Plasma TNF-α in Obese Diabetic and Obese Non-diabetic Patients and its Relation to Glycaemic Control

Azza O Alawad,¹ Tarig H Merghani,² Mansour A Ballal³

¹Department of Physiology, Faculty of Medicine, University of Al-Neelain, Khartoum- Sudan/ Email: azzaosman2@hotmail.com
²Department of Physiology, Faculty of Medicine, University of Tabuk, Tabuk, Saudi Arabia/ Email: t.hakim@ut.edu.sa
³Department of Physiology, Faculty of Medicine, University of Science and Technology, Khartoum- Sudan/ Mansour.ballal@yahoo.com

Correspondence to Azza O Alawad, Department of Physiology, Faculty of Medicine, University of Al-Neelain. Khartoum- Sudan/ Email: Azzaosman2@hotmail.com

ABSTRACT

Introduction: TNF-α impairs insulin signalling and induces insulin resistance. Previous studies described a correlation between plasma TNF-α and degree of obesity; however, few studies investigated its relation to glycaemic control. The aim of this study was to compare plasma TNF-α in obese type II diabetic patients and obese non-diabetic patients and to determine its relation to haemoglobin A1c and fasting blood sugar as laboratory indicators of glycaemic control.

Materials and methods: A random sample of 40 obese diabetic patients (cases) and 40 obese non-diabetic subjects (controls) were selected from patients attending for follow up of their diabetes in Gabir Abu-Eliz centre and from the co-patients respectively. Each participant was interviewed and examined clinically to exclude presence of acute or chronic medical illness.
Weight and height of each participant were measured using standardized weight and height scales. Haemoglobin A1c was measured using the “NycoCard Haemoglobin A1c test” (Axis - Shield/ Norway). TNF-α was measured using commercially available ELISA kits from ADIPO Bioscience/ USA. Fasting blood sugar was measured using one touch® glucometer (LifeScan Canada Ltd).

**Results:** Plasma TNF-α was significantly higher among cases than controls (P= 0.003). About two thirds (67%) of participants with low plasma TNF-α had normal values of haemoglobin A1c while the majority (58%) of those with high values had high values of haemoglobin A1c (P= 0.037). The relation between plasma TNF-α and fasting blood sugar in all participants was statistically insignificant (P= 0.515).

**Conclusion:** Plasma TNF-α was significantly higher among obese diabetics than obese non-diabetics. A significant relation was found between high plasma TNF-α and poorly controlled diabetes mellitus.

**INTRODUCTION**

Tumor necrosis factor alpha (TNF-α) is a potent inflammatory cytokine. It is produced chiefly by activated macrophages; however, it can be produced by other types of cells like lymphoid cells, mast cells, endothelial cells, adipose tissue, fibroblasts and neuronal tissue.\(^1\) It exerts many effects on various organs in the body. It acts in the hypothalamus to induce release of corticotrophin releasing hormone (CRH), inhibits appetite by acting on satiety centre and it is involved in the mechanism of fever. It also acts in the liver to stimulate the acute phase response, resulting in elevation of C-reactive protein (CRP) level in plasma and a number of other
mediators. It acts as a chemotactic substance for neutrophils and promotes their migration by increasing expression of adhesion molecules on endothelial cells and increasing vascular permeability. In addition, TNF-α can induce apoptosis, sepsis (through IL1 & IL6 production), cachexia, and inflammation; and it is able to inhibit tumorigenesis (causes necrosis of certain tumors) and activate the immune response to limit bacterial, fungal, parasitic and viral replication.\(^2\)-\(^7\) In addition to all these effects, it promotes serine-phosphorylation of insulin receptor substrate-1 (IRS-1); this impairs insulin signalling and induces insulin resistance.\(^8\)

