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# CORRELATION BETWEEN TNF- α AND INSULIN RESISTANCE IN NON-DIABETIC CENTRAL OBESE SUBJECTS: A STUDY FROM RAJASTHAN

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**ABSTRACT**

**Background:** To find out correlation between TNF- α and insulin resistance in non-diabetic central obese subjects.
**Materials and Methods:** The present study was conducted on the central obese subjects at the Department of Medicine, Maharana Bhapal Govt. Hospital, RNT Medical College, Udaipur. For this study 40 non-diabetic subjects were enrolled having central obesity. Plasma glucose was estimated by an enzymatic method called GOD/POD METHOD. Fasting serum insulin was determined by Radioimmunoassary (RIA) method. HOMA-IR(Insulin resistnace) was calculated in all subjects from fasting glucose and serum insulin level using the standard formula.. TNF-α was measured by TNF-α enzyme immunoassay. All data was statistically analyzed using SPSS software.
**Results:** Higher values of mean TNF- α level was observed for increasing BMI and waist circumference. A significant correlation was observed between TNF- α and BMI. TNF-α shows strong correlation with fasting insulin and HOMA-IR (P<0.001).
**Conclusion:** TNF - α can be used as suitable marker to investigate insulation resistance (IR).

**KEYWORDS :** TNF-α, Insulation Resistance (IR), BMI, HOMA-IR, Microvascular Inflammation, Metabolic vascular disease

## INTRODUCTION

Obesity is a condition in which the natural energy reserve, stored in the fatty tissue of humans and other mammals, is in increased to a point where it is a risk factor for certain health conditions or increased mortality. Obesity develops from the interaction of individual biology and the environment. Usually there is not only single cause for obesity. Biological as well as life-style factor contribute to this problem.[1] Generally, Obesity occurs due to consumption of higher amount of calories leading to imbalance between calories-in and calories-out.[2]

Genetic factors and some genetic disorders (e.g., Prader-Willi syndrome), environmental, social and other factors also strongly influence obesity.[3]Obesity is also supported by underlying illness[4] (e.g., hypothyroidism, Cushing's syndrome, a hormonal disorder, Polycystic ovary syndrome) and Certain medications (e.g., atypical antipsychotics, steroids, antidepressants) .[5]Moreover, various factors such as high glycemic diet (i.e., diet that consists of meals that give high postprandial blood sugar), inactivity and sedentary lifestyle, eating disorders (e.g., binge eating disorder), stressful mentality, insufficient sleep and consumption of alcohol also contribute of obesity.

In the clinical setting, obesity is typically evaluated by measuring BMI (body mass index), waist circumference, and evaluating the presence of risk Factors and comorbidities. BMI is a simple and widely used indicator for estimating obesity.[6] BMI, or Body Mass Index, was developed by the Belgian statistician and anthropometrist Adolphe Quetelet. It is calculated by dividing the subject's weight in kilograms by the square of his/her height in metres ($BMI = kg/m^2$).[7]

$$BMI = \frac{\text{Weight in kg}}{\text{Height in m}^2}$$

OR

$$[\ BMI = \frac{\text{Weight in pounds}\ ]\ x\ 703}{\text{Height in inches}}$$

According to WHO classification, as per BMI ranges, the obesity is classified as pre-obese, Obese class-I, Obese Class-II and Obese Class-III. When the waist-hip ratio exceeds 0.9 in men or 0.85 in women, then such obesity is termed as Central obesity(or 'apple-shaped' or 'masculine' obesity).It occurs when the main deposits of body fat are localised around the abdomen and the upper body. It commonly coexists with polycystic ovary syndrome (PCOS) and syndrome X. Central obesity is associated with a statistically higher risk of heart disease, hypertension, insulin resistance and diabetes mellitus type-2.

Obesity is recognized as a major health problem in both developed and developing countries. [8] The major health consequences associated with overweight and obesity are diabetes mellitus type 2, CAD, hypertension, gall bladder diseases, dyslipidaemia, cardiovascular diseases, osteoarthritis and insulin resistance. Obesity is both an individual clinical condition and is increasingly viewed as a serious public health problem.

