

SERUM TNF- α AND LEPTIN LEVELS IN GRADED OBESE DIABETIC WOMEN- FREE FROM MICRO AND MACRO VASCULAR COMPLICATIONS

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ABSTRACT

Asian Indians have more visceral fat and a greater insulin resistance. Obesity is associated with a low-grade inflammation of adipose tissue resulting in activation of the cytokine system which leads to exaggerated insulin resistance, impaired glucose tolerance and even vascular complications. Leptin, known to control the appetite has also been shown to regulate the glucose level. TNF- α also has been suggested as the link between obesity, diabetes and atherosclerosis. In our study we explored the level of leptin and TNF- α with BMI among the different BMI groups in females of type 2 diabetes. Seventy three diabetic females free from micro and macro vascular disorders were recruited. They were assessed for physical and chemical parameters like Body Mass Index, TNF- α and leptin. The relation looks more linear when regression line was plotted with leptin in the BMI range of 30-35. But this relation was lost (BMI>30-35) when TNF- α and BMI were plotted suggesting the practical limitations of TNF- α . Though the clinical appearance of vascular complications did not exist, the correlation between leptin and anthropometric variable clearly suggests a positive link between obesity (inflammation) and metabolic syndrome and it remains even before the appearance of clinical manifestation of the vascular complications. Thus a relation of leptin with the anthropometric variables along with the TNF- α in female diabetics without any micro and macro vascular complication has ensured the understanding of the interdependence of these markers in different levels of obesity.

Keywords: BMI, females, Leptin, TNF- α , vascular.

INTRODUCTION

Asian Indians have more visceral fat for any given BMI¹ and for any given body fat they have greater insulin resistance². Over the past several decades the global prevalence of overweight and obesity has increased substantially. These developments are also visible in developing countries like India. The new insight into obesity and health has come because of understanding of the adipocyte as an endocrine organ. As obesity sets in adipocytes enlarges leading to

dysregulation of the controlled mechanisms and accumulation of macrophages in the adipose tissue resulting in inflammation. Understanding of the biology of adipose tissue and, in particular, its secretory functions have intensely improved our knowledge of the pathophysiological link among the increase in the fat mass, namely obesity, insulin resistance and cardiovascular complications. The adipocytes or adipose tissue infiltrating macrophages are able to bring a low-grade

inflammation state that could play a central role in obesity and type 2 diabetes-related insulin resistance and cardiovascular complications. The protein leptin, known as a satiety hormone regulates appetite, energy homeostasis, glucose³ and lipid metabolism⁴⁻⁸. Morbid rates of leptin may provide a link between obesity, diabetes and increased cardiovascular risk. Obesity is characterized by hyperleptinemia⁹, while leptin levels decrease considerably during weight loss¹⁰. Plasma leptin levels is also known to correlate with body fat content⁴⁻⁶. The increase of fat cells in number and in size is coupled with an increase in leptin secretion⁹. TNF α (Tumor necrosis factor- α), a cytokine secreted by adipocytes, influences the energy balance and glucose homeostasis. TNF- α is also known to cause insulin resistance and plays a major role in the pathogenesis of obesity-linked type 2 diabetes^{11,12}. TNF- α levels are also increased in obesity and also exhibits gender difference¹³. Leptin and TNF- α levels are different in diabetic and/or obese subjects compared with non-diabetic, non-obese individuals and also been suggested as the link between obesity, diabetes and atherosclerosis¹⁴. Leptin is known to exhibit gender difference and is supported that its concentration is higher in females than males¹⁵. Although this might suggest a role for female sex hormones in the regulation of leptin secretion, women had higher levels than men even pre-pubertal¹⁶ and post-menopausal¹⁷ suggesting that testosterone in men may be the major determining factor for this gender based difference in leptin levels. This has made us to agree that females have a higher fat content than males. Hence we aimed in understanding the level of leptin and TNF- α in the graded obese diabetic female subjects free from micro and macrovascular disease.

MATERIALS AND METHODS

This study was a cross sectional study. 73 females were included in the study group. The study population was aged between 30-70 years. Persons with history of type 2 diabetics for at least one year, without any micro and macro vascular complication, on oral or on diet control, non-pregnant and free from usage of oral contraceptives were selected as the study group. Criteria for inclusion were –should have been recognized as diabetic for at least one year, should be on oral hypoglycemic drugs or diet control, free from diabetic neuropathy, nephropathy and retinopathy, free from any preexisting cardio vascular disease. Diabetic neuropathy was ruled out by filament test and Quantitative Sensory Testing (QST). Where a 10-gram monofilament is used to find the sensation

to the filament. Loss of sensation to this is considered as positive for diabetic neuropathy. QST are used to evaluate a sensory detection threshold or other sensory responses from supra-threshold stimulation. The common physical stimuli were (i) touch-pressure, (ii) vibration, and (iii) coolness, warmth, cold pain, and heat pain. Failure to identify these was considered positive for diabetic neuropathy.

