Al-Azhar Med. J . (Medicine). *Vol. 51(2), April, 2022, 1193 – 1198*
DOI: 10.21608/amj.2022.230481
https://amj.journals.ekb.eg/article_230481.html

STUDY OF SERUM TUMOR NECROSIS FACTOR ALPHA LEVEL IN PREDIABETICS AND TYPE 2 DIABETIC EGYPTIAN PATIENTS

By

Mahmoud Ezzat Abd El-Raouf Mohamed, Youssef Khalil Ahmed, Esam Mohammed Ghamry and Mohamed Abd El-Hamid Khedr*

Departments of Internal Medicine and Clinical Pathology*, Faculty of Medicine, Al Azhar University, Cairo

Mobile: 01128293785, **E-mail:** kingelteb@gmail.com

ABSTRACT

Background: Inflammatory cytokines have potential to enhance insulin resistance, prediabetes and hence type 2 diabetes mellitus (T2DM). Tumor necrosis factor alpha (TNF-α) is a cytokine that is released by adipocytes and inflammatory cells in response to chronic inflammation. The current research was taken to study the role of TNF-α in pathogenesis of prediabetes and new onset T2DM in Egyptian subjects.

Objective: Measurement of TNF-α level in prediabetics, treatment naive T2DM patients, and healthy persons to assess its possible role in management.

Subjects and methods: This study included 30 patients with treatment naive T2DM, 30 prediabetics and 30 control subjects. We assessed serum TNF-α level in all groups by enzyme linked immunosorbent assay (ELISA).

Results: Our study showed a significantly higher level of TNF-α in T2DM patients compared to prediabetics and control subjects. TNF-α showed positive correlation with HBA1c.

Conclusion: TNF-α is associated with concurrent prediabetes and T2DM and correlates positively with HBA1c .

Key words: T2DM, TNF-α and HBA1c.

INTRODUCTION

Metabolic and immune systems are among the most important systems required for survival, and there is a strong integration between regulation of metabolism and the immune responses. Chronic inflammation due to abnormal productions of cytokines and activation of inflammatory signaling pathways is closely associated with metabolic disorders such as obesity, insulin resistance, prediabetes and T2DM *(Alzamil, 2020).*

TNF-α was the first proinflammatory cytokine recognized for its involvement in pathogenesis of insulin resistance and T2DM. It has been reported that TNF-α decreases the expression of insulin regulated glucose transporter type 4 (GLUT4) which is located mainly in adipocytes, cardiac and skeletal muscles. Moreover, TNF-α can act as an inhibitor of peripheral insulin action by inducing serine phosphorylation of insulin receptor substrate-1 which leads to insulin resistance *(Akash et al., 2018).* Two

studies indicated that both patients with type 1 and type 2 DM have significantly elevated levels of serum TNF-*α (Liu et al., 2016* and *Qiao et al., 2017).*

Research about the role of TNF- α in type 2 diabetes mellitus is not settled yet and is still a subject of active research work. A recent systematic review investigated the association between TNF-α and type 2 diabetes mellitus and concluded that there is considerable heterogeneity between studies and further research is needed *(Liu et al., 2016).*

In this study, we aimed to look into the relation between the level of TNF-α and the prediabetes state, and the newly diagnosed T2DM.

SUBJECTS AND METHODS

The present study was a cross sectional case control study and was approved by the Research Ethical Committee. Oral and written consents were obtained from all patients and controls after a full explanation of the study.

This study was conducted on 90 Egyptian citizens ages ranged from 30 to 65 years, The studied subjects were recruited from Al-Hussein University Hospital. Subjects were classified into three equal groups:

- **Group A:** Included patients with newly diagnosed up to 6 months type 2 diabetes (treatment naive) diagnosed according to the latest American Diabetes Association (ADA) criteria.
- **Group B:** Included patients with prediabetes diagnosed according to the latest American Diabetes Association (ADA) criteria, with impaired fasting blood glucose or impaired glucose tolerance or impaired HbA1c.
- **Group C:** Included healthy subjects with no diabetes or prediabetes as a control group.

