Correlation of Tumour Necrosis Factor-α and Interleukin-6 with Anthropometric Indices of Obesity and Parameters of Insulin Resistance in Healthy North Indian Population

Niti Agarwal*, Anubhuti Chitrika**, J Bhattacharjee**, SK Jain*

Introduction

White adipose tissue (WAT) is now seen as a highly dynamic organ, being involved in a wide range of physiological and metabolic processes far beyond the paradigm of fuel storage. This changed perspective has occurred through the recognition that WAT is an endocrine organ, which secretes several major hormones including leptin and adiponectin together with a diverse range of other protein signals and factors collectively termed as adipokines. The group includes cytokines like tumour necrosis factor-alpha (TNF-α), Interleukins like IL-6, IL-8, IL-10, acute phase proteins, e.g., PAI-1, haptoglobin, CRP, serum amyloid A and inflammation related proteins like adiponectin, nerve growth factor (NGF) and monocyte chemotactrant protein-1 (MCP-1)1.

Previous reports suggest a positive association between components of the insulin resistance syndrome and the acute phase reactants including C-reactive protein, von Willibrand’s factor, fibrinogen, etc2,3. CRP is the most extensively studied inflammatory marker in prospective setting. The production of CRP by the liver is under the control of cytokines, which are elevated in acute infection states. Cytokines mainly IL-1, IL-6 and TNF-α exhibit major stimulatory effects on hepatic synthesis of acute phase proteins. IL-6 and TNF-α are soluble polypeptides acting as important hormonal regulators in immunoregulation, haematopoiesis and the inflammatory cascade4,5,6.

Since pathological conditions associated with insulin resistance syndrome were associated with high TNF-α, it was suggested that it might play an important role in the development of insulin resistance. It has since been demonstrated by many researchers that serum levels of TNF-α are elevated in obesity7-9 and that the levels fell after weight loss9. In contrast to this finding, there are reports that have not been able to demonstrate any relation between circulating TNF-α and obesity, insulin resistance, or impaired glucose tolerance10-13. Since the cytokines are difficult to measure, people have tried to establish association between insulin resistance/obesity and TNF-receptors and TNF-α mRNA. These studies have produced equally conflicting results with some showing increased expression14 while others were not able to provide any such evidence15.

Role of IL-6 in cardiovascular diseases is also well studied. There is a positive correlation between IL-6 and risk of future myocardial infarction16, unstable angina17 and death18. A report from the Rural Health Study has shown that elevated concentration of IL-6 predicts total cardiovascular mortality over a 5-year follow-up, the association being independent of vascular disease, smoking, and traditional risk factors18.

It has been demonstrated in various studies that circulating IL-6 levels are elevated in insulin resistant states such as obesity19, impaired glucose tolerance and type 2 diabetes20,21. Weight reduction by diet and exercise reduce plasma IL-6 levels22. Elevated IL 6 levels have been demonstrated to be associated with dyslipidaemia by some researchers24. In contrast to some of the existing evidence, few studies have found that IL-6 did not alter the effects of insulin on glucose homoeostasis25 and that no significant difference is seen in plasma TNF-α or IL-6 levels between obese and non-obese subjects25.

In the present study, we have studied the correlation of TNF-α and IL-6, with anthropometric indices of obesity, insulin resistance, and dyslipidaemia in healthy North Indian population.
Material and methods

The study was conducted at a tertiary care government hospital at Delhi, India. 101 healthy individuals (50 males and 51 females) (age group 19 - 60 years) were enrolled after obtaining their written consent. All volunteers went through a detailed medical history and examination to ensure that they did not suffer from any medical illness – acute or chronic – at the time of study. Pregnant females, current smokers, chronic alcoholics, subjects with past or present malignancy, gout, endocrine disease, rheumatoid arthritis, osteoarthritis, personal or family history (in first degree relatives) of diabetes mellitus or hypertension, history of chronic hepatitis, chronic renal failure, coronary artery disease, polycystic ovarian disease, any history of febrile illness or any major illness during previous 30 days, history of using oral contraceptives or other drugs affecting the metabolic profile (beta-blockers/antihypertensives/adrenergic drugs/steroids/other hormones), or any chronic inflammatory diseases and any evidence of infection were excluded from the study.

Anthropometric assessment included measurement of height, weight, waist circumference, hip circumference and skin fold thickness (subscapular, suprailiac, biceps, and triceps). All indices readings were taken in triplicate and the mean of three readings was used for analysis. Height was measured to the nearest 1 mm. Weight was measured to the nearest 1 kg using Beurer weighing machine (MS 01). The body mass index (BMI) was calculated as kg/height^2 (in metres). Waist circumference was measured midway between the iliac crest and lower-most margins of the ribs, and the hip girth was measured at the maximum circumference of buttocks with the subject wearing minimum clothes with feet placed together. Waist hip ratio was calculated using the formula: circumference at waist/circumference at hip.