Diabetes mellitus[8] is emerging as a major health problem associated with the obesity. A positive relation between obesity and the risk of type-2 diabetes has been established repeatedly the world over. There is a strong association of body weight with insulin resistance.[10] Higher BMI is associated with hyperinsulinemia and insulin resistance. Insulin resistance is one of the major aetiological factor for diabetes and the risk association of obesity with diabetes is largely mediated through insulin resistance.[11]

The insulin resistance that accompanies obesity promotes the development of hyperglycaemia by increasing hepatic glucose output and impairing uptake of glucose in peripheral tissue. Insulin resistance is an inability of insulin to produce its biological effect in insulin sensitive tissue. Major sites include skeletal muscle, adipocyte and liver. Insulin resistance results in metabolic syndrome which is associated with the risk of increased coronary and cardiovascular mortality. Subjects with abdominal or central obesity along with dyslipdemia, hypertension (BP > 130/85 mm Hg) and fasting blood glucose > 110 mg/dl are highly prone to metabolic syndrome. It has been observed in various studies that majority of non-diabetic patients having metabolic syndrome later develop diabetes.

Faced with multiple risk factors associated with the syndrome of diabetes mellitus, it is of paramount importance to identify for suitable marker to investigate insulation resistance. Many factors modulate insulin signaling such as TNF-α , interleukins and other inflammatory cytokine.Their increased secretion may be associated with insulin resistance. There are various studies in support of role of TNF-αas mediator of IR. TNF-α was first described as a protein inflammatory cytokine released from monocyte or macrophage in response to various infection. It was the first adipokine which was found to act as direct receptors of TNF-R1 and TNF-R2 in cell surface of most of the cells of all the adipokens.[14]

Normally, insulin binding causes tyrosine auto phosphorylation of intracellular part of insulin receptor and subsequent tyrosine phosphorylation of insulin reception substrate-1 (IRS1). But TNF-αcauses serine phosphorylation and thus prevents tyrosine phosphorylation cascade. It reduces insulin action in muscle cell and liver cell by prevention of tyrosine phosphorylation. Therefore three major insulin sensitive metabolic tissues in the body i.e. muscle, liver and fatty tissue are targeted and affected by TNF- α which in turn induces insulin resistance.

TNF- α reduces the availability of GLUT-4 in adipocyte by reducing

expression of GLUT-4. It also reduces lipoprotein lipase (LPL) expression and increased FFA. TNF-α affects the deposition of lipid adipose tissue by causing in appropriate apoptoses and dedifferentiation of adipocytes and preadipocytes. Thus TNF-α can be examined as a insulin resistance marker due to its significant property of inhibiting insulin signaling. [15,16,17,18,19]

The objective of this study was to find out correlation between TNF-α and insulin resistance in non-diabetic central obese subjects.

## MATERIALS AND METHODS

The present study was conducted on the central obese subjects at the Department of Medicine, Maharana Bhapal Govt. Hospital, RNT Medical College, Udaipur. For this study 40 non-diabetic subjects were enrolled having central obesity, waist circumference> 102 cm in men and >88 cm in women with waist : hip raitio > 0.9 in men and > 0.85 in women.

### 2.1 INCLUSION CRITERIA

Study subjects consisted of:

- Non-diabetic individuals with BMI>24 ( as per the classification of overweight and obesity by BMI from National heart, lung and blood Institute (North American Association for the study of obesity) .
- Persons with central obesity, waist circumference > 102 cm in men and >88 cm in women, waist hip ratio > 0.9 in men and >0.85 in women.

### EXCLUSION CRITERIA:

Exclusion Criteria were as under:

1. Evidence of Diabetes Mellitus coronary artery disease, stroke, TIA or Peripheral vascular disease by history and clinical exam.
2. Patient's suffering from ketosis, Malignancy, Collagen Vascular disease, serious hepatic, pulmonary or renal disease.
3. Patient's suffering from acute infectious.