Exclusion criteria:

- Patients with clinically significant renal, liver, neurological, endocrinal, cardiovascular, autoimmune, infection, any acute inflammation or other major systemic diseases including malignancy.
- Patients who received antibiotics, nonsteroidal anti-inflammatory drugs, corticosteroid, or cytotoxic drugs before taking blood for the study.
- Patients with the habits of smoking and/or alcohol use.

All patients were subjected to full history, clinical examination, laboratory investigations as fasting blood glucose, kidney and liver function tests, HBA1c, serum insulin and TNF-α. In all control and patient groups, 5 ml of venous blood were taken in plain tube and left to clot at room temperature for 20 minutes, then centrifuged, and the serum was separated and stored at -20°C until assessed for TNF-α using ELISA kits supplied by R&D Systems (Abingdon, United Kingdom).

Statistical analysis:

Data were analyzed by the computer software program Statistical Package for the Social Sciences (SPSS version 20, Chicago). Descriptive characteristics of the study patients were expressed as mean ± SD (standard deviation) and compared by a one way analysis of variance

(ANOVA) test. Tukey's test was used as post hoc test.

Kolmogorov–Smirnova and Shapiro–Wilk tests were used to see whether data follow normal distribution or not.

Probability (P-value) was considered significant at $P \leq 0.05$.

Pearson's correlation coefficient (R) test was used to assess the degree of association between two sets of variables.

RESULTS

As regard to TNF-α level, there was a statistically significant difference (p value <0.001) between T2DM and prediabetes groups, and prediabetes and control groups **(Table 1)**.

Table (1): Comparison between studied groups as regard TNF-α

[TNF-α (pg/ml)]	T2 DM Group *(n=30)*	Prediabetes Group *(n=30)*	Control Group *(n=30)*	ANOVA	p-value
Mean±SD	9.69±2.31	6.98±1.74	3.38±0.89	98.811	<0.001
Tukey's Test					
I vs. II		**I vs. III**		**II vs. III**	
<0.001		<0.001		<0.001	

n: number, p-value : Probability value, SD: Standard deviation.

Our data also showed that TNF-α level was positively correlated with glycemic profile (fasting blood glucose and HBA1c) in T2DM, prediabetes and control groups **(Table 2)**.

Table (2): Correlation between TNF-α with HbA1C and fasting glucose in each group

TNF-α	Correlation	T2 DM Group		Prediabetes Group		Control Group	
		HBA1c	FBG	HBA1c	FBG	HBA1c	FBG
Serum TNF-(pg/ml)	R	0.690	0.669	0.444	0.427	0.755	0.572
	p-value	<0.001	<0.001	0.014	0.019	<0.001	<0.001

p-value: Probability value, R: correlation coefficient

DISCUSSION

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine. It has since been found to be produced by various cell types including macrophages, lymphoid cells, endothelial cells, cardiac myocytes and adipose tissue *(Braumuller et al., 2013)*. It has been reported that TNF-α reduces the expression of insulin-regulated glucose transporter type 4 (GLUT4) which is located mainly in adipocytes and skeletal muscles *(Alzamil, 2020)*. TNF-α was believed to induce insulin resistance by inhibiting phosphorylation of insulin receptor substrate 1 (IRS-1) on insulin signaling cascade, Therefore, this marker is

suspected to be a possible mediator between insulin resistance and diabetes *(Smitka and Maresova, 2015)*. Our data showed that there was a significant increase in level of TNF-a in diabetic patients than prediabetics and control subjects, Also prediabetics have significantly higher level of TNF-a than the control subjects. The associations between TNF-a and insulin resistance, prediabetes or T2DM had been widely researched. Most studies found similar results of our data that subjects with insulin resistance, prediabetes, or T2DM have increased levels of TNF-a (Mirza et al., 2012 and Akash et al., 2018).