Triceps, biceps, suprailiac, and subscapular skinfold thickness were measured using skinfold calipers. All skinfolds were measured to the nearest 0.1 mm. All readings were taken on the right-side of the body with the subject standing in a relaxed condition. A mean of the three readings was recorded. Percentage body fat (%BF) was calculated using the standard equation of Durnen and Womersely.

All subjects underwent liver function tests, kidney function tests including uric acid, haemogram, detailed serum lipid profile (fasting) and 75 gm OGGT which included measurement of fasting glucose/2-hour post-prandial glucose and fasting insulin/2-hour post-prandial insulin. Glucose and lipids were measured by autoanalyser. Principle of glucose measurement was the glucose oxidase method. Total cholesterol, HDL-C and triglycerides were measured directly and LDL-C and VLDL-C calculated indirectly using Friedewald formulae. Cases found to be diabetic were excluded from the subsequent study. All subjects underwent measurement of IL-6 and TNF-α.

Two ml of venous blood sample was collected in a plain vial under sterile conditions. The samples in plain vials were allowed to clot at room temperature. The clotted blood sample was then centrifuged for 5 minutes. The serum was then stored at -20° C till serum insulin was batch analysed.

The serum insulin was estimated by Sandwich ELISA method. Read on the Vmax ELISA Reader and the absorbance of each well recorded at 450 nm (versus 650 nm) and 490 nm (versus 650 nm) using polychromatic model. The values of more than 25 IU/L was taken as hyperinsulinaemia.

TNF-α was measured using diacline (Sandwich ELISA) commercial kit for research use only. The absorbance was read on a spectrophotometer using 450 nm as the primary wavelength with 620 nm reference wavelength. IL-6 was measured using DIACLONE (Sandwich ELISA) kit for research use only. The absorbance was measured on a spectrophotometer using 450 nm as primary wavelength with 620 nm as reference wavelength.

21 subjects selected randomly from the study population underwent insulin suppression test by Modified Horano Method to study the metabolic clearance rate (MCR) of glucose and insulin clearance rate (ICR).

Statistical analysis was performed using Microsoft excel and SPSS softwares.

Results

BMI did not correlate significantly with either of the inflammatory markers studied in the total study group or...
in either sexes individually. The inflammatory markers did not show any trend with BMI quartiles and the median values of TNF and IL-6 in the total study population. BMI quartiles in females showed a rising trend with TNF but no such trend was observed with IL-6. No such trend was seen in the males for TNF or IL-6 (Fig. 1).

The study population was divided into two groups of obese and non-obese based on the modified WHO criteria of the Asia Pacific region\textsuperscript{26}, obesity being defined as BMI $\geq 25$ kg/m$^2$, and the median levels of TNF and IL-6 compared. The TNF level in the obese was higher than those in non-obese (11.25 pg/ml vs. 9.375 pg/ml) (Fig. 2). However, the levels of IL-6 were higher in the non-obese group.

No significant correlation of WHR with any of the inflammatory markers was found in the females, males, or the total study population. The WHR quartiles however showed a rising trend of TNF and a declining trend with IL-6 in females. No such trend was observed with mean levels of cytokines in males (Fig. 3).

WC was not found to be significantly correlated with either TNF or IL-6 in either groups. Median values of TNF were studied with obesity using waist circumference as criteria (males $> 94$ cm and females $> 80$ cm) and a rising trend was observed with obesity defined by this criterion (Fig. 4 and 5). However, median values of IL-6 showed a declining trend with obesity defined by waist circumference. Median values of IL-6 in non-obese males was 37.5 while in obese males was 11.5 pg/ml. In females, IL-6 in non-obese was 57.75 and in obese was 20.75 pg/ml.

None of the parameters were significantly correlated with HC. TNF and IL-6 were not found to be significantly associated with PBF in either of the groups. TLC
TNF-α correlated significantly with fasting insulin, fasting GIR (negatively) and HOMA-IR in the total study population with p-value of < .05 in each. None of the other parameters correlated significantly with TNF-α. In males, it correlated significantly negatively with diastolic blood pressure only. In females, only fasting blood sugar was found to be significantly correlated with TNF-α with a p-value of < .05. TNF levels increase with increasing insulin quartiles and showed a rising trend with increasing fasting blood sugar (Fig. 6).

