insulin and IL-8 level remains within normal range in non-diabetic obese group when compared with the control group.

From these data, it was indicated that the effect of type-2 diabetes mellitus on adiponectin level was more pronounced than the effect of obesity and it is effect was potentiated by obesity.

Synergistic effect of obesity and type-2 diabetes mellitus on the level of insulin, where there was no significant elevation in serum insulin when diabetic group alone or obese group alone was compared with control group, but there was significant elevation of insulin level observed when diabetic obese group compared with non-diabetic non-obese (control) group.

Correlation between serum adiponectin, insulin and IL-8 levelin type-2 diabetic patients and control group.

The spearman correlations and scatter plots were used to evaluate bivariate relationship of serum adiponectin with insulin and IL-8 in diabetic and control groups. We found that there was significant negative correlation between serum adiponectin and serum insulin [$r = -0.304$, $p = 0.012$], **(table-5), (figure-1)** in diabetic group, and there was no significant correlation in control group. There was no significant correlation between adiponectin and IL-8, as shown in **(table-5)**.

Negative correlation between adiponectin and serum insulin level may be explained by the theory which state that insulin is a very important regulator of adiponectin gene expression, where some authers found adecrease in adiponectin gene expression after more prolonged exposure to insulin [21].

There was no significant correlation between serum insulin level and IL-8 level

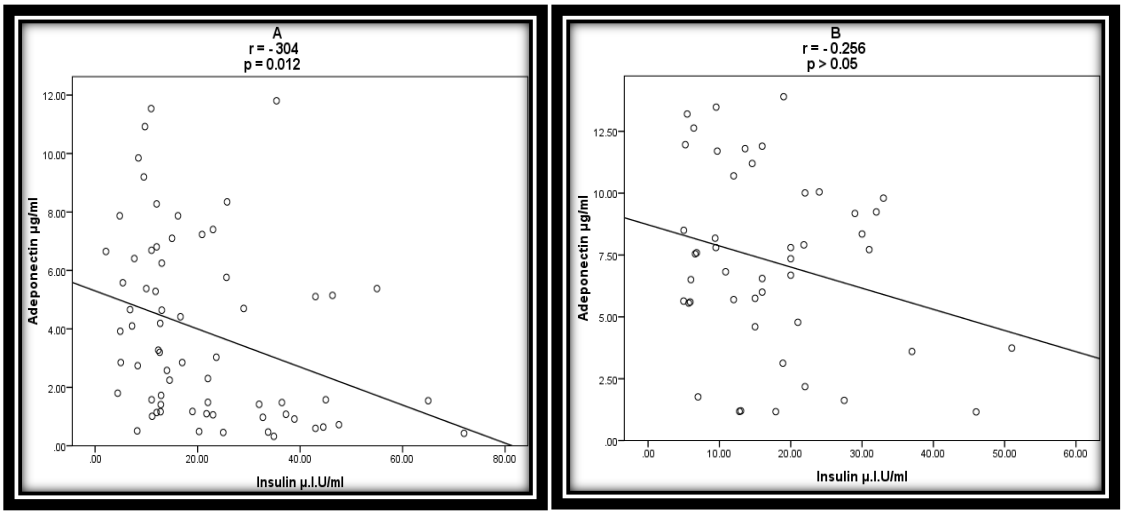
in type-2 diabetic patients and in control group as illustrated in **(table-6)**.

Table 5.Correlation of serum adiponectin with insulin and IL-8 level in type-2 diabetic patients and control group

Parameters	Diabetic patients		Control	
	r	P	r	p
Insulin	-0.304	0.012	-0.256	NS
IL-8	0.027	NS	0.136	NS

Table 6.Correlation of serum insulin with IL-8 level in type-2 diabetic patients and control group

Parameters	Diabetic patients		Control	
	r	P	r	p
IL-8	0.114	NS	0.184	NS



(Figure-1) Correlation of serum adiponectinwith insulin in type-2 diabetic patients (A) and in the control group (B)

Correlation of serum hormones (adiponectin, insulin) and IL-8 Level with the body mass index (BMI) in type-2 diabetic patients and control group

The spearman correlations and scatter plots were used to evaluate bivariate relationship of serum adiponectin, insulin and IL-8 with the BMI in type-2 diabetic patients and in non-diabetic (control) subjects. In diabetic patients serum adiponectin show significant negative correlation with BMI [r = -0.314, p = 0.010], **(table-7) (figure-2)** serum insulin demonstrate significant positive correlation with BMI [r = 0.313, p = 0.017], **(table-7) (figure-3)**, serum IL-8 show no significant correlation with BMI. Whereas in control group significant positive correlation of insulin with BMI [r =0.292, p=0.046], **(table-7) (figure-3).**

Adiponectin secretion, in contrast to secretion of other adipokines, is paradoxically decreased in obesity, this may be attributable to inhibition of adiponectin gene transcription by inflammatory and angiogenic factors secreted by hypertrophic adipocytes [(22)]. These results demonstrate that adiponectin level decreased as BMI increased, but these reductions are significant and more

pronounced in diabetic group, than in control group, which may be due to accumulation the effects of type-2 diabetes mellitus and obesity.

Serum insulin levels elevated in both groups as BMI increased, these may explain the fact that says the insulin resistance is one of the most important metabolic consequences of obesity, and the pathophysiological mechanisms behind these are probably a combination of the toxic metabolic effects of free fatty acids and adipokines [(23)].

