

Research Article

Comparison of Tumor Necrosis Factor Alpha and Insulin Resistance in Obese versus Non-obese Type 2 Diabetic Patients

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Abstract | Type 2 diabetes mellitus (T2DM) and insulin resistance (IR) are linked to each other. Obesity and T2DM are states of low-grade chronic inflammation, which result in increased levels of inflammatory markers such as C-reactive protein (CRP), interleukins 6 (IL-6) and tumor necrosis factor alpha (TNF- α). Relation of TNF- α with obesity induced IR and T2DM is unclear as results obtained from different studies are very controversial.

Objective: This study was designed to compare TNF- α levels and insulin resistance in obese and non-obese type 2 diabetics.

Methodology: A cross sectional comparative study was conducted in diabetic clinic of Mayo Hospital, Lahore. We determined and compared TNF- α levels and insulin resistance in 90 subjects where there were 50 obese patients with T2DM and 40 were non-obese Type 2 diabetic patients. TNF- α and serum insulin levels were determined using ELISA. Insulin resistance was calculated using HOMA-IR. Comparison between groups was performed using independent sample t-test. The P value ≤ 0.05 was considered statistically significant.

Results: Mean HOMA-IR and TNF- α values were significantly (p-value <0.01) higher in obese diabetics (17.13 ± 8.77) and (10.96 ± 4.69), respectively when compared to non-obese Type 2 diabetic patients (3.40 ± 5.05) and (3.49 ± 2.36) respectively. Mean HOMA-IR in males was 6.52 ± 7.03 and in females was 12.85 ± 10.54 (p-value 0.006).

Conclusion: Increased inflammation in obese diabetics explains the role of tumor necrosis factor alpha in insulin resistance induced by obesity. Weight reduction in obese individuals will help in reducing TNF- α levels and to improve insulin sensitivity in T2DM.

Received | January 02, 2016; **Accepted** | January 10, 2018; **Published** | April 07, 2018

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Citation | Khurram, R. 2018. Comparison of tumor necrosis factor alpha and insulin resistance in obese versus non-obese type 2 diabetic patients. *Annals of King Edward Medical University*, 24(1): 81-85.

DOI | <https://doi.org/10.21649/akemu.v24i1.2333>

Keywords | Insulin Resistance, Type 2 Diabetes Mellitus, Obesity, Tumor Necrosis Factor Alpha

Introduction

In Type 2 diabetes mellitus (DM), blood sugar levels are increased due to defects in insulin secretion and/or insulin action ⁽¹⁾. The prevalence of DM in the world was estimated to be around 171 million people

in the year 2000, which is expected to be more than 366 million in the year 2030 ^(2,3). While in Pakistan, the prevalence of DM was about 5.2 million in the year 2000. It will be up to 13.9 million by the year 2030 ⁽²⁾. The prevalence of type 2 DM is about 90% of all cases of diabetes ⁽¹⁾.

Type 2 diabetes mellitus (T2DM) develops mostly in overweight and obese persons and is directly linked to insulin resistance⁽³⁾. It was revealed by National Health Survey Of Pakistan that 25% of the population is suffering from obesity⁽⁴⁾. Adipose tissue not only stores the excess calories but also actively secrete several substances such as fatty acids, hormones and cytokines that can act in a paracrine or endocrine fashion⁽⁵⁾. Obesity is the major cause of many metabolic disorders, which are characterized by the chronic inflammation and are linked to T2DM and insulin resistance⁽⁶⁾.

TNF- α is remarkably higher in obese people and it may serve as important risk factor for future development of T2DM and may prove a novel target for the therapeutic intervention⁽⁵⁾. TNF- α is a pro-inflammatory adipokine and is released by macrophages of adipose tissue. It can cause insulin resistance by several mechanisms^(5,7).

Literature review shows that TNF- α is related to insulin resistance such as study by Bertin et al⁽⁸⁾ has found out that body mass index (BMI) is associated with TNF- α but not with blood glucose levels. Study by Bhatti et al⁽⁹⁾ showed that TNF- α has no significant relation to insulin resistance, body mass index and abdominal circumference. Similarly, Sujaita et al 2010⁽¹⁰⁾ in their study found that serum TNF- α was neither associated with insulin, homeostatic model assessment-insulin resistance (HOMA-IR) nor with obesity parameters in local population. Another study by Swaroop et al⁽¹¹⁾ showed that inflammation plays an important role in the progress of insulin resistance mostly in males with increased BMI. Study by Rajajajeswear⁽⁵⁾ found that TNF- α concentration was significantly high in obese T2DM and has strong correlation with BMI.