Arbitrarily taking HOMA-IR of 4.0 as cut-off, the median

<table>
<thead>
<tr>
<th>p-value</th>
<th>TNF</th>
<th>IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>.761</td>
<td>.056</td>
</tr>
<tr>
<td>WHR</td>
<td>.389</td>
<td>.066</td>
</tr>
<tr>
<td>PBF</td>
<td>.508</td>
<td>.691</td>
</tr>
<tr>
<td>WC</td>
<td>.884</td>
<td>.122</td>
</tr>
<tr>
<td>HC</td>
<td>.363</td>
<td>.296</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>.031</td>
<td>.113</td>
</tr>
<tr>
<td>Post-prandial insulin</td>
<td>.078</td>
<td>.822</td>
</tr>
<tr>
<td>FBS</td>
<td>.975</td>
<td>.598</td>
</tr>
<tr>
<td>PPBS</td>
<td>.648</td>
<td>.963</td>
</tr>
<tr>
<td>Fasting GIR</td>
<td>.025</td>
<td>.118</td>
</tr>
<tr>
<td>Post-prandial GIR</td>
<td>.058</td>
<td>.400</td>
</tr>
<tr>
<td>MCR</td>
<td>.933</td>
<td>.067</td>
</tr>
<tr>
<td>ICR</td>
<td>.941</td>
<td>.140</td>
</tr>
<tr>
<td>Homa-IR</td>
<td>.033</td>
<td>.176</td>
</tr>
<tr>
<td>SBP</td>
<td>.998</td>
<td>.329</td>
</tr>
<tr>
<td>DBP</td>
<td>.719</td>
<td>.019</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>.627</td>
<td>.268</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>.078</td>
<td>.779</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>.771</td>
<td>.184</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>.884</td>
<td>.700</td>
</tr>
</tbody>
</table>

Significantly correlated with PBF only in the males.
values of TNF and IL-6 showed that TNF-α levels are higher in subset with greater insulin resistance in the total population and females. IL-6 levels were also found to be higher in subset with higher insulin resistance. TNF-α levels are higher in patients with impaired glucose tolerance (Fig. 7, 8, 9). Similarly median TNF levels showed rising trend with IGT in all the subgroups considered (Fig. 10, 11, 12). TNF-α did not correlate significantly with any of the lipid
parameters in either of the groups. TNF levels show a rising trend when dyslipidaemia is defined as triglycerides as cut-off. However, no such expected trend is observed when HDL-C cut-off values are used (Fig. 13).

IL-6 correlated significantly with PP GIR in the total study population and males only. None of the other parameters correlated significantly with IL-6 in any of the study groups. One important thing which is noted is that although not significantly correlated, the correlations with fasting and postprandial insulin and sugar and HOMA-IR are negative.

IL-6 correlated significantly with triglycerides and HDL/ LDL ratio in females. In males and the total study population none of the lipid parameters were found to be significantly correlated with IL-6. With dyslipidaemia, IL-6 surprisingly showed a declining trend.

**Discussion**

**Obesity**

In our study conducted in healthy north Indian population, we found no significant correlation between serum levels of TNF-α or IL-6 with any of the anthropometric measures of obesity. However, when we studied the mean TNF-α levels with increasing quartiles of BMI and WHR, we found a rising trend. No trend could be demonstrated for IL-6. Similar to our findings, Carvalho *et al* found no differences related to the peripheral expression of the cytokines were found among the eutrophic, euthrophic with high percentage body fat and overweight groups.

Our findings are consistent with the findings of Pincelli *et al* and Kern *et al*, who also found no correlation between serum TNF-α values and obesity. Our findings are in accordance with the study conducted by Tseigos *et al* who also found no significant difference in plasma IL-6 levels between obese and non-obese subjects. Choi *et al* found a negative correlation (though insignificant) between IL-6 and obesity.

In contrast to our findings, there are studies, which have demonstrated a significant positive correlation between IL-6 and obesity. In the Whitehall II study, which followed-up subjects for 11 years, the mean increases in CRP and IL-6 were 0.08 (95% CI, 0.07 - 0.09) mg/litre and 0.04 (95% CI, 0.03 - 0.05) pg/ml per 1 kg increase in body
weight during follow-up. In Asian Indians, Yudkin et al found a ten-fold higher levels of IL-6 in slum dwellers despite their lower waist hip ratio, when compared with villagers. However, they also found a three times higher level of IL-6 in middle class subjects (who had a higher waist hip ratio), as compared with villagers. Elevated plasma leptin, hsCRP, IL-6, and FFA concentrations are associated with obesity and not necessarily with the type 2 diabetic state.

