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Correlation between Tumor Necrosis Factor–Alfa and Anti-tyrosine Phosphatase with Obesity and Diabetes Type 2

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Abstract

This study was done to find a correlation between adipokines such as tumor necrosis- alpha (TNF- α) and autoantigens such as anti-tyrosine phosphatase (IA2) with obesity and type 2 diabetes mellitus (T2DM). For this purpose, one hundred forty adult males were selected. 90 of them were diagnosed with type 2 diabetes and 50 healthy males). The subjects were divided into four groups. Group 1 had healthy controls with body mass index (BMI) between 18.5-25, group 2 had individuals who suffered from obesity only (BMI above 30), group 3 consisted of diabetes patients and group 4 had individuals who were diagnosed with both obesity and diabetes. The enzyme immunoassay was intended for quantification determination of TNF in serum. The results found a significantly ($p \leq 0.001$) higher concentration of TNF- α (21.01 ± 1.27 pg/ml) in patients suffering from both obesity and diabetes in comparison with the control (5.33 ± 0.67 pg/ml), obese patients (12.63 ± 1.35 pg/ml) and diabetes patients group (13.32 ± 0.83 pg/ml). Chi-square analysis found a significantly ($p \leq 0.001$) higher prevalence of abnormal TNF- α in males suffering from both obesity and diabetes, 73.33% compared with diabetes non-obese males 31.1%. A significant positive correlation ($p \leq 0.01$) was found between TNF- α with BMI ($r = 0.65$) and HbA1c ($r = 0.57$). No significant differences in IA2 concentration were observed between all groups. Also no significant correlation was observed between IA2 with BMI and HbA1c.

Keywords: TNF- α ; IA2; HbA1c; T2DM; Adipokines; Obesity

العلاقة بين عامل نخر الورم-الفا و مضاد الفوسفاتيز التيروسيني مع السمنة و السكري من النوع الثاني

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الخلاصة

تم اجراء هذه الدراسة لايجاد العلاقة بين الحركيات الخلوية الدهنية مثل عامل نخر الورم- α TNF و المستضدات الذاتية مثل مضادالتايروسين فوسفاتيز ي IA2 في مرضى السمنة و السكرى من النوع الثانى. لهذا الغرض تم اختيار مائة واربعين ذكورا بالغين فى هذه الدراسة (90 منهم شُخصوا كمرضى سكرى من النوع الثانى و 50 ذكورا لم يعانون من السكرى). تم تقسيم العينات الى اربع مجاميع. المجموعة 1 مجموعة السيطرة الاصحاء مع مؤشر كتلة الجسم BMI ما بين 18.5 الى 25 ، المجموعة 2 عانوا من السمنة (مؤشر كتلة الجسم اكثر من 30)، المجموعة 3 عانوا من السكرى و المجموعة 4 عانوا من السمنة و السكرى معا. تم استخدام طريقة المقايسة المناعية الانزيمية لتقدير كمية α -TNF فى المصل. اوجدت النتائج زيادة معنوية ($p \leq 0.001$) فى تركيز α -TNF (1.27 ± 21.01 بيكوغرام/امل) فى المرضى الذين يعانون من السمنة و السكرى معا مقارنة مع مجاميع السيطرة (0.67 ± 5.33 بيكوغرام/امل) ، مرضى السمنة (12.63 ± 1.35 بيكوغرام/امل) و مرضى السكرى فقط (0.83 ± 13.32 بيكوغرام/امل). اظهرت نتائج اختبار مربع كاي X^2 زيادة معنوية ($p \leq 0.001$) فى انتشار α -TNF الغير الطبيعى فى الذكور المصابين بالسمنة 73.33% و السكرى معا مقارنة مع الذكور المصابين بالسكرى بدون السمنة 31.1%. تم ايجاد علاقة طردية معنوية $p \leq 0.01$ بين α -TNF و مؤشر كتلة الجسم ($r = 0.65$) و كذلك مع HbA1c ($r = 0.57$). لم يتم ايجاد اية فروقات معنوية فى تركيز IA2 بين المجاميع كلها و لم يتم ايجاد علاقة معنوية بين IA2 مع مؤشر كتلة الجسم و كذلك مع HbA1c.

