INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

Volume: 2: Issue-1: Jan-Mar -2011 IJABPT *ISSN 0976-4550*

TUMOR NECROSIS FACTOR–ALPHA IN THE DEVELOPMENT OF INSULIN RESISTANCE IN TYPE 2 DIABETES MELLITUS

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ABSTRACT : Obesity and Insulin resistance are very frequent disorders and are described as the dominant risk factors for cardiovascular diseases. Recent research suggests that TNF-alpha, an adipocytokine plays a key role in the development of insulin resistance. Aim of the study was to estimate TNF-alpha levels and to investigate the association of TNF-alpha with the various factors associated with insulin resistance like Body mass index, Waist Hip Ratio, fasting insulin etc. A total of 100 subjects attending out patient department of General Medicine , Narayana Medical College , Nellore were selected for the study.50 patients were diagnosed as type-2 diabetics and 50 subjects were taken as normal healthy controls who attended for a general check-up. Anthropometric and biochemical measurements were analyzed. Serum Insulin and TNF-α were measured using chemiluminescence method and ELISA method respectively. Homeostasis model assessment score was used to gauge the level of insulin resistance. Our Results showed significant elevation in TNF-alpha levels in diabetic subjects and correlations between TNF-alpha Body Mass Index and HOMAIR were significant**.** All correlations were stronger for females compared to males.

Key words: TNF-alpha, Insulin Resistance, Type-2 Diabetes Mellitus

INTRODUCTION

The development of the concept that T2DM is an inflammatory condition,0 is an exciting and novel approach to the understanding of this condition [Calina popa,et al,2007]. Recent work in the area of obesity has confirmed that obesity is a state of low grade chronic inflammation, as indicated by increased concentrations of C-reactive protein, TNF, interleukins and other inflammatory markers identified in the blood of obese people [G.Imeno R.E, et al, 2005]. Several endocrine, paracrine and autocrine mediators derived from adipose tissue play essential roles in the regulation of adipocyte functions , in particular those related to insulin action [Jose.L.G, et al, 2005].One significant complication of obesity includes the strong association with IR , which represents the greatest risk factor for T2DM [D.Dixon,et al,2004]. The level of insulin resistance is known to be associated with increased occurrence of myocardial infarction, stroke and peripheral vascular disease [Misra A,et al,2002]. Pro-inflammatory cytokines were found to be increased in patients with T2DM [Mishima Y,et al, 2001]. TNF- appears to play a key role in the pathogenesis of obesity induced IR and T2DM [Skolnik E, et al 1996]. TNF-alpha alter insulin sensitivity by triggering different key steps in the insulin signaling pathay (Bastard J.P, et al 2006).

The activation of proinflammatory pathways after exposure to TNF induces a state of IR in terms of glucose uptake in myocytes and adipocytes that impairs insulin signaling at the level of the Insulin Receptor Substrate proteins [Iria Nieto Vazquez, 2008]. Increased TNF-α production has been observed in adipose tissue derived from obese rodents or human subjects and TNF-α has been implicated as a causative factor in obesity-associated insulin resistance and the pathogenesis of type 2 diabetes[Aguirre, 2000]. and also current evidence suggests that administration of exogenous TNF-α to animals can induce insulin resistance, whereas neutralization of TNF-α can improve insulin sensitivity [Hotamisligil G.S, 1995]. Since recent studies indicate that agents which improve insulin sensitivity may be of great value in the treatment of type 2 diabetes mellitus, it is of interest to test whether insulin resistance and its clinical correlates can be reversed by therapies aimed at neutralization of TNF-α. The aim of this study was to analyze the involvement of TNF-alpha in the development of insulin resistance in T2DM and to study the association of TNF with BMI, WHR AND IR in T2DM and an attempt to elucidate the gender differences in the same.

MATERIALS AND METHODS

The study was carried out on a total of 100 subjects , recruited from the out patient department of General Medicine , Narayana Medical college and Hospital, Nellore, Andhra Pradesh. Out of 100 subjects 50 were newly diagnosed diabetic patients (men=28, women=22) and 50 (men=24, women=26)were normal healthy subjects who attended for their periodic health checkup.All individuals were subjected to a complete medical evaluation by a Physician including a full medical history and physical examination . Informed written consent was obtained from all the subjects. Both males and females between 25 to 55 years of age were included in the study.Individuals with severe inflammatory or infectious diseases , cancers , subjects with evidence of severe hepatic or renal disease , Persons on insulin or other medications that could affect glucose metabolism and pregnant or lactating women were excluded from the study.

Anthropometric measurements

Height (cm), waist and hip circumference(cm) were noted using a measuring tape (to the nearest 0.1 cm). Waist Circumference was measured at the mid point between the lower border of rib cage and the iliac crest , and Hip circumference was measured at the level of Trochanter, the widest part of the hip region . Weight(kg) was measured to the nearest 0.1Kg using a weighing machine simultaneously. Obesity was defined as BMI> 30Kg/m^2 .

Methods

Plasma glucose was analyzed by glucose oxidase method using Human kits (GmbH) by automated chemistry anaslyser , Humaster -300 (GmbH , Germany). Serum insulin was estimated in automated Chemiluminescence immuno assay [Beckman coulter, Virginea] using kits by Bayer diagnostics. Serum TNF-α concentrations were measured by using sandwich ELISA kit method (e- Bioscience, Bender med systems) which has an inter assay co-efficient of variations of 7.5- 10.4% and a lower limit of detection 0.5pg/ml. All samples were processed and examined according to principles of good laboratory practice at Central laboratory , Narayana Medical College and Hospital, Nellore.