But studies by Zahorska Markiewicz et al, Laimer et al, Yudkin et al and Berberoglu, observed elevated serum TNF-α in obesity. Dandona et al found that obesity is associated with increased plasma TNF-α concentrations in women and the levels fall with weight loss. Esmaillzadeh et al found those with the high TG and waist circumference phenotype had higher circulating levels of CRP, TNF-α, IL-6 and E-selectin compared with those with normal WC and normal serum TG levels and these correlations persisted despite adjustment for body mass index attenuated the associations, but all were still statistically significant.

Though ELISA method for measurement was considered superior, some other authors have also reported inconsistent results with IL-6 and CRP being significantly correlated with BMI (r = 0.42 and r = 0.55), but MCP-1 and TNF-α were not (r = -0.07 and r = 0.06).

Insulin resistance

Our study did not demonstrate any significant correlation between circulating TNF-α or IL-6 and the markers of insulin resistance studied (FBG, PPBG, GIR, HOMA-IR). Median TNF levels showed a rising trend when studied in increasing quartiles of fasting serum insulin. Bluhler et al and Muller et al found that plasma levels of TNF-α are not elevated in insulin resistant obese individuals with impaired glucose tolerance. Hube et al also did not find significant difference in the levels of TNF-α between obese individuals with and without impaired glucose tolerance/NIDDM. In study conducted by Choi et al in elderly, non-smoking and non-diabetic Korean women, serum proinflammatory cytokine TNF-α and IL-6 concentrations were neither increased in subjects with IGT nor closely correlated with the components of the metabolic syndrome.

Our findings are in contrast with studies which have demonstrated a significant positive correlation between IL-6 and insulin resistant states.

In our study when the median values of subjects with post-OGTT blood sugar > 140 and those with normal GTT were compared, it was observed that subjects with IGT had a higher median TNF. The findings are in accordance with those of Tsigos et al who showed that, although no significant difference in plasma TNF-α levels exists between obese and non-obese subjects overall. TNF-α levels were significantly elevated in obese subjects with 2 h glucose level of more than 140 mg/dl compared with other obese and non-obese controls.

Lipids

We did not find any significant correlation between lipid parameters and TNF or IL-6, apart from significant correlation between IL-6 and triglycerides. Using the cut-off values proposed by NCEP to define dyslipidaemia in metabolic syndrome, we could not demonstrate any positive trend. In fact, surprisingly we observed a negative trend between dyslipidaemia and IL-6 similar to the findings of Choi et al. In their study among adolescents, Carvalho et al found significant correlation between IL6 and triglycerides.

TNF-α has limited half-life and is difficult to measure in large scale epidemiological studies. Since the action of TNF-α and IL-6 may be autocrine or paracrine, it is possible that circulating levels of measured TNF-α may not reflect the true biological activity of the cytokines. Also, the bioavailability and/or action of circulating TNF in obese might depend upon the circulating TNF receptors.

Although, IL-6 has been described as a pro-inflammatory cytokine, there is evidence that suggests a role of IL-6 in counteracting the manifestations of the inflammatory response. IL-6 has been shown to increase insulin resistance and enhance lipogenesis, while at the same time there are studies which demonstrate its role in promoting lipolysis.

Also, the dispersion of the TNF and IL-6 levels in our study was very wide. The TNF levels varied from undetectable levels to 111.25 pg/ml (excluding one value of 225 pg/ml) and IL-6 ranging from 1.75 pg/ml to 148 pg/ml.
levels were not significantly different in the two sexes. The levels of TNF and IL-6 in various studies have not been consistent. They have been found to be high (55.3 ± 14.28 pg/ml in controls and 42.2 ± 12.81 pg/ml in females) to low (2.10 ± 19 pg/ml and 1.65 ± 18 pg/ml in obese and controls) (.25 pg/ml in men and .02 pg/ml in women) and even undetectable levels. From our study, we could not establish any significant role of TNF-α or IL-6 in relation to obesity or insulin resistance.

From the observations and results of our study, we conclude that there is no significant role of IL-6 in obesity or insulin resistance. And these parameters have no clinical utility in north Indian population.

References


11. Pincelli AI, Brunani A, Scacchi M et al. The serum concentration of tumour necrosis factor alpha is not an index of growth hormone-or obesity-induced insulin resistance. Horm Res 2001; 55: 57-64.


13. Muller S, Martin S, Koenig W et al. Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors. Diabetologia 2002; 45: 805-12.


29. Fransson EL, Batty GD, Tabák AG et al. Association between change


35. Bluher M, Kratzsch J, Paschke R, Plasma levels of tumour necrosis factor-α, angiotensin II, growth hormone, and IGF-I are not elevated in insulin-resistant obese individuals with impaired glucose tolerance. *Diab Care* 2001; 24: 328-34.


37. Pradhan AD, Manson JE, Rifai N *et al.* C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001; 286: 327-34.


