Evaluation of TNF-α and IL-6 Levels in Obese and Non-obese Diabetics: Pre- and Postinsulin Effects

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Abstract

Background: Type 2 diabetes (T2DM) mellitus is a serious implication of obesity. The effect of insulin therapy on levels of inflammatory markers among obese and non-obese diabetics has been inadequately studied. **Aim:** The study aimed to analyze the preinsulin and postinsulin levels of tumor necrosis factor α (TNF-α) and IL-6 in nonobese and obese T2DM patients. **Materials and Methods:** We assessed TNF-α and IL-6 levels in healthy controls (n=10) and diabetic patients (obese and nonobese; n=20 each) and analyzed the postinsulin effect on TNF-α and IL-6 levels after 24 and 48 weeks. TNF-α and IL-6 levels were also correlated with fasting plasma glucose of obese and nonobese diabetic patients after insulin therapy. **Results:** There is augmentation of TNF-α and IL-6 levels in diabetic patients and augmentation is more in obese than in nonobese diabetics. The obese group showed a significant decrease (P value<0.05) after 24 weeks of insulin therapy and an extremely significant decrease (P<0.001) in TNF-α and IL-6 levels after 48 weeks of therapy. The nonobese group showed an extremely significant decrease (P<0.001) in TNF-α and IL-6levels after 24 and 48 weeks both. **Conclusion:** There is augmented inflammation in diabetes and it is more in obese diabetics. Insulin therapy tends to counter this inflammation, but the response is delayed in obese diabetics.

Keywords: Diabetes, Inflammation, Obesity

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Introduction

The trends in the prevalence of obesity documented over the last few decades in our country have been alarming, with morbid obesity affecting 5% of our country's population. Hippocrates was among the pioneer scientists who recognized obesity as a medical disorder rather than just being a cosmetic problem leading to many other comorbid conditions such as dyslipidemia, hyperinsulinemia, diabetes, hypertension, cardiovascular diseases and inscribed that "Corpulence is not only a disease itself, but the harbinger of others."

Type 2 diabetes mellitus (T2DM) is a heterogeneous group of metabolic disorders characterized by chronic

hyperglycemia and is a serious implication of obesity.[1] As individuals become obese and their adipocytes enlarge, adipose tissue undergoes molecular and cellular alterations affecting systemic metabolism. First, macrophage numbers in adipose tissue increase with obesity,[2] where they apparently function to scavenge older adipocytes. Second, several proinflammatory factors are produced in adipose tissue macrophages with increasing obesity. In fact, adipose tissue macrophages are responsible for almost all adipose tissue tumor necrosis factor α (TNF-α) expression and significant amounts of interleukin-6 (IL-6).[3] Concentrations of acute-phase response markers and mediators of inflammation such as cytokines like TNF-α and interleukin-6 are also raised in people with T2DM. This finding has led to the suggestion that raised concentrations of pro-inflammatory cytokines and the resultant acute-phase response may underlie much of the metabolic clustering due to obesity and diabetes mellitus. Therefore it can be conjectured that inflammation is thought to contribute to the development of insulin resistance, a significant sequel of obesity and is evident by studies describing role of TNF-α in mediating insulin resistance in obese patients.[4,5]

Insulin therapy has a breakthrough role in breaking this vicious cycle of obesity and diabetes as it not only controls chronic hyperglycemia but also regulates inflammatory process central to diabesity (conjoined term for diabetes and obesity) due to its anti-inflammatory property.[6] However the effect of insulin therapy on levels of inflammatory markers and difference if any among obese and non-obese diabetics has been inadequately studied. Based on above-given background information, the pre- and postinsulin (24 weeks and 48 weeks) effects on circulating levels of TNF-α and IL-6 in obese and non-obese diabetics were studied.

Materials and Methods

This was a prospective cohort study on 40 patients with T2DM, aged 45–65 years. Patients, who did not show improvement in glycemia levels with oral hypoglycemic drugs, were started with insulin therapy and were receiving statins were included in the study. Participants were divided into two cohorts, obese diabetic and nonobese diabetic (*n*=20 each), and obesity was defined as BMI>30. The average duration of diabetes was 21 months in obese and 34 months in nonobese participants. The study also included age- and sex-matched 10 normal healthy volunteers as controls. Insulin dose was titrated by clinician, by measuring fasting plasma glucose (FPG) and modifying the dose accordingly. Patients were monitored during the study by FPG at monthly intervals, but FPG at 0, 24 and 48 weeks only were considered for analysis.