A structured questionnaire was used to collect socio demographic from the subjects willing to participate in this study. History of smoking, alcohol, dietary pattern, occupation and physical activity was also recorded. A complete medical history including hypertension, heart disease, diabetes mellitus peripheral arterial disease, cerebrovascular, therapy undertaken and duration of illness was obtained for all the subjects. Anthropometric data were obtained including height and weight. Weight was measured using a standard spring balance. The subjects were weighted with light clothes on and without shoes. BMI (body mass index) of all the subjects was calculated using standard formula. WHR (waist hip ratio) was calculated by measuring the waist girth as the minimum circumference between iliac crest and lower costal margin while hip girth was measured at the maximum width over the greater trochanter.

A through clinical examination of all the subjects was done including neurological examination. Blood pressure was measured in right upper limb in sitting position noting both systolic and diastolic pressures after a rest of 10 minutes with random zero sphygmomanometer.

Fasting blood sample (defined as no caloric intake for at least 8 hours) was obtained for determing fasting serum insulin, fasting plasma glucose and serum lipid profile including of total cholesterol, HDL-cholestrol, LDL- cholesterol by standard glucose oxidase method. Postprandial blood glucose was collected 2 hours after standard breakfast of the patient.

Plasma glucose was estimated by an enzymatic method called GOD/POD METHOD using the setup manufactured by sigmat Diagnostic (India)Ltd. In this method glucose is oxidized by the enzyme Glucose Oxidase (GOD) to give D-Gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of enzyme peroxidase (POD) oxidizes phenol, which combines with 4-aminoantipyrine to produce a red colored quinoneimine dye. The intensity of colour produced as proportional to the glucose concentration of the sample.

Fasting serum insulin was determined by Radioimmunoassary (RIA) method using I125 tracer. HOMA-IR(Insulin resistnace) was calculated in all subjects from fasting glucose and serum insulin level using the standard formula.

The basic principle of RIA method is governed by the competition between a radioactive and a non- radioactive antigen for a fixed number of antibody binding site. The amount of I125 labelled insulin bound to the antibody is inversely proportional to the concentration of unlabelled insulin present in the sample. The separation of free and bound antigen is easily and rapidly achieved by using a double antibody system.

The insulin resistance was thus calculated from fasting blood glucose and fasting serum insulin level by HOMA (Homeostatis Model Assessment) method using the following formula:

$$\text{HOMA-IR} = \{ \text{Insulin X Glucose}/18)\} /22.5$$

where Insulin is fasting Insulin in un/ml and Glucose is fasting Glucose in mg/dl. Cholesterol and Triglycerides were measured by standard enzymatic methods using Stat Fax semi-autoanalyser.

TNF- α was measured by Immunotech TNF-α enzyme immunoassay (ELISA IM1211, IM11121 methods). TNF- α values of various samples were obtained by interpolation from a standard curve performed in the same assay as that of the sample. A plot was obtained with TNF-α concentration of the standards on horizontal axis and absorbance of all the samples on the vertical axis. Corresponding TNF-α concentration of all the samples were obtained by reading off the interpolated values on the horizontal axis.

All data was statistically analyzed using SPSS software. Parametric data were expressed as mean value ± standard deviation (SD) and categorical variables as percentage. "p" value <0.05 was considered significant.

## RESULTS

Results obtained for all the 40 subjects are compiled in this section. Table no.1 provides distribution of study Subjects based on Body Mass Index for all the subjects included in this study.

**Table No. 1 : Distribution of Study Subjects Based on Body Mass Index**

| Parameter | BMI 25-29.9 | BMI 30-34.99 | BMI 35 & Above |
|---|---|---|---|
| Female | 3 | 7 | 7 |
| Male | 1 | 12 | 10 |
| Total | 4 | 19 | 17 |

This table shows the distribution of study subjects on the basis of body mass index. Total no. of subject with BMI 25-29.9 were 4, among whom 1 was male & 3 were females. Total no. of subject with BMI 30-34.99 were 19, among whom 12 were male & 7 were females. In the remaining 17 subjects with BMI ≥35, 10 were males & 7 were females Table-2 depicts comparison of clinical & asymptomatic characteristics of the study subjects.