Obesity, which is one of the most prevalent and serious health problems worldwide, is associated with insulin resistance and type 2 diabetes mellitus. Insulin resistance resulting from obesity is also linked to a wide array of other pathophysiological abnormalities including hypertension, hyperlipidemia, atherosclerosis, and polycystic ovarian disease.\(^9\) TNF-α may play an important role in the pathogenesis of obesity and insulin resistance.\(^10\) Studies in both obese animal models and human subjects suggest that elevated production of TNF-α by adipose tissues is associated with decreased insulin sensitivity\(^11,12\) The altered TNF-α expression in adipose tissues is also positively correlated with the degree of obesity and the level of hyperinsulinemia.\(^11,13\) Furthermore, TNF-α-deficient mice were protected from obesity-induced insulin resistance and hyperlipidemia.\(^14\)

Haemoglobin A1c is a subtype of haemoglobin A. Here glucose molecules react with the terminal amino acid in the beta chains of haemoglobin A forming glycated haemoglobin (or haemoglobin A1c). In individuals with poorly controlled diabetes, its quantity becomes higher
than normal. Once a haemoglobin molecule is glycated, it remains until it is hydrolyzed in the spleen with the breakdown of the red blood cell after completion of its life-span. Quantity of haemoglobin A1c within a red blood cell reflects the average level of glucose to which the cell has been exposed during its life-cycle. Measuring glycated haemoglobin assesses the level of control of diabetes mellitus over the previous four weeks to three months. Normal Hb A1c is about 5% of total haemoglobin in adults. It was recommended that a threshold of ≥6.5% HbA1c could be used to diagnose diabetes.\textsuperscript{15,16}

Persistent elevations in blood sugar (and, therefore, haemoglobin A1c) increase the risk for the long-term vascular complications of diabetes. Although TNF-α has been linked to insulin resistance in obese individuals, there is paucity of data regarding its relation to haemoglobin A1c and to overall diabetes control. This study was conducted to evaluate plasma level of TNF-α among obese diabetic and obese non-diabetic patients and to assess its relation to level of hemoglobin A1c and fasting blood sugar among these patients.

**MATERIALS AND METHODS**

This is a case control study conducted in Jabir Abu-Eliz centre for diabetes control in the period from January to August 2012. A random sample of 40 obese diabetic patients (cases) and 40 obese non-diabetic subjects (controls) were selected from patients attending for follow up and from the co-patients respectively. Inclusion criteria for both cases and controls were adult, body mass index ≥ 30, non-smoker, normotensive, afebrile and with no symptoms or signs of acute or chronic infection. Cases were known diabetics for at least one year whereas controls were non-
diabetics. Both groups were matched according to age, sex, height, weight and area of residence. An anonymous interviewer-based questionnaire, requesting information about present health status and diabetes control was applied to each participant. Clinical examination was carried out to exclude signs of acute or chronic medical abnormalities. Height and weight of each participant was measured using standardized height and weight scales. Body mass index (BMI) was calculated as weight (in kilograms)/height (in meters$^2$). Chemically clean and sterile disposable needles, syringes, swabs and EDTA containers were used for blood collection. Five micro litre of blood was taken for Haemoglobin A1c measurement using the “NycoCard Haemoglobin A1c test” (Axis -Shield/ Norway); with a measuring range of 4-15% HbA1c. About 3 ml of blood was centrifuged for plasma separation. Plasma samples were frozen and stored at -20 °C for biochemical analysis. TNF-α was measured using commercially available quantitative sandwich enzyme-linked immuno-sorbent assay (ELISA) kits according to instructions of the manufacturers. The analyses were performed with 96-well microtiter plate human ELISA kits for TNF-α (from ADIPO Bioscience/ USA). Test sensitivity was 3.9 pg/ml. Fasting blood sugar was measured using one touch® glucometer (LifeScan Canada Ltd). The research conforms to the ethical principles of medical research developed by the World Medical Association Declaration of Helsinki. Ethical clearance was given by the Research Committee (Faculty of Medicine/ University of Al-Neelain). Approval was obtained from the Ministry of Health. Written consents were obtained from each participant before entry into the study. All data obtained with questionnaire and biochemical analysis were analyzed using the Statistical Package for the Social Sciences (SPSS) version 19. The chi square test was used to test distribution of categorical
variables. The differences between test and control groups were assessed with the student's t test. Statistical significance was accepted when P value is ≤ 0.05.