These results show no significant correlation of serum IL-8 with BMI, although some previous study shows significant positive correlation between serum IL-8 and BMI [(24)].

Conclusions

The major finding in the present study was demonstrated that a decreased serum adiponectin level was associated with obesity and type-2 diabetes mellitus, so we can conclude that adiponectin as a hormone plays an important role in prevention of obesity and insulin resistance, and increase insulin sensitivity.

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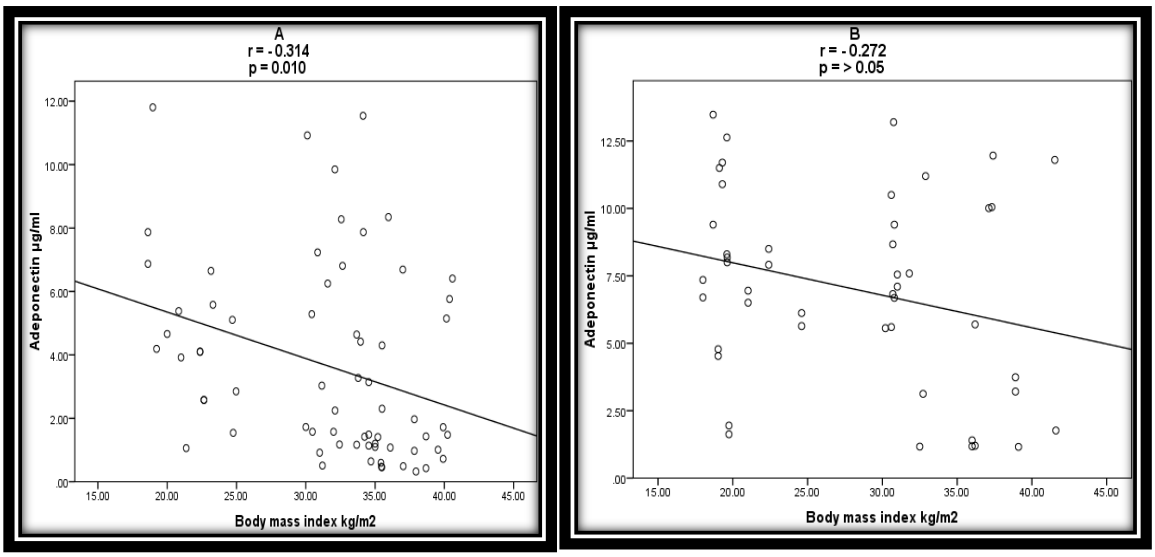
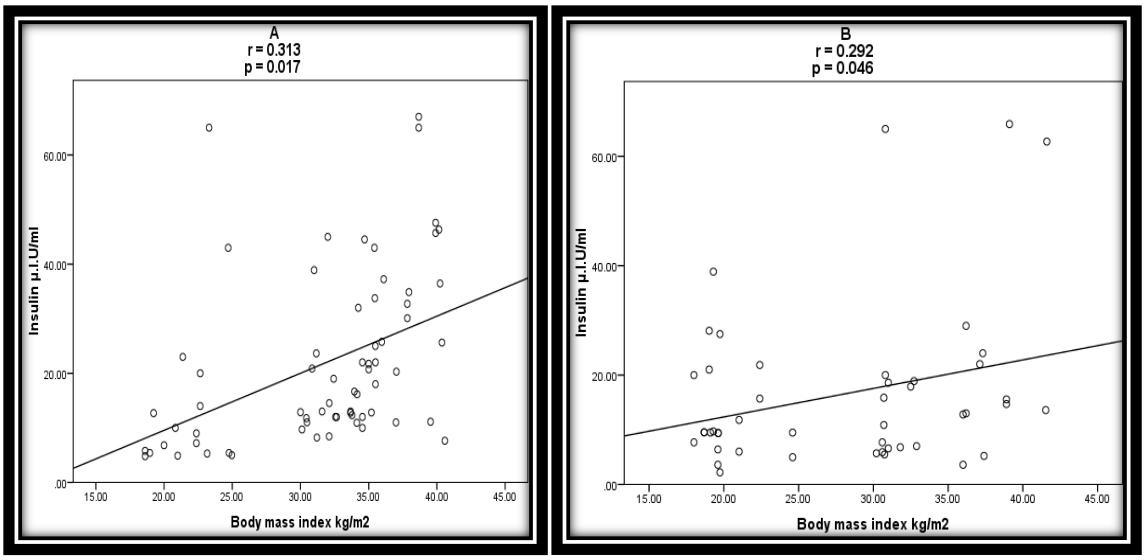
Table 7. Correlation of serum hormones (adiponectin, insulin) and IL-8 level with the body mass index (BMI) in type-2 diabetic patients and control group

Parameters	Diabetic patients		Control	
	r	p	r	P
Adiponectin	-0.314	0.010	-0.207	NS
Insulin	0.313	0.017	0.292	0.046
IL-8	0.138	NS	0.118	NS



(Figure-2) Correlation of serum adiponectin with BMI in type-2 diabetic patients (A) and the control group (B)



(Figure-3) Correlation of serum insulin with BMI in type-2 diabetic patients (A) and the control group (B)

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