Patients, who had a history of any acute or chronic disease other than T2DM, either at present or in recent past, or were taking oral hypoglycemic drugs/ACE inhibitors, were excluded from the study, to minimize possible confounding of results. All the patients to be included in the study were informed about the objectives of this study and any potential benefits or losses. A standardized patient consent form, approved by Institutional Ethics Committee, was used for this purpose. Serum samples were collected before the start of insulin treatment and after 24 and 48 weeks. The levels of TNF-α and IL-6 were measured in each sample.

TNF-α immunoassay
The concentration of TNF-α in various serum samples of diabetes patients and healthy controls was determined by use of a commercial ELISA Kit (R and D Systems).

This assay employed the quantitative sandwich enzyme immunoassay technique. The cut-off or lower limit of sensitivity was 4.4 pg/ml. The intraassay and interassay coefficients of variation for TNF were 5.2% and 7.4%, respectively.

IL-6 assay
Detection and quantitative measurement of IL-6 in serum were done by AviBion Human IL-6 ELISA kit (Orgenium Laboratories, Finland). The minimum detectable concentration (MDC) was estimated to be 2 pg/ml. The intraassay and interassay coefficients of variation for IL-6 were 9.4% and 8.6%, respectively.

Statistical analysis
The data were expressed as mean ± standard deviation. Paired *t* test was done to compare TNF-α and IL-6 between two groups (obese and non-obese). Analysis of variance (One- way Anova) was used to compare TNF-α and Il-6 levels in the same set of groups (either obese or non-obese) at two different times. Pearson's coefficient of correlation was done to assess association between TNF-α and Il-6 levels with glycemic status after insulin therapy in obese and nonobese diabetics. Statplus software was used for statistical analysis. *P* values <0.05 were considered significant while *P* value <0.001 were extremely significant.

Results

TNF-α and Il-6 levels in normal healthy, diabetic nonobese, and diabetic obese patients
We measured TNF-α and Il-6 levels [Table 1] in patients samples (*n*=20 from each group, non-obese diabetics and obese diabetics) a healthy normal controls (*n*=10). As evident from this study a negligible level of TNF-α (4.46 pg/ml) and IL-6 (4.98 pg/ml) was recorded in normal healthy control. Samples of similar age group of nonobese diabetic patients devoid of any insulin treatment (i.e., preinsulin samples) showed an ~20-fold (87.8 pg/ml; *P*<0.001) augmentation in TNF-α levels and ~7-fold (34.9 pg/ml; *P*<0.001) augmentation in IL-6 levels of preinsulin nonobese diabetics compared to healthy controls. Next, we probed the levels of TNF-α and IL-6 as patients. A tremendous augmentation was observed in the levels of TNF-α and IL-6 by ~25-fold (112.1 pg/ml; *P*<0.001) and ~8.7-fold (38.2 pg/ml; *P*<0.001) respectively compared to healthy controls.

Table 1: TNF-α and IL-6levels in obese patients, before treatment, after 24 and 48 weeks							
Status	Normal control	Nonobese diabetics			Obese diabetics		
		Preinsulin	24 weeks	48 weeks	Preinsulin	24 weeks	48 weeks
TNF-α (pg/ml)	4.46±1.42	87.9±4.9	68.5±2.9	24.7±2.3	112.2±11.0	105.5±7.3	57.2±2.8
IL-6 (pg/ml)	4.98±1.23	34.9±4.1	25.5±2.1	12.3±1.0	38.2±3.8	36.0±2.4	20.4±1.4

## Effect of duration of insulin treatment on TNF-α and Il-6 levels in nonobese and obese diabetic patients

Thereafter, the effect of insulin treatment of nonobese diabetic patients on the expression of TNF-α and IL-6 was probed after 24 and 48 weeks of insulin administration. A decrease by ~1.28 and ~1.44 fold each was recorded with TNF-α and IL-6 ($P<0.001$) after 24 weeks. Interestingly, after 48 weeks of insulin administration to nonobese diabetic patients, an appreciably high-magnitude decrease by ~3.63-fold ($P<0.001$) and 2.82-fold ($P<0.001$) was observed for TNF-α and IL-6 respectively.