RESULTS

Table 1 describes characteristics of cases (n=40) and controls (n=40) in the study group. Male: Female ratio was 1:1.5 in both cases and controls. Statistical analysis showed insignificant difference between the two groups in age (P= 0.564), height (P= 0.094), weight (P= 0.486) and body mass index (P= 0.328). Table 2 shows comparison between cases and controls in plasma TNF-α. High values (≥ 5pg/ml) were found in the majority (55%) of obese diabetic patients whereas low values (< 5pg/ml) were found in the majority (77%) of obese non-diabetic subjects (P= 0.003). About two thirds (67%) of participants with low plasma TNF-α had normal values of haemoglobin A1c while the majority (58%) of those with high values had high values of haemoglobin A1c (table 3). This finding was statistically significant (P= 0.037). Table 4 shows that the relation between plasma TNF-α and fasting blood sugar in all participants is statistically insignificant (P= 0.515).
Table 1: Characteristics of cases and controls in the study group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Study group</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Cases</td>
<td>35</td>
<td>50</td>
<td>45.03</td>
<td>5.09</td>
<td>0.564</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>35</td>
<td>50</td>
<td>44.33</td>
<td>5.69</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>Cases</td>
<td>1.49</td>
<td>1.81</td>
<td>1.63</td>
<td>0.08</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>1.55</td>
<td>1.83</td>
<td>1.66</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Cases</td>
<td>69</td>
<td>105</td>
<td>85.00</td>
<td>7.70</td>
<td>0.486</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>78</td>
<td>105</td>
<td>86.52</td>
<td>11.35</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Cases</td>
<td>30.02</td>
<td>40.37</td>
<td>32.04</td>
<td>2.08</td>
<td>0.328</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>30.04</td>
<td>39.85</td>
<td>32.29</td>
<td>2.06</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Plasma TNF-α among obese diabetic and obese non-diabetic subjects in the study group

<table>
<thead>
<tr>
<th></th>
<th>TNF-α</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 5 pg/ml</td>
<td>≥ 5 pg/ml</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Obese Diabetic</td>
<td>18 (45%)</td>
<td>22 (55%)</td>
<td>40 (100%)</td>
</tr>
<tr>
<td>Obese Non-diabetic</td>
<td>31 (77%)</td>
<td>9 (33%)</td>
<td>40 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>49 (61%)</td>
<td>31 (39%)</td>
<td>80 (100%)</td>
</tr>
</tbody>
</table>

P= 0.003
Table 3: Plasma TNF-α in relation to haemoglobin A1c among all participants in the study group

<table>
<thead>
<tr>
<th>Haemoglobin A1c</th>
<th>TNF-α &lt; 5pg/ml n (%)</th>
<th>TNF-α ≥ 5pg/ml n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6.5%</td>
<td>33 (67%)</td>
<td>13 (42%)</td>
<td>46 (57%)</td>
</tr>
<tr>
<td>≥ 6.5%</td>
<td>16 (33%)</td>
<td>18 (58%)</td>
<td>34 (43%)</td>
</tr>
<tr>
<td>Total</td>
<td>49 (100%)</td>
<td>31 (100%)</td>
<td>80 (100%)</td>
</tr>
</tbody>
</table>

P=0.037

Table 4: Plasma TNF-α in relation to fasting blood glucose among all participants in the study group

<table>
<thead>
<tr>
<th>Fasting blood glucose</th>
<th>TNF-α &lt; 5pg/ml n (%)</th>
<th>TNF-α ≥ 5pg/ml n (%)</th>
<th>Total n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 126 mg/dl</td>
<td>32 (65%)</td>
<td>17 (55%)</td>
<td>49 (61%)</td>
</tr>
<tr>
<td>≥ 126 mg/dl</td>
<td>18 (35%)</td>
<td>13 (45%)</td>
<td>31 (39%)</td>
</tr>
<tr>
<td>Total</td>
<td>49 (100%)</td>
<td>31 (100%)</td>
<td>80 (100%)</td>
</tr>
</tbody>
</table>