Indices

The Homeostasis Model Assessment (HOMA - IR) method was used for the calculation of Insulin resistance. This method has been validated as a reliable measure of insulin resistance in vivo in humans .

HOMA IR method closely mirrors the glucose clamp technique in the assessment of insulin sensitivity [Bonora et al 2000]. Higher HOMA scores denoted lower insulin sensitivity and greater insulin resistance.

$$\text{HOMA IR} = \frac{\text{Fasting Serum insulin (}\mu\text{U/ml) X Fasting Plasma Glucose (mM/l)}}{22.5}$$

Body mass index [BMI], defined as body mass in kilograms divided by the square of height in metres, was calculated.

Waist Hip ratio (WHR) was calculated as waist circumference divided by hip circumference.

Statistical analysis was performed using SPSS-13 software version. Spearman's correlation analysis was used to analyze the data. Spearman's rho < 0.05 was considered as statistically significant.

RESULTS

Table 1 summarizes the base line characteristics of both study and control subjects . Fasting insulin levels and HOMA scores were significantly higher in all Diabetic patients when compared to control subjects. The mean TNF- alpha concentration was statistically significant between cases and controls [p< 0.001] . Table 2 shows the means and standard deviations of various parameters investigated between male(n=28) and female(n=22) subjects. S.TNF alpha concentration was significantly elevated in females than in males. Table 3 shows the results of correlation between plasma TNF-alpha levels and main Anthropometric and clinical variables. There was a significant positive correlation between TNF-Alpha and measures of obesity as BMI ,WHR and HOMA IR in subjects with T2DM.

Table 1: Baseline characteristics of the study and control groups

VARIABLES	CONTROLS	CASES	P VALUE
n	50	50	NA
M/F	24/26	22/28	NA
Age (years)	41.34 ± 5.15	51.42 ± 11.36	NA
BMI(kg/m^2)	21.3 ± 1.6	25.12 ± 4.21	0.0001
FBS(mmol/lit)`	8.58 ± 17	82.54 ± 3.12	0.000
Insulin µg/ml	9.09 ± 7.68	19.1 ± 24.63	0.0072
HOMA IR	4.587 ± 0.94	7.4 ± 10.8	0.065
TNF-α	22.94 ± 25.2	215.8 ± 119.58	0.000

Statistical difference according to ManWhitney U test. P<0.05 is significant

Table 2 : Mean and SD values of variables between male (n=28) and female(n=22) diabetics

PARAMETER	SEX	MEAN	STANDARD DEVIATION	P VALUE
Age (years)	M F	52.32 49.81	11.88 11.55	0.458
BMI(kg/m^2)	M F	24.74 25.66	1.49 6.18	0.481
FBS(mmol/l)	M F	8.98 7.97	4.09 2.93	0.337
Serum Insulin (µU/ml)	M F	17.39 21.27	21.98 28.04	0.586
Serum TNF-α (pg/L)	M F	186.17 252.09	62.14 160.78	0.044
HOMA IR	M F	7.50 7.41	11.23 10.68	0.977

P< 0.05 is significant

Table -3 Correlation between TNF-alpha levels with BMI, WHR, HOMAIR and Insulin Resistance within cases.

Variable	TNF – α	
	r value	P value
BMI	0.383	0.006
WHR	0.075	0.607
Insulin	0.265	0.063
HOMA IR	0.172	0.233

P < 0.05 is significant by spearmans correlation.

DISCUSSION

Insulin resistance is a wide spread feature of common atherogenic diseases including obesity, type2 diabetes mellitus, Hypertension which are linked to cardiovascular diseases (Nobuhiko togashi et al 2002). Though the mechanism of IR is not yet fully understood many studies indicate that TNF-alpha plays a major role in the pathogenesis of obesity induced IR resulting from an interaction with insulin signaling pathway. Thus the recognition of IR seems to have investigational and clinical relevance in identifying subjects at high risk of type2 DM and CVD. In our study TNF-alpha levels correlated positively with fasting blood sugar and insulin levels in both study and control subjects. In our study we found a strong Correlation between TNF-alpha and BMI suggesting the role of TNF-alpha in obesity induced IR.

The first evidence that adipocytes from obese animals express markedly increased amounts of TNF-alpha was shown by Hotamisligil, et al in 1993 . later data have shown that TNF-alpha is also expressed in human adipose tissue and that its plasma concentration in obese subjects is decreased after weight loss[Hotamisligil, et al, 1995]. According to Ziccardi, et al 2002, there was a significant positive correlation between TNF-alpha and BMI . Invitro studies on human cell lines have confirmed that when exposed to TNF-alpha adipocytes become insulin resistant[Saghizadeh M,et al, 1997]. Mishihama et al, found that s. TNF-alpha inobese type2 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-alpha may play a role in the pathophysiology of type2 Diabetes mellitus.

Conclusion

The results of our study suggest that TNF-alpha is an important mediator of IR in the pathogenesis of obesity related IR and anti- TNF-alpha agents might serve as a new therapeutic target in treating obesity induced Diabetes mellitus.

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