1. Introduction

Diabetes mellitus is considered a global disease that affects around 1 in 11 people in the world [1]. Some estimates suggest that about a third of the global population will have diabetes by 2050 [2]. Compared to other chronic conditions, diabetes also has high morbidity and mortality rates [3]. Annual mortality associated with diabetes is estimated at four million [4], which is mainly due to the increased incidence of diabetes complications, especially cardiovascular diseases [5]. Continuous updates on diabetes diagnosis and treatment are essential in reducing the health and economic burden on individual patients and the wider community because of its severity [6]. Despite the existence of several pharmacological groups for anti-diabetic agents, there is an annual report on their indications and use [7]. Strong evidence exists that obesity treatment may prolong the progression from prediabetes to type 2 diabetes. It has also been shown to be effective in treating type 2 diabetes. To enhance glycemic regulation and reduce the need for glucose-lowering drugs, moderate and sustained weight loss has been shown to be a key factor. According to the American Diabetes Association, with a minimum of 3-5% weight loss, clinical advantages can be seen [8].

Due to excessive cytokine synthesis and activation of inflammatory signaling pathways, chronic inflammation is linked to metabolic disorders such as obesity, insulin resistance and T2DM. [9]. Adipose tissue is a metabolically active organ that can be the cause of low-grade chronic inflammation in obese individuals. Chronic inflammation occurs before the onset of T2DM and plays a significant role in its pathogenesis [10]. TNF- α was the first proinflammatory cytokine to be identified as playing a role in insulin resistance development and T2DM. TNF- α has been shown to reduce the expression of GLUT4, an insulin-regulated glucose transporter found primarily in adipocytes, skeletal and cardiac muscles. Furthermore, by inducing serine phosphorylation of insulin receptor substrate-1, TNF- α can act as an inhibitor of peripheral insulin action, leading to insulin resistance [11].

According to two recent meta-analysis both type 1 and 2 DM patients have significantly elevated serum TNF- α levels, thus indicating a positive correlation with insulin resistance [12, 13]. The amount of visceral fat in obese T2DM patients is related to TNF- α plasma levels which are not influenced by an acute decrease in blood glucose levels in poorly regulated diabetic patients [14].

As compared to young-onset type 1 DM, adult-onset autoimmune diabetes is a diverse illness with a lower genetic load, a less severe autoimmune response and minor metabolic decompensation at the beginning (T1DM). For at least 6 months after diagnosis, majority of patients with adult-onset autoimmune diabetes do not require insulin treatment [15]. IA-2 belongs to the protein tyrosine phosphatase family and is one of the most important autoantigens in diabetes [16]. In a cohort of 5,330 individuals with T2DM, the autoimmune diabetes study reported that the prevalence of being positive for IA2 autoantibodies was 4.5% [17]. The present study aimed to assess the correlation between adipokines such as TNF- α and autoantigen such as IA2 with obesity and type 2 diabetes mellitus.

2. Materials and Methods

2.1. Subjects

The study included 140 males and who were divided into four groups:

- 1- Control (non-diabetes and non-obese): 25 healthy males.
- 2- Non-diabetes obese: 25 males.
- 3- T2DM (non-obese): 45 males.
- 4- T2DM (obese): 45 males.

Informed consent was obtained from both the control and patient groups to fill out the study protocol sheet before vein puncture. The patients were diagnosed according to the concentration of blood glucose and HbA1c. Clinical examination was performed under the supervision of physicians who specialized in diabetes and endocrinology, and their classification into groups was according to criteria [18]. Data collection took place in the teaching hospitals of Fallujah and the Center of Endocrinology and Diabetes. The ages of the males ranged between 30-60 years. The samples of the study were taken following the Helsinki Declaration of 1975, as revised in 2000, and approved by the Human Ethical Committee of Salahaddin University, College of Science, Biology department, and numbered 4S/320.

2.2. Collection of Samples

For each subject, 10 ml of blood was obtained by vein puncture. The samples collected were split into two parts of 2 and 8 ml. The first part was placed in a tube containing EDTA. HbA1c was calculated using this blood that was processed in less than three hours. The second part was centrifuged at 3000 rpm for ten minutes to collect the serum which was then stored at -20 degrees Celsius until it was analyzed.

2.3. Measurement of Body Mass Index

Body mass index (BMI) is a weight-relative calculation that is based on the height and weight of an individual. It is calculated by dividing an individual's body mass by the square of their height, with the result expressed in kg/m². In the process of developing "social physics" Belgian Polymath Adolphe Quetelet devised this index between 1830 and 1850 [19]. The current value settings for these individuals are as follows: a BMI of 18.5 to 25 indicates the optimum weight, a BMI lower than 18.5 implies underweight, number above 25 indicates overweight and the number above 30 indicates obesity [20].

