ORIGINAL ARTICLE

CORRELATION OF TUMOUR NECROSIS FACTOR-α WITH OBESITY INDUCED INSULIN RESISTANCE IN TYPE 2 DIABETES MELLITUS

Farhat Ijaz, Rana Khurram Aftab*, Syed Muhammad Zubair*, Rana Rakhshan Aftab**

Department of Physiology, CMH Lahore Medical College, *King Edward Medical University, **Rashid Latif Medical College, Lahore, Pakistan

Background: Obesity is a major cause of many metabolic disorders characterized by chronic inflammation that is linked with T2DM and insulin resistance. Tumour Necrosis Factor Alpha (TNF-α) is remarkably higher in obese people. The aim of this study was to determine the levels of TNF-α and its correlation with insulin resistance and body mass index in type 2 diabetic patients. **Methods:** This study was conducted at Diabetic Clinic of Mayo Hospital, Lahore. TNF-α levels were determined and correlated with insulin resistance and body mass index in 90 obese type 2 diabetics. TNF-α and serum insulin were determined using ELISA. Insulin resistance was calculated by HOMA-IR. Pearson correlation test was used to correlate TNF-α with Insulin Resistance and body mass index, and $p \leq 0.05$ was considered statistically significant. **Results:** There was a strong positive correlation of fasting insulin and HOMA-IR with TNF-α ($r=0.986$, $p<0.001$). There was also strong positive correlation of TNF-α with BMI. **Conclusion:** Increased inflammation in obese diabetics explains the role of TNF-α in insulin resistance caused by obesity. Weight reduction in obese individuals will help in reducing TNF-α levels and to improve insulin sensitivity in T2DM.

Keywords: Insulin Resistance, Obesity, Type 2 Diabetes Mellitus, Tumour Necrosis Factor Alpha

INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are the emerging diseases of this century. These two represent chief public health problem in the world and are associated with potentially threatening comorbidities. The incidence of overweight and obesity is growing rapidly in the world, especially in developing countries.[1] There is an important correlation between these two conditions. Meta-analysis of different studies regarding link between T2DM and obesity indicated the highest relative risk (RR) with waist circumference and body mass index (BMI) in both genders.[2] All T2DM patients are not obese. Likewise, all obese patients are not diabetics. But a large number of type 2 diabetics are overweight/obese, and many overweight or obese people are diabetics. Excessive weight gain, obesity and T2DM can be prevented by change in lifestyle, avoiding sedentary habits and excessive energy intake. Both conditions have insulin resistance and dyslipidemia such as high triglyceride (TG's) and low high-density lipoprotein-C (HDL-C). The hereditary base of human obesity that leads to insulin resistance and type 2 diabetes mellitus is multifactorial. Recent clinical strategies have recognized the role of workout and exercise in the prevention and treatment of diabetes mellitus.

National Health Survey of Pakistan revealed that 25% of population suffers from obesity.[3] The high prevalence of obesity is leading towards decreased life span by its complications such as T2DM and cardiovascular disease (CVD). Obesity is the major cause of many metabolic disorders which is characterized by the chronic inflammation that is linked with T2DM and insulin resistance.[4] Tumour Necrosis Factor Alpha (TNF-α) is remarkably higher in obese people. 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, released by macrophages of adipose tissue. It can cause insulin resistance by several mechanisms.[6] The release of adipocytokines from tissue macrophages of adipose tissue points to chronic inflammatory condition that plays an important role in the development of insulin resistance and T2DM.[7]

Literature review shows that studies are done to determine the role of TNF-α and insulin resistance (IR) in obesity and T2DM.[8,9] But results obtained from different studies are very controversial and relationship of TNF-α with obesity induced IR and T2DM is still unclear. The objective of this study was to determine the correlation of TNF-α levels with insulin resistance and body mass index in patients of type 2 diabetes mellitus.

METHODOLOGY

It was a cross-sectional study conducted from July to December 2016 at Diabetic Clinic of Mayo Hospital, Lahore after approval from the Institutional Review Board. Sample size of 90 patients was estimated by sample size determination in health studies using 90% power of test, 5% level of significance. Convenient sampling technique was used for data collection. Registered and physician diagnosed cases of T2DM irrespective of gender, age 30–75 years were offered to be enrolled, while patients on insulin therapy, Type 1 diabetics, Metformin or Thiazolidinedione users, asthmatics and smokers were excluded from the study. Informed written consent was taken from subjects. They were called next day with a 12-hour overnight fast.

Height of the subjects was recorded in meters and weight in Kg. Five ml venous blood was taken for fasting blood glucose, fasting insulin levels and TNF-α levels. It was centrifuged and stored at -20 °C. Insulin resistance was calculated by HOMA-IR method (homeostatic model assessment).