It has been reported that high levels of inflammatory cytokines including TNF-a appeared in early stages of T2DM and were capable of predicting the development of type 2 diabetes through increasing insulin resistance which agreed with our data that TNF-a significantly increased in prediabetic stage of T2DM than in control subjects (Bashir et al., 2020).

Interestingly, we found a positive correlation between TNF- and body mass index (BMI), and also with insulin resistance in either diabetic or prediabetic groups There data were consistent with a study in KSA which found a significantly higher TNF-α levels in obese diabetic patients compared to nonobese diabetic patients and their elevated TNF-α levels showed a significant positive correlation with insulin resistance *(Alzamil, 2020)*.

On the other side, one study showed decreased levels of TNF- a in T2DM compared to healthy controls, These results were against our data and most of other studies data in this field, The author of this study interpreted that this discrepancy could be attributed to duration of the diseases as the majority of patients included in this study had a long disease duration (greater than 5 years), small sample size, and the differences in age and sex of the studied groups, although our studied subjects were new onset T2DM within 6 months of diagnosis before any medical treatment and in prediabetes stage *(Al-Shukaili et al., 2013)*.

Our data showed that TNF-α increased before onset of T2DM as in our prediabetic group There was a significant increase in serum levels of TNF-α than in control group indicating low grade inflammation and increase in TNF-α contributes to the pathogenesis of insulin resistance, and type 2 diabetes: These data were comparable to data from a study who found increased serum TNF-α concentrations in type 2 DM and IGT subjects *(Gupta et al., 2015)*.

On the other hand, a study on Korean subjects had not found any association of TNF-a with impaired glucose tolerance group. However, the authors explained that in an important point. They mentioned that they did not extensively Studied TNF-α in the prediabetes group of patients and subjects in prediabetes group were not obese *(Choi et al., 2014)*.

REFERENCES

1. **Akash MH, Rehman K and Liaqat A. (2018):** Tumor necrosis factor-alpha role in development of insulin resistance and pathogenesis of type 2 diabetes mellitus. Journal of Cellular Biochemistry, 119(1):105-110.
2. **Al-Shukaili A, AL-Ghafri S, Al-Marhoobi S, Al-Abri S, Al-Lawati J**

and Al-Maskari M. (2013): Analysis of inflammatory mediators in type 2 diabetes patients. International Journal of Endocrinology, 97(5):56-62.

3. **Alzamil H. (2020):** Elevated serum TNF-α is related to obesity in Type 2 Diabetes Mellitus and is associated with glycemic control and insulin resistance. Journal of Obesity, 2020(1):176-184.
4. **Bashir H, Showkat AB, Majid S, Hamid R, Kou RK, Rehman MU, Bhat JA, Qadir K and Masood A. (2020):** Role of inflammatory mediators (TNF-α, IL-6, CRP), biochemical and hematological parameters in type 2 diabetes mellitus patients. India Med J Islam Repub Iran., 34(5):119-126.
5. **Braumuller H, Wieder T, Brenner E, Aßmann S, Hahn M and Alkhaled M. (2013):** T-helper-1-cell cytokines drive cancer into sentence. Journal of Nature, 494 (7437): 361-365.
6. **Choi KM, Lee J, Lee KW, Seo JA, Oh JH and Kim SG. (2014):** Comparison of serum concentrations of C-reactive protein, TNF-a and IL-6 between elderly Korean women with normal and Impaired Glucose Tolerance. Diabetes Res Clin Pract., 64(1):99-106.
7. **Gupta A, Ten S and Anhalt H. (2015):** Serum levels of soluble TNF-a receptor 2 are linked to insulin resistance and glucose tolerance in children. Pediatric Endocrinol Metab., 18(1):75-82.
8. **Liu C, Feng X, Li Q, Wang Y, and Hua M. (2016):** Adiponectin, TNF-α and inflammatory cytokines and risk of type 2 diabetes: a systematic review and meta-analysis. Cytokine, 86(2):100–109.
9. **Mirza S, Hossain M and Mathews C. (2012):** Type 2-diabetes is associated with elevated levels of TNF-alpha, IL-6 and adiponectin and low levels of leptin in a population of Mexican Americans: a cross-sectional study. Cytokine, 57(1):136-142.
10. **Qiao YC, Chen YL and Panetal YH. (2017):** The change of serum tumor necrosis factor alpha in patients with type 1 diabetes mellitus: a systematic review and meta-analysis. PLoS One, 12(4):129-137.
11. **Smitka K and Maresova D. (2015):** Adipose Tissue as an Endocrine Organ: An update on pro-inflammatory and anti-inflammatory microenvironment. Prague Med Rep., 116(2):87-111.