2.4. Estimation of HbA1c

HbA1c is a blood test to find the average plasma glucose over the period of past 8 to 12 weeks [21]. It can be done at any time of day and does not necessitate any kind of special planning, such as fasting. These characteristics have made it the favored tool for determining glycemic regulation in diabetics. The HbA1c is determined by using Cobas c311 Hitachi/Roche, Germany device. The Roche Diagnostic Cobas C311 analyzer is an automated software-controlled analyzer for clinical chemistry analysis. It is designed for both quantitative and qualitative *in vitro* determinations using a vast array of assays for analysis. The Cobas C311 analyzer uses serum/plasma to perform photometric assays and ion-selective electrode measurements.

2.5. Estimation of Serum TNF- α

The enzyme immunoassay is intended for TNF- α quantification in plasma, serum and culture supernatant. This is one of the immunological step sandwich-type assays. In the presence of a second anti-TNF- α monoclonal antibody linked to alkaline phosphatase, samples and calibrators are incubated in a microtiter plate coated with the first monoclonal anti-TNF- α . The wells are washed after incubation and the bound enzymatic activity is detected by adding a chromogenic substrate. The color strength is proportional to the amount of TNF- α in the sample.

2.6. Estimation of Serum IA2

The Anti-IA2 ELISA assay kit is a quantitative enzyme immunoassay for autoantibodies to protein tyrosine phosphatase in human blood serum.. IA2 Ab from the sample binds to IA2 coated on a microtiter plate in the first stage. IA2-Biotin binds to this complex in the second stage. The level of IA2 Abs in serum correlates with the amount of bound IA2-Biotin. Washing removes unbound IA2-Biotin by adding streptavidin-peroxidase and a colorogenic substrate (TMB), and reading the optical density at 450 nm.

2.7. Statistical Analysis

Analysis of data was performed by using SPSS (Version 17). Results were expressed as mean \pm S.E. Analysis of variance (ANOVA) and Duncan's post-hoc tests were used for comparing the TNF- α and IA2 between the groups. Fisher's chi-square test was used to compare the incidence of an abnormal concentration of TNF- α and IA2 between the groups. Pearson's correlation (r) was used to find the relationship between TNF- α and IA2 with BMI and HbA1c. A p-value of less than 0.05 was considered to be statistically significant.

2. Results and Discussion

The results presented in **Table 1** show the general criteria of the participants who were used in the current study. No significant differences in the ages appeared between all studied groups. Non-diabetes and T2DM obese groups were found to have significantly ($p \leq 0.001$) higher BMI when compared with control and T2DM non-obese groups. No significant differences in obesity and diabetes durations were observed between both obese and diabetes groups accordingly. Regarding the HbA1c and fasting blood sugar, both diabetes groups showed a higher significant concentration in the HbA1c $p \leq 0.01$ and fasting blood sugar $p \leq 0.001$ when compared with control and non-diabetes obese groups.

Table 1 - General criteria of the control, obese and diabetes groups.

	Control	Nondiabetes Obese	T2DM Nonobese	T2DM Obese	P-value
Age (years)	47.23 \pm 8.45 ^a	40.67 \pm 6.56 ^a	50.38 \pm 10.68 ^a	45.80 \pm 5.26 ^a	NS
Body mass index	23.12 \pm 1.36 ^b	32.57 \pm 1.27 ^a	22.28 \pm 1.87 ^b	32.67 \pm 1.88 ^a	0.001
Duration of obesity (years)	–	3.20 \pm 0.50 ^a	–	3.60 \pm 0.80 ^a	NS
Duration of diabetes (years)	–	–	5.00 \pm 0.30 ^a	4.80 \pm 0.60 ^a	NS
HbA1c %	4.45 \pm 0.78 ^a	5.35 \pm 1.00 ^a	8.24 \pm 1.68 ^b	8.86. \pm 1.34 ^b	0.01
Fasting blood sugar (mg/dL)	95.28 \pm 10.57 ^a	100.85 \pm 8.76 ^a	150.45 \pm 12.70 ^b	165.46 \pm 15.80 ^b	0.001

p-value \leq 0.05 considered significant. NS= non-significant. Post-hoc Duncan's- test: no differences between groups with the same letter.

The results presented in Table 2 show a significant ($p \leq 0.001$) increase in TNF- α concentration in obese and diabetes groups (12.63 ± 1.35 pg/ml in non-diabetes obese), (13.32 ± 0.83 pg/ml in T2DM non-obese) when compared with the healthy control group (5.33 ± 0.67 pg/ml). A higher TNF- α concentration ($p \leq 0.001$) was observed in T2DM obese (21.01 ± 1.27 pg/ml).

Table 2 - The value of TNF- α in control, obese, and diabetes patients.