Considering above-mentioned results obtained from different studies on the role of TNF- α and IR are highly controversial and our understanding is incomplete. Only fewer studies have been conducted in south Asian population especially in Pakistan regarding inflammatory markers and insulin resistance in T2DM. However, relationship of TNF- α with obesity induced IR and T2DM is still unclear. Therefore, the present study was designed to determine and compare the levels of TNF- α and insulin resistance in obese and non-obese type 2 diabetic patients. These findings will help to establish the role of inflammato-

ry markers with insulin resistance in obese-mediated diabetics in Pakistani population. It will generate new data for our population directly affecting the management of patients with diabetes. Additionally, it will help researchers to develop methods for reducing TNF- α and IR state. Prevention of type 2 diabetes and its related complications is the utmost importance for present period as the prevalence of T2DM is continuously increasing.

This study was aimed to determine and compare TNF- α levels and insulin resistance in obese versus non-obese type 2 diabetics.

Materials and Methods

A cross sectional comparative study was conducted in Diabetic Clinic of Mayo Hospital, Lahore after approval from the Institutional Review Board. The study duration was 6 month from July 2015 to December 2015.

Sample size of 90 patients was estimated by using 90% power of test, 5% level of significance with expected mean value of TNF- α in obese type 2 diabetic group and non obese diabetic group as 215.8 (5) and 168.5⁽⁵⁾, respectively. Non-probability convenient sampling technique was used.

Registered and physician diagnosed cases of T2DM irrespective of gender, age between 30-75 years were offered to be enrolled. While patients on insulin therapy (identified on history), Type 1 diabetics, drugs which can decrease insulin resistance e.g. Metformin, Thiazolidinediones users for last 6 months, asthmatic patients for last 5 years (TNF- α levels are higher in lung disorders) and smokers on the basis of history as smoking effect the inflammatory pathways (taking 20 packs of citrates/year for last two years) were excluded.

Informed written consent was taken from patients and included patients were called next day with a 12-hour overnight fasting. Stadiometer and weight machine was used for height in meters and weight in Kg. BMI was calculated by formula; weight (kg)/height (m²). On the basis of BMI, they were divided into 2 groups. Cut-off value of BMI was 25kg/m². For laboratory parameters such as tumor necrosis factor alpha (TNF α), fasting blood glucose and fasting insulin levels, 5ml of venous blood was taken by aseptic measures

Table 1: Descriptive statistics of Study parameters in type 2 diabetic patients

	Mean	S.D	Range	Minimum	Maximum
Age (years)	50.63	9.89	43.00	32.00	75.00
Height (m ²)	1.61	0.11	.42	1.43	1.85
Weight (kg)	71.01	12.32	55.00	45.00	100.00
Body mass index (kg/m ²)	27.45	5.19	24.97	16.14	41.11
Duration of disease (years)	3.18	1.27	5.00	1.00	5.00
Fasting Blood Glucose (mg/dl)	164.68	31.11	180.00	60.00	240.00
Serum Insulin (F) μ IU/ml	26.96	24.55	109.42	1.78	111.20
HOMA-1R	11.02	10.03	44.00	.70	44.70
TNF- α (pg/ml)	7.64	5.38	21.82	.00	21.82

post 12 hour fast. It was centrifuged and stored at -20°C. Serum was divided into three batches (one batch for fasting blood sugar and lipid profile, 2nd batch for TNF- α , 3rd batch for fasting insulin level). Insulin resistance was calculated by HOMA method (homeostatic model assessment), which is validated as reliable measure of insulin sensitivity.

Formula of HOMA – IR = fasting blood glucose \times fasting insulin /22.5

Insulin was determined using enzyme amplified sensitivity immunoassay (EASIA) (INS-EASIA, catalogue no. KAP1251, DIAsource ImmunoAssays S.A, Belgium). The range of insulin levels was 5 to 19 μ IU/ml. While TNF- α was determined using immunoenzymatic assay (TNF- α -EASIA, catalogue no. KAP1751, DIAsource ImmunoAssays S.A, Belgium). The detectable concentration was ranged between 4.6 and 12.4 pg/ml. ELISA kits were run on AMP Platos R 11 microplate reader in CENUM laboratory, Mayo Hospital Lahore. Fasting blood glucose was measured by glucose oxidase method. All information collected was entered in a specially designed proforma.

