STATUS OF TNF-α AND INSULIN IN OBESE AND DIABETIC OBESE SUBJECTS OF WESTERN LUCKNOW

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ABSTRACT

Obesity has become a matter of quality to health care administrators. The busy lifestyle of people made them prefer fast food instead of taking healthy food. But the people are not aware that fast food habit converts to the disease like obesity, type 2 diabetes mellitus (T2DM), dyslipoproteinemia etc. This case control study had been carried out in department of Physiology in collaboration of Department of Biochemistry and Medicine, Era's Lucknow Medical College & Hospital, Lucknow to explore status blood sugar fasting (BSF), tumor necrosis factor-α (TNF- α), Insulin by standard spectrophotpmetric kit methods, blood pressure (BP) as well as anthropometric measurements with the help of suitable instruments and equipments in Control group, Obese group and Obese with type 2 diabetic group. Values of all above parameters were found increased in obese group with respect to control group and values of all these parameters were found increase in obese with type 2 Diabetes mellitus group with respect to obese group. Thus it is clear that obesity is risk factor for T2DM, Dyslipoproteinemia and coronary artery disease (CAD).

Received on : 24-05-2018
Accpected on : 01-07-2018

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KEYWORDS: Obesity, Anthropometric measurement, Type 2 diabeted mellitus, Triglycerol, Adipose tissue.

INTRODUCTION

Obesity is defined as excess body weight (>20% of ideal weight) due to accumulation of fat. (1-5) In the recent years, however, two more parameters have found in frequent use waist/hip ratio and waist circumference. (6) In many populations all these three parameters have been found to be related where as in some population's waist/hip ratio and waist circumference have been found a better expression of obesity as central obesity is better predictor of risk. It has been stressed by WHO and other workers that these parameters should be assessed in different populations because categorization may differ from population to population due to differing standards of nutrition, environmental variants, genetic disposition and finally unavoidable abdominal adiposity. (7) She stated that these Indian standards were prepared with the help of 200 experts. As per revised guidelines every second person in Delhi fulfills criterion for obesity or has excess of abdominal fat.

The etiology of simple obesity is multifunctional and multimechanistic. (8) The fundamental defect in this condition is lopsided energy management by body: more calories consumed than spent. Besides genetic inclination, the putative social, behavioral and environmental courses are increased consumptions of high energy foods particularly fast foods, increased frequency of food intake, indiscriminate selection of foods, especially refined foods excess of trans fat and higher glycemic load carbohydrates. The social aspects influencing the caloric intake are overzealous advertising of sweetened beverages and foods. Sedentary life is another determing factor. For example T.V., movies and computers and more confinement at home encourages less walking and physical exercise. (4) All these factors in concert tend to alter metabolism and appetite. (9) The production of TNF-α, a pro-inflammatory adipocytokine is noticeably enhanced in obesity. (10) TNF-α is a pleotropic cytokine with diverse functions and occurs in many pathological diseases like cancer, cardiovascular disease, type 2 diabetes mellitus etc. (11) It is produced by macrophages in response to inflammation, endotoxemia and cancer and plays a key role in the pathogenesis of peripheral insulin resistance in obesity. TNF-α inhibits tyrosine kinase activity at the insulin receptor level and cause obesity induced insulin resistance. (12-13) Many studies have shown increased serum levels of TNF-α in obese patients in comparison with lean subjects. (14) Emerging clinical data shows that, inflammation precedes the development of clinically overt diabetes and also predicts the subsequent cardiovascular events. (15) TNF-α may serve as an inflammatory biomarker and as an important risk indicator for the future development of type 2 diabetes mellitus and provide a novel target for therapeutic intervention. (16) This study was undertaken to estimate the TNF-α levels in type 2 diabetes mellitus and to analyse the association with the anthropometric (Body

Mass Index; BMI and Waist Hip Ratio; WHR) and clinical variables (fasting glucose and insulin) related to insulin resistance (IR), in obese and obese with diabetes.