Next, the insulin-induced effects in obese diabetic patients on the expression of TNF-α and IL-6 was probed after 24 weeks and 48 weeks of insulin administration. A decrease by ~1.1 fold each was recorded with TNF-α and IL-6 respectively ($P<0.05$) after 24 weeks of insulin administration. Similarly, a decrease by ~2.0 fold ($P<0.001$) and ~1.86 fold ($P<0.001$) was observed in TNF-α and IL-6 levels respectively after 48 weeks of insulin administration.

Correlation of TNF-α and IL-6 with FPG levels in nonobese and obese diabetics after insulin therapy

A positive correlation was found between postinsulin TNF-α and IL-6 with FPG levels of non-obese and obese diabetics after 24 and 48 weeks. The correlation coefficient (R) for non-obese diabetics between TNF-α with FPG levels was 0.97 and 0.98 at 24 and 48 weeks respectively [Figure 1a and b] while for IL-6 with FPG, it was 0.95 and 0.97 [Figure 1c and d]. However, obese diabetics had a correlation coefficient of 0.93 and 0.96 at 24 and 48 weeks respectively for TNF-α with FPG levels [Figure 2a and b] for IL-6 with FPG, it was 0.87 and 0.95 [Figure 2c and d].
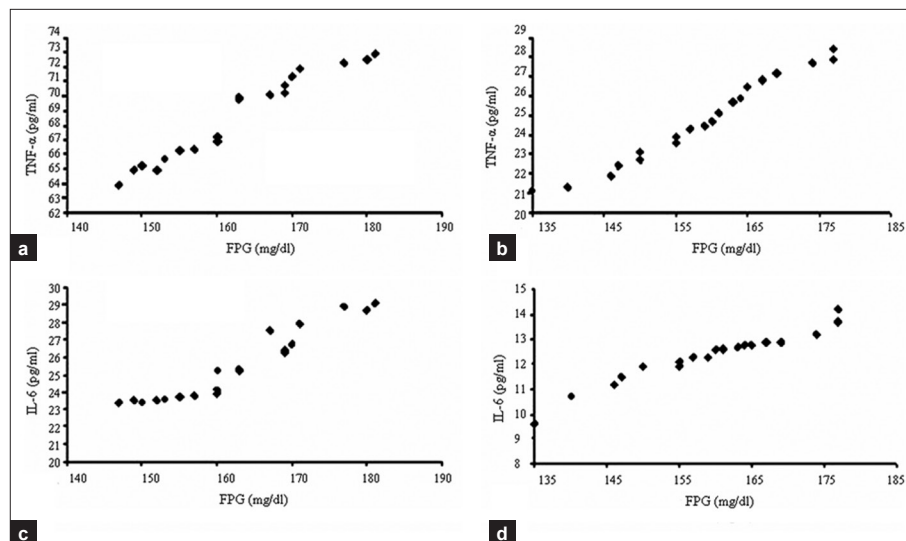
Figure 1(a-d): Postinsulin TNF-α (pg/ml) and IL-6 (pg/ml) correlation with FPG (mg/dl) in nonobese diabetics (detailed description in text)
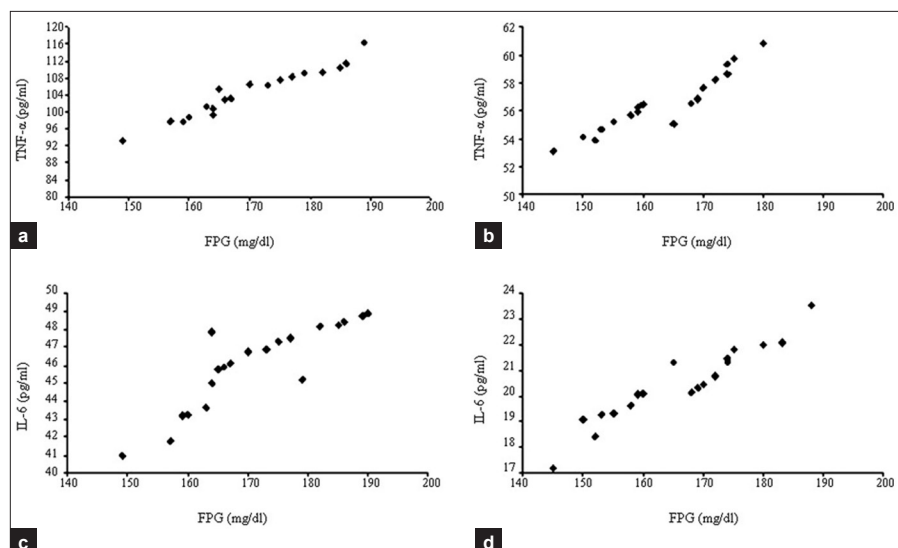
Figure 2(a-d): Postinsulin TNF-α (pg/ml) and IL-6 (pg/ml) correlation with FPG (mg/dl) in obese diabetics (detailed description in text)