All the collected data was analyzed using SPSS. Quantitative variables such as age, height, weight, age, TNF- α , IR, fasting blood glucose were presented as mean \pm S.D. While qualitative variables such as gender and obesity were presented as frequency and percentages. P- value \leq 0.05 was taken as significant. Independent sample t-test/Mann Whitney U-test were used for comparison between two groups. Confounders such as age and gender were controlled by stratification.

Results

A total of 110 patients were evaluated from diabetic

outpatient department of Mayo Hospital, Lahore that were eligible for inclusion in the study and laboratory testing. Eight patients declined consent because they had previously been involved in other research studies earlier in the year. Six patients failed to appear in the first visit for sample collection in fasting state. The number of patients evaluated was 96. However during sample collection, 6 samples were noted to be haemolysed hence discarded. A total of 90 active patients were analysed for the clinical and laboratory outcomes.

In this study, mean age of patients was 50.63 \pm 9.89 years with 26(28.89%) male and 64(71.11%) female patients. Mean \pm standard deviation of HOMA-IR and TNF- α were 11.02 \pm 10.03 and 7.64 \pm 5.38 pg/ml, respectively. There were 50 (55.56%) obese and 40 (44.44%) non-obese patients (Table 1).

Mean fasting glucose was higher in obese type 2 diabetic patients (171.32 \pm 86) as compared to non-obese diabetics (156.78 \pm 27.16), p-value = 0.030. Moreover mean fasting insulin was also statistically higher in obese patients (41.55 \pm 22.37) when compared to non-obese patients (8.71 \pm 11.49), p-value < 0.01. Similarly mean HOMA-IR and TNF- α was also higher in obese diabetics as compare to non-obese diabetic patients, p-value < 0.01 (Table 2).

Discussion

TNF- α is hypothesized to link obesity to insulin resistance. Studies in human and animal models have showed that TNF- α expression in the adipose tissue is considerably raised in obesity⁽¹²⁾ and decreased after weight loss⁽¹³⁾. In this study, we determined and compared TNF- α levels and insulin resistance in obese versus non-obese type 2 diabetics.

Table 2: Comparison of Biochemical Parameters between obese Type 2 diabetics and non-obese Type 2 diabetic patients

Type 2 Diabetics	BMI	n	Mean	S.D	p-value
Fasting Blood Glucose (mg/dl)	Obese	50	171.00	32.86	0.030 ^a
	Non-Obese	40	156.78	27.16	
Fasting Insulin (μIU/ml)	Obese	50	41.55	22.37	<0.01 ^b
	Non-Obese	40	8.71	11.49	
HOMA-1R	Obese	50	17.13	8.77	<0.01 ^b
	Non-Obese	40	3.40	5.05	
TNF-α (pg/ml)	Obese	50	10.96	4.79	<0.01 ^b
	Non-Obese	40	3.49	2.36	

Independent sample t-test was applied for comparison; Mann Whitney U test was applied (as data was not normal)

Our study found higher levels of TNF-α, fasting insulin and HOMA-IR in obese T2DM patients as compare to non-obese T2DM as reported in several studies (5,12,13). The TNF-α and HOMA-IR were found to be higher in females as compare to males. The most possible reason for high levels of TNF-α and HOMA-IR in females is due to raised BMI reported in females as compare to males in our population and unequal ratio of male/female. While study by Bhatti et al, found higher levels of TNF-α in males as compare to females, but HOMA-IR were more in females than males. But this study was only done on obese subject. Non-obese subjects and type 2 diabetics were not included in this study (9).

Our results demonstrated that TNF-α was increased in both diabetic groups but TNF- α was more in obese than non-obese type 2 diabetics. Same results were obtained in study conducted by Nilsson et al (14) in which TNF-α levels were increased 23% in lean T2DM and 51% in obese T2DM subjects. Katsuki et al (14,2) reported that TNF-α is elevated in obese T2DM but not in lean T2DM. According to Hotamisligil et al (12), body weight reduction in obese individuals is also associated with a reduction in TNF-α levels and in improved insulin sensitivity. Our present results clearly demonstrated that circulating TNF-α levels were significantly elevated in obese T2DM compared to non-obese type 2 diabetic.

Study on Indian population by Goyal R et al in 2012 (15) found that TNF-α levels were very high in obese type 2 diabetics but in our study the mean values of TNF-α levels were less than their values. But same findings like our study were obtained in international

studies done in Caucasians.