Groups	TNF- α (pg/ml)
G1: Control	5.33 ± 0.67^a
G2: Non-diabetes obese	12.63 ± 1.35^b
G3: T2DM non-obese	13.32 ± 0.83^b
G4: T2DM Obese	21.01 ± 1.27^c
p-value	0.001

The results are expressed as means \pm standard errors. p -value ≤ 0.05 considered significant.

Post-hoc Duncan's- test: no differences between groups with the same letter.

Chi-square analysis showed that G3 had a significantly ($p \leq 0.001$) higher prevalence of abnormal TNF- α (31.3%) compared with the G1 (0.0%) as shown in Table 3. On the other hand, a higher prevalence of abnormal TNF- α was recorded with the G4 (73.3%). This value is significantly higher ($p \leq 0.001$) than that of G3 (31.1%) as shown in Table 4 and G2 (20.0%), as shown in Table 5. These results agree with the findings of Alzamil [22] who found that TNF was significantly higher in T2DM patients than in the controls. TNF levels in blood were substantially higher in obese diabetic patients than in nonobese diabetic and obese nondiabetic patients. According to two recent meta-analysis, both type 1 and type 2 DM patients have significantly elevated serum TNF, which has a positive relationship with insulin resistance [12, 13].

Table 3 - The prevalence of TNF- α in control and T2DM non-obese groups.

TNF- α	G1	G3
With abnormal value (>15.6 pg/ml)	0.0% (0)	31.1% (14)
With normal value (<15.6 pg/ml)	100.0% (25)	68.9% (31)

Fishers' chi-square = 9.722 p -value = 0.001

Table 4 - The prevalence of TNF- α in T2DM non-obese and T2DM obese groups.

TNF- α	G3	G4
With abnormal value (>15.6 pg/ml)	31.1% (14)	73.3% (33)
With normal value (<15.6 pg/ml)	68.9% (31)	26.7% (12)

Fishers' Chi-square = 18.421 p -value = 0.001

Table 5- The prevalence of TNF- α in non-diabetes obese and T2DM obese groups.

TNF- α	G2	G4
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With abnormal value (>15.6 pg/ml)	20.0% (5)	73.3% (33)
With normal value (<15.6 pg/ml)	80.0% (20)	26.7% (12)

Fishers' chi-square = 16.076 p- value = 0.001

TNF plasma levels are linked to the amount of visceral fat in obese T2DM patients and are not affected by acute blood glucose lowering in poorly regulated diabetic patients [14]. TNF- α has been linked to insulin resistance and T2DM, both of which are linked to obesity in previous studies [23-25]. TNF- α , which is formed by activated macrophages, CD4+ lymphocytes, natural killer cells, neutrophils, mast cells, eosinophils and neurons, is a cytokine that plays a role in systemic inflammation and often causes an acute phase reaction [26]. TNF can contribute to the pathogenesis of type 2 diabetes and obesity by inducing insulin resistance through direct effects on the insulin signaling pathway [27]. In malignant diseases, TNF- α is linked to weight loss, hypermetabolism and resting energy expenditure as an endogenous cause [28].

TNF- α increased in both diabetic types but it was higher in obese type 2 diabetics than non-obese type 2 diabetics, according to our findings. Same results were obtained by Ijaz *et al.* [29]. Reduced body weight is linked to lower TNF- α levels and increased insulin sensitivity in obese people [30]. TNF- α inhibits insulin secretion by inducing amylin expression in the β -cell, resulting in an increase in amylin to insulin ratio in β -cell and probably an excess of amylin over insulin secretion. As amylin accumulates as amyloid, it can play a role in β -cell destruction in type 2 diabetes. Furthermore, since an increase in the circulating amylin/insulin ratio has been linked to insulin resistance which suggests that TNF- α may play a role in the relation between obesity and type 2 diabetes [31].

The results that are presented in Table 6 show non-significant differences in serum IA2 concentration between all groups.

Table 6 - The value of IA2 in control, obese and diabetes patients.

Groups	IA2 (IU/ml)
G1: Control	4.31 \pm 0.88 ^a
G2: Non-diabetes obese	4.76 \pm 0.87 ^a
G3: T2DM non-obese	5.81 \pm 0.47 ^a
G4: T2DM obese	6.00 \pm 0.74 ^a
p-value	0.06

The results are expressed as means \pm standard errors. p-value \leq 0.05 considered significant.

Post-hoc Duncan's- test: no differences between groups with the same letter

Analysis of the data with chi-square showed no significant differences in abnormal IA2 prevalence between G1 and G3 as shown in Table 7, and between G3 and G4 as shown in Table 8. Also, no significant differences were observed between G2 and G4 as presented in Table 9.