P=0.515
DISCUSSION

It is well known that both obesity and TNF-α are associated with insulin resistance and development of type 2 diabetes mellitus. TNF-α promotes insulin resistance by disrupting the insulin receptor-mediated signalling.\(^8\) Other proposed mechanisms include down-regulation of genes that are required for normal insulin action, direct effects on insulin signaling and induction of elevated free fatty acids via stimulation of lipolysis.\(^18\) whereas the exact mechanism of obesity mediated insulin resistance is still debatable.\(^19\) Obesity may be associated with higher levels of TNF-α;\(^20\) however, the concept that TNF-α, acting via its receptors, is a major contributor to obesity-associated insulin resistance was not supported by many studies.\(^21\) On the contrary, weight loss in obese patients was found to be associated with reduced TNF-α production and ameliorated insulin resistance.\(^22\) On the other hand, significantly increased peripheral insulin sensitivity was observed in obese animals by neutralizing TNF-α.\(^11\) In this study, plasma TNF-α was significantly higher among obese diabetics than obese non-diabetics. This might indicate an existence of a mechanism by which plasma TNF-α rises in diabetic patients and probably causes insulin resistance and type 2 diabetes mellitus. The rise in plasma TNF-α cannot be explained by obesity alone since both diabetics and non-diabetics were obese. On the other hand, the elevation in plasma TNF-α might be caused by hyperglycaemia. Hyperglycaemia is a known cause of inflammation\(^23\) followed by increased production of acute phase proteins and pro-inflammatory cytokines, including TNF-α.\(^24,25\) Monocytes taken from diabetic patients produce higher levels of TNF-α in comparison with control monocytes taken from healthy subjects.\(^26\) In a previous study, high TNF-α was found in hyperglycaemic patients with gestational diabetes; however, the researchers thought that TNF-α might be involved in the pathogenesis of hyperglycaemia.
through induction of insulin resistance.\textsuperscript{27} In consistence with that, hypoglycaemia was reported as one of the complications of treatment with TNF-α inhibitors.\textsuperscript{28} In this study, although TNF-α was significantly higher among obese patients with poorly controlled diabetes mellitus, as indicated with high haemoglobin A1c, its relation to fasting blood sugar was statistically insignificant. Many mechanisms were proposed to explain how hyperglycaemia may induce monocyte production of TNF-α;\textsuperscript{29} however, induction of hyperglycaemia following insulin resistance that is caused by TNF-α is well documented.\textsuperscript{8,10-12}

Haemoglobin A1c gives clue about overall control of diabetes mellitus during the previous few weeks before measurement. Values $\geq$ 6.5\% indicate poorly controlled diabetes mellitus. In this study, the majority of patients with plasma TNF-α $\geq$ 5 pg/ml had poorly controlled diabetes mellitus that was indicated with high values of haemoglobin A1c ($\geq$ 6.5\%). This could be explained by the fact that TNF-α increases insulin resistance, resulting in worsening of diabetes and further elevation in haemoglobin A1c.\textsuperscript{8} In a previous study, observation of plasma TNF-α in type 2 diabetic patients for two years revealed continuous increase in its values that is associated with rise in haemoglobin A1c,\textsuperscript{30} indicating a positive relation between plasma TNF-α and poor control of diabetes mellitus.

In conclusion, this study showed that plasma TNF-α was significantly higher among obese diabetic patients compared to obese non-diabetics. A significant relation was found between high TNF-α in plasma and poorly controlled diabetes mellitus indicated with high haemoglobin A1c. Further research is needed to investigate whether hyperglycaemia is a cause or a result of high plasma TNF-α in diabetic patients.
REFERENCES


7- Rink L, Kirchner H. Recent Progress in the Tumour Necrosis Factor-Alph Field. International Archives of Allergy and Immunology 1996; 111 (3): 199-209.