دراسة مستوي عامل نخر الورم ألفا في الدم للمرضي المصريين المصابين بمرحلة ما قبل السكري ومرضي السكري من النوع الثاني

محمود عزت عبدالرؤوف، يوسف أحمد خليل، عصام محمد غمري، محمدعبدالحميد خضر*

قسمي الباطنة العامة و الباثولوجيا الإكلينيكية*، كلية الطب، جامعة الأزهر، القاهرة

E-mail: kingelteb@gmail.com

خلفيـــة البحـــث: الســـيتوكينات الالتهابيـــة لـــديها القـــدرة علـــي زيـــادة مقاومـــة الانســـولين وبالتـــالي تســـبب مرحلـــة ماقبـــل الســـكري و مـــرض الســـكرمن النـــوع الثـــاني. وعامـــل نخرالـــورم ألفـــا هـــو الســـيتوكين الّـــذي يـــتم اطلاقـــه مـــن الخلايـــا الدهنيـــة والخلايـــا الالتهابيـــة إســـتجابة للالتهـــاب المزمن.

الهـــدف مـــن البحـــث: قيـــاس مســـتوى عامـــل نخـــر الـــورم ألفـــا فـــي مرضـــي مـــا قبـــل الســـكر، مرضـــي الســـكر مـــن النـــوع الثـــاني الحـــديثي الاكتشـــاف والأشـــخاص الأصـــحاء لتقيـــيم دوره المحتمل في تشخيص ومتابعة المرضي.

المرضـــي وطـــرق البحـــث: أجريـــت هـــذه الدراســـة علـــى ثلاثـــين مـــن مرضـــى مرحلـــة مـــا قبـــل الســـكر وثلاثـــين مـــن مرضـــي الســـكر مـــن النـــوع الثـــاني وثلاثـــين مـــن الأفـــراد الأصـــحاء المتطـــوعين كمجموعـــة ضـــابطة. وقـــد تـــم قيـــاس مســـتوى عامـــل نخـــر الـــورم ألفـــا بالـــدم بطريقـــة المقايسة المناعية المرتبطة بإنزيم (الإليزا) للمجموعات الثلاثة.

النتـــائج: أظهـــرت دراســـتنا ارتفاعـــا ملحوظـــا فـــي مســـتوي عامـــل نخـــر الـــورم ألفـــا فـــي مرضـــي النـــوع الثـــاني مـــن الســـكرمقارنة بمرضـــي مرحلـــة مـــا قبـــل الســـكر والمجموعـــة الضـــابطة، كمـــا أظهـــرت أن مســـتوي عامـــل نخـــر الـــورم ألفـــا يتناســـب بشـــكل ايجـــابي مـــع معـــدل الســـكر التراكمي في جميع المجموعات.

الاســـتنتاج: مســـتوي عامـــل نخـــر ورم ألفـــا يزيـــد فـــي مرضـــي مرحلـــة مـــا قبـــل الســـكر ومرضـــي السكر من النوع الثاني ويرتبط ايجابيا مع معدل السكر التراكمي.

الكلمـــات الدالـــة : مـــرض الســـكر مـــن النـــوع الثـــاني، عامـــل نخـــر الـــورم ألفـــا و معـــدل الســـكر التراكمي.