Insulin was determined using Enzyme Amplified Sensitivity Immunoassay (EASIA) (INS-EASIA, catalogue No. KAP1251, DIA source Immuno Assays SA, Belgium). The range of insulin levels was 5–19 µIU/ml. TNF-α was determined using immuno-enzymatic assay (TNF-α-EASIA, catalogue No. KAP1751, DIA source Immuno Assays SA, Belgium). The detectable concentration was between 4.6 and 12.4 pg/ml. Kits were run on AMP Platos R 11 micro plate reader at Centre for Nuclear Medicine (CENUM) Laboratory, Mayo hospital Lahore. Fasting blood glucose was measured by glucose oxidase method. All information collected was entered in a specially designed proforma.

Collected data was entered into SPSS-21 for analysis. Quantitative variables like age, height, weight, TNF-α, IR, fasting blood glucose were presented as Mean and Standard Deviation, and qualitative variables like gender and obesity were presented as frequency and percentages. Correlation was evaluated using Pearson correlation test, and $p \leq 0.05$ was taken as significant.

RESULTS

Mean age of patients was 50.63±9.89 years in 26 (28.89%) male and 64 (71.11%) female subjects. The mean values of height, weight and BMI were 1.161±0.11 m², 71.01±12.32 Kg and 27.45±5.19 Kg/m² respectively. Mean±SD of HOMA-IR and TNF-α were 11.02±10.03 and 7.64±5.38 pg/ml respectively. Average fasting Glucose and fasting insulin levels were 164.68±31.11 mg/dl and 29.96±24.55 µIU/ml respectively (Table-1).

Table-2 shows associations between serum TNF-α and study parameters with Pearson correlation coefficient. In Type 2 diabetics, there was a strong positive correlation of fasting insulin, HOMA-IR with TNF-α ($p < 0.001$). There was also significant positive correlation between TNF-α and BMI ($p < 0.001$).

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

Variable	Mean±SD	Min	Max
Age (Year)	50.63±9.89	32	75
Height (m²)	1.61±0.11	1.43	1.85
Weight (Kg)	71.01±12.32	45	100
Body mass index (Kg/m²)	27.45±5.19	16.14	41.11
Duration of disease (Year)	3.18±1.27	1	5
Fasting Blood Glucose (mg/dl)	164.68±31.11	60	240
Serum Insulin (F) µIU/ml	26.96±24.55	1.78	111.2
HOMA-1R	11.02±10.03	0.7	44.7
TNF-α (pg/ml)	7.64±5.38	0	21.82

Table-2: Correlation of HOMA-IR and BMI with TNF-α in T2DM

Bivariate correlation	Tumor Necrosis Factor Alpha	
	r	p
Fasting Insulin	0.55[*]	<0.001[*]
HOMA-1R	0.54[*]	<0.001[*]
Body Mass Index	0.68	<0.001[*]

*significant

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[10] and decreased after weight loss[11].

In our study, Serum insulin and HOMA-IR were strongly positively associated with TNF-α as same was stated in other international studies.[12] It is well recognized that adipose tissue is source of nearly all TNF-α.[5,13] TNF-α levels also showed positive correlation with BMI. There is not direct relationship between inflammatory marker and generalized obesity. The role of abdominal obesity along with generalized obesity has direct link with increased insulin resistance and indirectly associated with higher cytokines levels.[14,15]

Similar to our study, same results were obtained by Katsuki *et al*[16]; they did not find a connection between HOMA-IR and TNF-α in obese T2DM. Mayazaki *et al*[17] in their study determined the possible role of increased TNF-α levels in type 2 diabetics and their relation to the severity of insulin resistance of whole body. Their results proved significantly higher circulating TNF-α concentrations in T2DM, but it was not correlated with the degree of whole body insulin-mediated glucose disposal (Rd). There is possibility that with type 2 diabetes progression other pathogenic factors responsible for obesity deterioration[18], play an important role in insulin resistance and make the correlation between serum TNF-α concentration and insulin resistance doubtful.

This fact is also established from *in vitro* studies that when human cells are exposed to TNF-α, adipocytes become insulin resistant.[19] Study conducted by Mishima *et al*[20] found that TNF-α in obese type 2 diabetics depend on the degree of their insulin resistance but not on BMI. Finally, besides several indirect lines of evidence our data suggests that TNF-α may play a role in the pathophysiology of type 2 diabetes mellitus.

As Pakistani population has higher incidence of obesity, our population is more susceptible to its deadly effects. The outcome of insulin therapy on levels of inflammatory markers amongst obese and non-obese diabetics has not been studied sufficiently. There is necessity to find out the effect of insulin therapy to neutralize and/or reduce the levels of TNF-α.

The small sample size and the limited observation period do not allow a definitive conclusion from 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 work on type 1 diabetes mellitus to find out the effect of inflammatory markers in early age.

CONCLUSION

TNF-α plays an important role in insulin resistance linked with obesity and T2DM in humans. Early detection of TNF-α especially in obese individuals having T2DM may help the physicians for better management, which in turn will improve quality of life.

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Address for Correspondence:
Dr Rana Khurram Aftab, Assistant Professor, Department of Physiology, King Edward Medical University, Lahore, Pakistan. **Cell:** +92-321-2491550
Email: drranakhurram81@outlook.com