Due to higher incidence of obesity in our country, Pakistani population is more prone to its lethal effects. The effect of insulin therapy on levels of inflammatory markers among obese and non-obese diabetics has been inadequately studied. There is need to find out the effect of insulin therapy to neutralize and/or reduce the levels of TNF-α. There is also need to do further investigations at the gene level in detail so that we can get conclusive inference from the above observations.

The small size of the sample and the limited observation period do not allow a definitive conclusion of these data. A more comprehensive study with the large population and long period would be more informative. This study is performed on type 2 diabetics. There is need to do research on type 1 diabetes mellitus to find out the effect of inflammatory markers in early age.

Conclusion

TNF-α levels are more in obese diabetics as compare to non-obese diabetics. Our current observations add further support to the evidence that TNF-α plays an important role in insulin resistance linked with obesity and T2DM in humans.

Author's Contribution

Farhat Ijaz: Designed the study, collected data and wrote the article.

Rana Khurram Aftab and Samia Jawed: Equally contributed in helping the first author.

References

1. American diabetes association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2015 Jan; 38(Supplement 1): S8-S16 <https://doi.org/10.2337/dc15-S005>
2. Nadeem A, Naveed AK, Hussain M.M, Raza SI. Correlation of inflammatory markers with type 2 diabetes mellitus in Pakistani patients. JPMI 2013; 29(3):267-71.
3. Younis BB, Ahmad R. Patients with diabetes mellitus and their management – A local scenario. Annals of KEMU 2014;16(4):237-41.
4. Afzal S, Qaiser F, Saeed I, Malik K. Metabolic

- syndrome according to ATP₃ guidelines in public sector diabetic clinic in Pakistan. *EMBASE* 2014;8(10):95-101.
5. Rajarajeswari D, Ramalingam K, Krishnamma M, Krishna TS. Association of TNF- α with obesity in type 2 diabetes mellitus. *IJPBS* 2011;2(2):352-5.
 6. Hamada D, Maynard R, Schott E, Drinkwater CJ, Ketz JP, Kates SL, Jonason JH, Hilton MJ, Zuscik MJ, Mooney RA. Suppressive effects of insulin on tumor necrosis factor-Dependent early osteoarthritic changes associated with obesity and type 2 diabetes mellitus. *Arthritis Rheum* 2016; 68:1392-1402. <https://doi.org/10.1002/art.39561>
 7. Popa C, Netea MG, Van Riel PL, Van der Meer JW, Stalenhoef AF. The role of TNF- α in chronic inflammatory conditions, intermediary metabolism and cardiovascular risk. *JLR*. 2007;48(4):751-62. <https://doi.org/10.1194/jlr.R600021-JLR200>
 8. Bertin E, Nguyen P, Guenounocu M, Durlach V, Potron G, Leutenegger M. Plasma levels of tumor necrosis factor-alpha (TNF-alpha) are essentially dependent on visceral fat amount in type 2 diabetic patients. *Diabetes Metab*. 2000;26:178-82.
 9. Bhatta SA, Aziz S, Shaikh NA, Hussain T. Insulin resistance and inflammatory markers in obesity. *PJMS* 2012;28(1):101-4.
 10. Sujata RM, Sudha SD, Suresh DM. Role of adipocytokines in insulin resistance: studies from urban western Indian population. *Ind J Diabetes Metab* 2010;18 :35-42.
 11. Swaroop J.J, Rajarajeswari D, Naidu JN. Association of TNF α with insulin resistance in type 2 diabetes mellitus. *IJMR* 2012; 135:127-30. <https://doi.org/10.4103/0971-5916.93435>
 12. Kern P, Saghizadeh M, Ong J, Bosch R, Deem R, Simsolo R. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Invest* 1995;95: 2111-9. <https://doi.org/10.1172/JCI117899>
 13. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* 1995;95:2409-15. <https://doi.org/10.1172/JCI117936>
 14. Nilsson J, Jovinge S, Niemann A, Reneland R, Lithel H. Relation between plasma tumor necrosis factor alpha and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 1998;18:1192-202. <https://doi.org/10.1161/01.ATV.18.8.1199>
 15. Goyal R, Faizy AF, Siddiqui SS, Singhai M. Evaluation of TNF- α and IL-6 levels in obese and non obese diabetics: Pre and postinsulin effects . *NAJMS* 2012;4(4):180-4. <https://doi.org/10.4103/1947-2714.94944>