**Table No. 2: Clinical Parameter of Study Subjects**

| Data | F | M | Grand Total |
|---|---|---|---|
| Age | 35.59±9.91 | 39.26±9.42 | 37.70±9.68 |
| Height (cm) | 1.52±0.05 | 1.68±0.05 | 1.61±0.09 |
| Weight (kg) | 78.47±10.81 | 96.70±9.67 | 88.95±13.56 |
| BMI | 33.85±3.36 | 34.34±2.83 | 88.95±3.04 |
| Waist Circumference | 103.00±10.55 | 109.87±6.98 | 106.95±9.22 |
| Hip Circumference | 112.35±9.01 | 109.70±7.39 | 110.83±8.11 |
| Waist Hip ratio | 0.91±0.04 | 1.00 | 0.96±0.06 |
| SBP | 125.65±5.44 | 133.48±6.91 | 130.15±7.38 |
| DBP | 78.71±5.05 | 84.00±4.55 | 81.75±5.40 |
| FBS (mg/dl) | 101.41±10.28 | 101.43±8.39 | 101.43±9.11 |
| PPBS(mg/l) | 143.71±11.62 | 147.04±13.81 | 145.63±12.87 |
| Fasting insulin(uIU/L) | 26.05±10.74 | 23.±6.41 | 24.68+8.48 |
| HOMA | 6.46±2.37 | 5.95±1.72 | 6.17±2.01 |
| TNF-alpha | 58.06±29.49 | 51.87±36.52 | 54.50±33.45 |

This table depicts comparison of clinical & asymptomatic characteristics of the study subjects. It was seen that mean±SD age of

the female subject in the study was 35.59±9.91 & that of male subject was 39.26±9.42 years which are comparable. Females has BMI value 33.85±3.36 whereas males has more i.e. 34.34±2.83. The mean waist circumference in female was 103.00±10.55 & in the men it was 109.87±6.98. While hip circumference is higher in females 112.35±9.01 as compared to males 109.70±7.39. Likewise WHR is more in males 1.00±0.04 than in females 0.91±0.04. The mean systolic blood pressure in males is 133.48±6.9 & in females it was 125.65±5.44. The mean fasting blood sugar in female 101.41±10.28 and in males was 101.43±8.39, fasting insulin levels were 26.05±10.74 in females & 23.67±6.41 in females. HOMA insulin resistance was calculated 6.46±2.37 in female & 5.95±1.72was males. The TNF alpha level were 58.06±29.49 in females & 51.87±36.52 in males.Table-3 provides comparison between TNF- alpha and BMI of various subjects.

**Table No. 3: Comparison between TNF-alpha & BMI**

| BMI | TNF- α |
|---|---|
| 25-29.99 | 25.25±16.36 |
| 30-34.99 | 51.68±31.40 |
| 35 & above | 64.53±35.17 |
| Mean | 54.50±33.45 |

Above table shows that in BMI 25-29.99 the mean TNF value are 25.25±16.36 while 30-34.99 it was 51.68±31.40 whereas in BMI 35 & above TNF value was 64.53±35.17. The mean TNF level progressively rose in a direct relation to the increase of BMI.

Table-4 provides comparison between TNF- alpha and HOMA IR of various subjects.