Table 7 - The prevalence of IA2 in control and T2DM non-obese groups

	G1	G3
With abnormal value (>30 I.U./ml)	0.0% (0)	2.2% (1)
With normal value (<30 I.U./ml)	100.0% (25)	97.8% (44)

Fishers' chi-square = 0.564 p- value = 1.000

Table 8 - The prevalence of IA2 in T2DM non-obese and T2DM obese groups.

	G3	G4
With abnormal value (>30 I.U./ml)	2.2% (1)	2.2% (1)
With normal value (<30 I.U./ml)	97.8% (44)	97.8% (44)

Fishers' chi-square = 0.564 p- value = 1.000

Table 9 - The prevalence of IA2 in non-diabetes obese and T2DM obese groups

	G2	G4
With abnormal value (>30 I.U./ml)	0.0% (0)	2.2% (1)
With normal value (<30 I.U./ml)	100.0% (25)	97.8% (44)

Fishers' chi-square = 0.564 p- value = 1.000

Testing for islet autoantibodies as a part of the diagnostic assessment in T2DM is significant to a large number of adult patients, as it may contribute to the progression rate of insulin resistance [32]. Differences in study design and inclusion criteria (such as age at the time of diagnosis, sex, mode of recruitment, number and type of autoantibodies tested, and sensitivity and specificity of autoantibody assays), as well as ethnicity, could explain the global variation in autoantibody positivity in patients with T2DM. In addition, the rising prevalence of T2DM in particular populations may have an impact on the frequency of autoantibody-positive in T2DM patients [33]. In patients with newly diagnosed type 1 diabetes, 62.9% of IA2 antibody tests came back positive [34]. While the autoimmune diabetes study reported that the prevalence of positive for IA2 autoantibodies in T2DM was 4.5%. These results agree with the findings of our study in which approved that the prevalence of the antibody against IA2 in T1DM is more frequent than in T2DM.

In the present study, a strong positive correlation was found between the serum TNF- α concentration with both HbA1c and BMI as shown in Table 10, while a non-significant correlation was found between IA2 with TNF- α , HbA1c and BMI.

Table 10 - Pearson's correlation (r) between TNF- α and IA2 with HbA1c and BMI.

	IA2	HbA1c	BMI
TNF- α	0.23	0.57 **	0.65 **
IA2	0	0.18	0.34

** Correlation is significant at $p \leq 0.01$

It has been proved that both obesity and diabetes are linked to an increase in TNF- α . These results are in line with the findings of Rajarajeswari *et al.*[23] who found that TNF-levels to

be substantially higher in obese T2DM patients and that there was a clear association between BMI and TNF-levels which were in contrast with the results of Bhatta *et al.* [35] who observed no significant correlation between TNF- α with insulin resistance and BMI. The role of TNF- α in insulin resistance caused by obesity is explained by increased inflammation in obese diabetics. Obese people can reduce their TNF levels and increase their insulin sensitivity by losing weight [29]. Obese mice have TNF- α overexpression in their adipose tissues, hence creating a direct connection between obesity, T2DM and chronic inflammation [36]. TNF- α is an essential factor in systemic inflammation. Furthermore, it has been shown to increase liver's glucose and triglycerides production while also causing insulin resistance due to its metabolic effects. TNF- α plays a role in obesity and T2DM by causing a substantial decrease in peripheral glucose uptake in response to insulin [37]. Also, the duration of the diabetes disease is related to TNF- α increase. Patients with 121-180 months of the disease have a higher concentration of serum TNF- α when compared with 1-120 months of disease [38].

Finally and according to the interpretation of our data and results, the adipokines such as TNF- α are significantly correlated with obesity and T2DM. The concentration of serum TNF- α and the prevalence of the positive or abnormal value of them in obese T2DM is significantly higher than that of the control and obese non-diabetes. Regarding IA2, no correlation was recorded between IA2 with obesity and T2DM. These results proved that antibodies against IA2 were not found in T2DM. While other investigations and research demonstrate that anti-IA2 is more frequent and is related to T1DM.

Conclusions

The present study concluded that TNF- α is strongly correlated with obesity and diabetes type 2. Also, obese diabetes patients have more TNF- α concentration compared to non-obese diabetes males. No correlation was found between antibodies against IA2 with obesity and TD2M

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Conflict of Interest

The authors declare no conflict of interest.

Human Ethics Declaration

The samples of the study were taken following the Helsinki Declaration of 1975, as revised in 2000, and approved by the Human Ethical Committee of Salahaddin University, College of Science, Biology department, and numbered 4S/320.

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