Due to aforesaid reasons resulting in the tilted and excess energy intake, the body starts gaining weight. This gain is practically confined to accumulation of fat in adipocytes which are centre of adiposity from where aberrant signals originate to initiate various abnormal biochemical outcomes resulting in insulin resistance, metabolic syndrome, diabetes, CVD and others. (17) The struggle begins between physiological and unphysiological forces and diseases set in when physiological processes are overwhelmed. The single most important process regulating "Energy Homeostasis" is "Glucose Homeostasis" in blood and tissues. The major consumers of glucose are peripheral tissue cells. Insulin is undisputedly a key regulator of this process. However, there are an array of hormones and cytokines to counter check its regulatory function. Unfortunately in obesity these counter switches get disturbed. Among these TNF- is one of the major switches. (18) It gradually diminishes insulin potency in the peripheral tissues with the result cells do not obediently respond to commands of insulin for flow of glucose from exracellular milieu inside the cell. This refusal of the cells to listen to the commands of insulin is known as "Insulin Resistance". Initially beta-cells send more insulin to combat this situation causing "Hyper insulinemia". However, the capacity of beta-cells is limited. Gradually they start getting exhausted and beta-cell dysfunction develops. While these three processes are in progress numerous other unfavorable factors such as proinflammatory cytokines, mitochondrial stress through altered redox status intervene along with TNF-. In chronic and persistent obesity, insulin alone fails to combat the opposite forces culminating in multiple abnormalities.

The above proposed hypothesis of text position among TNF-, insulin resistance is quite sound and appealing but its veracity is not proven in all populations or in all patients.

Study Design

This case control study had been designed to evaluate the interconnectivity among TNF , insulin and insulin resistance by examining these parameters in: a) normal subjects b) normal obese subjects (simple obesity) and c) Type 2 Diabetes Mellitus Obese (T2DM Obese) Patients.

MATERIALS AND METHODS

The study was carried out on 90 subjects attending outpatient department of general medicine, Era's Lucknow Medical College & Hospital, Sarfaraz Ganj, Hardoi Road, Lucknow. 30 obese subjects (M = 15, F = 15), 30 T2DM Obese patients (M = 15, F = 15) and 30 normal healthy control (M = 15, F = 15) who attended for their periodic health checkups. All individuals were subjected to a complete medical evaluation by a physician including a full medical history and physical examination. Both males and females between 25-65 years of age were included in the study. Patients with evidence of acute or chronic inflammatory or infectious disease, cancer, persons on insulin, or other medications that could affect glucose metabolism and pregnant or lactating women were excluded from the study. (19)

Collection of blood sample:

Blood sample had collected from median antecubital vein followed by overnight fasting, for biochemical estimations in fluoride (sodium fluoride and potassium oxalate, 5.4 mg NaF and 3.0 mg K-oxalate in each vial) and plain vials respectively.

Anthropometric measurement:

Height (cm), Weight (kg), Waist and hip circumferences (cm) were noted using a measuring tape to the 0.1 cm. Waist circumference was measured at the midpoint between the lower border of rib cage and the iliac crest. Hip circumference was measured at the level of trochanter, the widest part of the hip region. Weight (kg) was measured to the nearest 0.1 kg using a weighing machine simultaneously. Waist hip ratio (WHR) was calculated as waist circumference divided by hip circumference. BMI was calculated as weight (kg) divided by height (m^2). Obesity is defined as BMI > 30kg/ m^2. (20-25)

Biochemical estimations

Glucose: Blood glucose was estimated by the standard enzymatic kit method. (26)

TNFα and **Insulin** will be estimated by the standard ELISA kit method.