**Table No. 4: Comparison between TNF-alpha & HOMA IR**

| BMI | TNF- | HOMA |
|---|---|---|
| 25-29.99 | 25.25±16.36 | 3.87±1.16 |
| 30-34.99 | 51.68±31.40 | 5.88±1.87 |
| 35 & Above | 64.53±35.17 | 7.03±1.86 |
| Mean | 54.50±33.45 | 6.17±2.01 |

Table-4 shows comparative presentation of TNF & HOMA-IR values for various subjects. In BMI 25-29.99 subjects, the TNF- was 25.25±16.36 & insulin resistance was 3.87±1.16. In BMI 30-34.99 subjects, TNF- was 51.68±31.40 & insulin resistance was 5.88±1.87. In BMI 35 & above subjects, TNF- value was 64.53±35.17 & insulin resistance was 7.037±1.86. Thus obtained results clearly indicates that the intensity of insulin resistance progressively increased in the subject with increasing body mass index.

Table No. 5 provides correlation coefficient values between TNF-α values and various anthropometrical & biochemical parameters.

**Table No. 5 : Correlation coefficients for TNF-α & various Anthropometrical & Biochemical Parameters**

| Parameters | Correlation 'r' value | P value |
|---|---|---|
| TNF- and Waist Circumference | 0.423 | <0.002 |
| TNF- and Hip Circumference | 0.407 | <0.002 |
| TNF V/s Fasting Insulin | 0.508 | <0.001 |
| TNF V/s HOMA-IR | 0.581 | <0.001 |

## DISCUSSION:

The study population measures average BMI values 37.70±9.68 years. The percentage of male to female was 57.50 and 42.50, respectively. The anthropometrical measures revealed BMI values in these subjects averaging (33.85±3.36) in females and 34.34±2.83 in males. The female population had waist circumference of 103.00±10.55 and males had 109.87±6.98.

Higher values of mean TNF-α level was observed for increasing BMI and waist circumference. These finding are similar to the study conducted by PA Kern et al. who examined the correlation between TNF-α and insulin resistance in lean and obese subjects. They also observed that the relationship between TNF-α and IR in obese subjects was significantly high level (7.5 folds) as compared to non-obese subjects. Serum level of TNF-αwas positively correlated with BMI in obese subjects in their study.

We also observed a significant correlation between TNF-α and BMI. Overweight and obese subjects had significantly higher serum level of TNF-α which progressively increases with degree of obesity or inflammatory state . Monica Bullo et al. studied relation between TNF-α , leptin adipose tissue expression and low grade systemic inflammation. This report determined the relationship between inflammation and degree of adiposity. It was found that TNF-α levels were significantly higher in the subjects with higher values of adiposity and BMI. Our results are also in agreement with this study. Moreover, we observed that two subjects in this study with central obesity had higher level of TNF-alpha but low values of insulin resistance. So in these subjects TNF-alpha can be interpreted to have more association with central obesity than insulin resistance. It indicates that obesity represents an inflammatory state and TNF expression increases in adipocytes of central obese person.

Our results compiled in table-5 shows a statistically significant co-relationship (P<0.002) between TNF-α and increased waist and hip circumference. TNF-a in our study shows strong correlation with fasting insulin and HOMA-IR (P<0.001).

## SUMMARY AND CONCLUSION:

The present study was conducted on the central obese subjects at the Department of Medicine, Maharana Bhapal Govt. Hospital, RNT Medical College, Udaipur with the aim to find out correlation between TNF-a and insulin resistance in non-diabetic central obese subjects. For this study 40 non-diabetic subjects were chosen having central obesity. The study population ranged from age of 18-60 years with a mean age of 37.70±9.68 years. The clinical and anthropometric data was obtained for all the subjects. The insulin resistance (IR) values of all the subjects were calculated from fasting blood glucose and fasting serum insulin level by HOMA (Homeostatis Model Assessment) method. The values were then compared with BMI of all the subjects. The values of TNF-alpha were also compared with the value of HOMA-IR in these 40 central obese subjects.

Our study has shown the potential of TNF-alpha as a marker of microvascular inflammation in the patients of central obesity. Our results strongly advocates TNF-α as a suitable marker of metabolic vascular disease like diabetes mellitus, atherosclerosis, etc. . Thus measurement of TNF-α in obese individuals may lead to the better understanding of pathogenesis of diabetes and other chronic diseases

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