Discussion

It has been hypothesized that T2DM is a manifestation of an ongoing acute-phase response that is primarily characterized by alterations of the so-called acute-phase proteins, such as C-reactive protein (CRP).[7,8] Elevated levels of IL-6, which is the main stimulator of the production of most acute-phase proteins, increase the risk of diabetes.[9-11] However, in addition to IL-6, other cytokines, such as IL-1 and TNF-α are central mediators of inflammatory reactions. It is well known that cytokines operate as a network in stimulating the production of acute-phase proteins. Obesity, as well as hyperglycemia, contributes to the rise of these inflammatory markers.

In the present study attempts were made to probe the difference, if any, between nonobese and obese diabetic patients with respect to pro-inflammatory markers like TNF-α and IL-6 and effect of insulin treatment on them. We have not come across any study in which levels of inflammatory markers were assessed before and after insulin treatment in obese diabetics.

TNFα and IL-6 are the major cytokines produced by adipose tissue and their circulating levels are increased in diabetics. It is noteworthy to point out here that our results clearly demonstrate preinsulin nonobese diabetics to express augmented TNF-α and IL-6 when compared to normal controls. As expected and in accordance to earlier studies, we also observed obese diabetics to exhibit a higher magnitude of TNF-α and IL-6 expressions when compared to nonobese diabetics.[12-14] Previous studies have shown that adipose tissue is the main source of circulating TNF-α.[15,16]

The most striking finding of the present study was the insulin-induced downregulation of TNF-α and IL-6 in nonobese and obese diabetic patients. Apart from the above, the other important observation made in this study was of the degree of suppression of TNF-α and IL-6 after insulin therapy, which was more marked in nonobese compared to obese diabetics. This could be explained by plausible role of the TNFα-system in the low-grade systemic inflammation associated with obesity and a supplementary autocrine role in the production of other pro-inflammatory cytokines such as IL-6 from adipose depots.[17] The insulin-induced suppression or downregulation after 24 weeks and 48 weeks in TNF-α and IL-6 at the protein level is highly exciting. A recent study also indicates that there is a relationship between duration of insulin therapy and an acute-phase response. This supports the notion that the relationship between TNF-α and IL-6 and insulin action may, therefore, be mediated by adiposity.[18] We did not study the relation between individual BMI and levels of TNF-α and IL-6.

The rising incidence of obesity in our country has made the Indian population susceptible to deleterious effects of obesity. There is paucity of data on effects of insulin therapy on inflammation associated with diabesity in Indians; therefore an effort is made to understand interplay of T2DM, inflammation, and obesity after various durations of insulin therapy, in this study. However, further in-depth investigations at the gene level are warranted in order to reach a conclusive inference from the above observations.

Conclusions

There is increased inflammation in obese diabetics in comparison to nonobese diabetics, as shown by greater elevation of TNF-α and IL-6 levels in the former. Insulin treatment leads to reduction in levels of these inflammatory markers in both obese as well as nonobese diabetic patients, but the response is blunted as well as delayed in obese diabetics. In summary, the findings of the present study may help in better understanding the responsiveness of insulin therapy in obese and nonobese diabetic patients.

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