Insulin resistance will be calculated by formula given below-

- HOMA INDEX = Fasting Insulin concentration (Unit/ml) x Fasting Glucose concentration (mili mol/l)/ 22.5
- Normal young subjects have an Insulin resistance of 1. (27)

All samples were processed and examined according to principles of good laboratory practice at clinical biochemistry

Statistical analysis: One-way-analysis of variance (ANOVA- Newman's student test) was performed by comparison of values. All hypothesis testing were two-tailed. P <0.05 was considered statistically significant and the results were expressed as mean ± SD. The Graph pad INSTAT 3.0 software was used to carried out the statistical analysis. (28)

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RESULTS

Variables → Groups ↓	BMI (Kg/ m²)	BSA (m²)	WHR	BP (mm Hg)		BSF (mg/dl)	TNF-α (pg/ml)	HOMA-IR	Insulin (μUnit/ml)
				SBP (mm Hg)	DBP (mm Hg)				
Control (n=30)	21.79 ±2.36	1.66 ±0.16	0.85 ±0.04	117.53±4.65	78.20 ±4.21	90.17 ±7.86	21.31 ±10.46	30.01 ±11.20	7.57 ±3.05
Obese (n=30)	31.69* ±4.07	1.98* ±0.16	0.89NS ±0.03	134.47* ±8.04	86.57* ±4.21	94.10* ±7.25	47.47* ±14.26	60.20* ±16.06	15.35* ±3.69
T2DM Obese **(n=30)**	33.45* ±3.84	2.05* ±0.14	0.87NS ±0.03	141.67*± 7.04	90.27* ±4.48	185.50* ±36.31	195.03* ±32.63	304.08* ±85.33	36.82* ±6.85

Table-1: Association of TNF- in obesity and Obesity with T2DM

Values expressed as mean ± SD of 30 subjects. Obese group was compared with control, T2DM Obese Patients were compared with Obese,*p<0.001, NS = Not Significant.

DISCUSSION

Pre-existing studies of human and animal models have indicated that, TNF-α expression in the adipose tissues is significantly elevated in obesity. (19-20, 29) In our study TNF-α concentration was significantly high in obese T2DM than in non obese subjects. Our results demonstrated that, increased level of TNF-α were associated with increased level of glucose in T2DM and was related to the degree of obesity. Nilksson et al reported that, the plasma TNF-α levels were increased by 23% in lean T2DM compared to 51% in obese T2DM subjects with more severe insulin resistance. (21–25, 30) Katsuki et al reported that, TNF-α is elevated in obese T2DM but not in lean T2DM.14, 31 According to Hotamisligil et al body weight reduction in obese individuals is also associated with a reduction in TNF-α level and in improved insulin sensitivity. Our present results clearly demonstrated that circulating TNF-α level were significantly elevated in T2DM compared to normal healthy subjects particularly in obese subjects, and is strongly correlated with BMI. Our observation is consistent with numerous previous studies which have documented a strong correlation between TNF-α and BMI. (22-24, 31) Elevated levels of TNF-α were also found to predict cardiovascular events with diabetes from the nurses' health study. (25) All these data provide strong associative evidence supporting subclinical inflammation as a unifying factor accelerating the progression of Insulin resistance and T2DM. Our data suggest a possible role of TNF-α in the pathophysiology of Insulin Resistance particularly in obese individuals (29-31).

CONCLUSION

The definition of the cutoff value for "normal" BMI in a population would depend on identifying the risk association with a disorder strongly associated with BMI. Further, such type of studies will specially help health workers and clinicians to suggest health and therapeutic regimen in a particular population. Needless to say, this study in due course of time is expected to be of great practical relevance to the students and staff of Era University.

ACKNOWLEDGEMENT: One of us (Vishnu Kumar) is grateful to the Director Academics, Era's Lucknow Medical College and Hospital, Lucknow for their guidance and support.

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How to cite this article : Singh S., Kumar V., singh K., Karoli R., Mahdi F., Status Of Tnf-α And Insulin In Obese And Diabetic Obese Subjects Of Western Lucknow. EJMR2018;5(1):1-5.