Diabetic retinopathy: Ophthalmoscopy (funduscopy or fundoscopy) is used to diagnose diabetic retinopathy during a dilated eye exam. It is done as part of an eye examination or routine physical examination. It is crucial in determining the health of the retina and the vitreous humor. A positive case was not included in the study.

Diabetic neuropathy: Microalbuminuria: spot-check samples were used along with the albumin/creatinine ratio (ACR). Any subjects with ACR ≥ 3.5 mg/mmol (female) or ≥ 2.5 mg/mmol (male), was excluded.

Pre existing CVD: Cardiac stress test (or Cardiac diagnostic test) is used to rule of any pre-existing CVD. Here the stress response is induced by exercise. ie walking on a tread mill, any diagnose of CVD, will rule out the criteria as a subject for the study.

Written informed consent was obtained from the selected subjects. 5 ml of blood in the fasting state was drawn and the separated serum was stored at -30°C in the department of Biochemistry, Yenepoya Medical College and was used within 45 days for the estimation of Leptin and TNF- α with Ray biotech kit using ELISA instrument at the department of biochemistry, Yenepoya Medical College. Anthropometric variables like height and weight were measured as per the standard procedure. Measurements of the weight to the nearest 0.1 kg by a weighing machine and height to the nearest of 0.1 cm by an anthropometer rod were done. BMI was calculated as weight (in kg) divided by height in meter square.

Statistic Analysis

Data was analyzed in Microsoft Excel and SPSS software. Value of individual parameter was expressed as mean and bar chart was plotted. The relation between leptin and BMI was showed using lineartrend line.

RESULT

The study group was divided based on their BMI (Table 1). It was seen that major share of the female study population were having a BMI in the range of pre-obese and obese. When the study population was plotted based on their cytokine value it was seen that the level of TNF-

α and leptin increased as the BMI increased (figure 1). Further as the BMI was plotted based on the groups with the leptin, we saw more linearity in BMI range >30 (figure 2).when

similar plot was done with the TNF- α , the linearity was lost in the BMI range >30 (figure 3).

Table 1: Representation of the diabetic female study population based on their BMI

BMI (kg/m ²)	Females	
	N	%
<18.50 Underweight	-	-
18.50-22.99 Normal range	1	1.37
23.0-24.99 Over weight	7	9.59
25.0 -29.99 Pre-obese	23	31.51
≥ 30.00 Obese	42	57.54

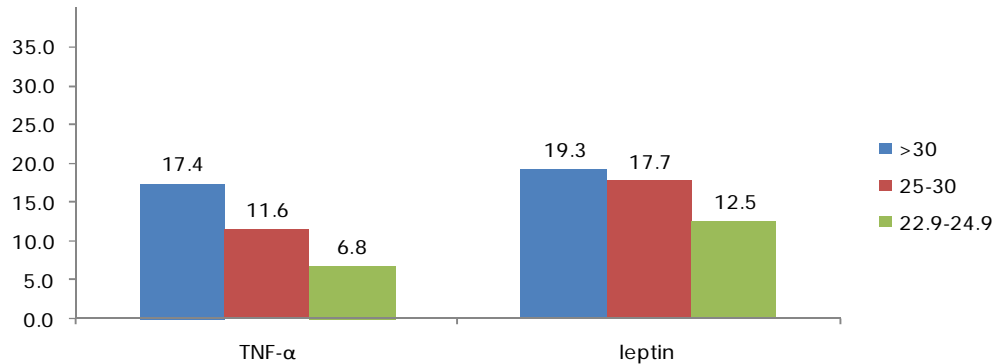


Fig. 1: Adipokine levels of different grades of BMI from the female diabetic study population

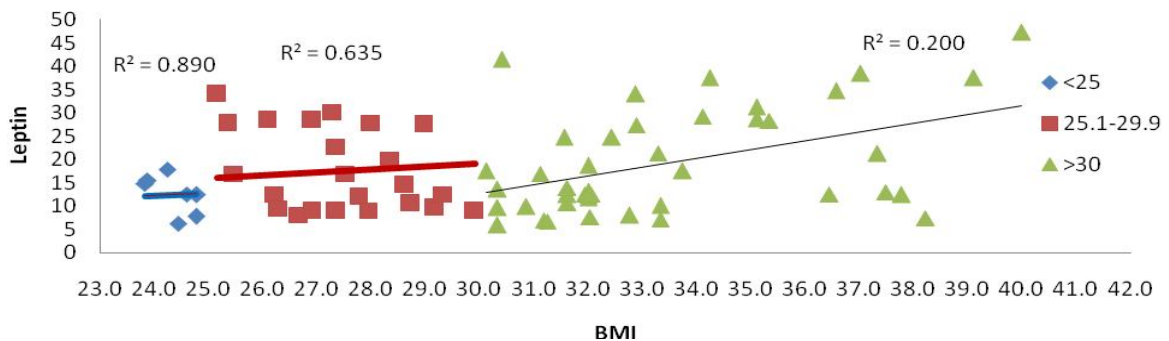


Fig. 2: Relationship of Leptin levels with different levels of BMI in female diabetic study population

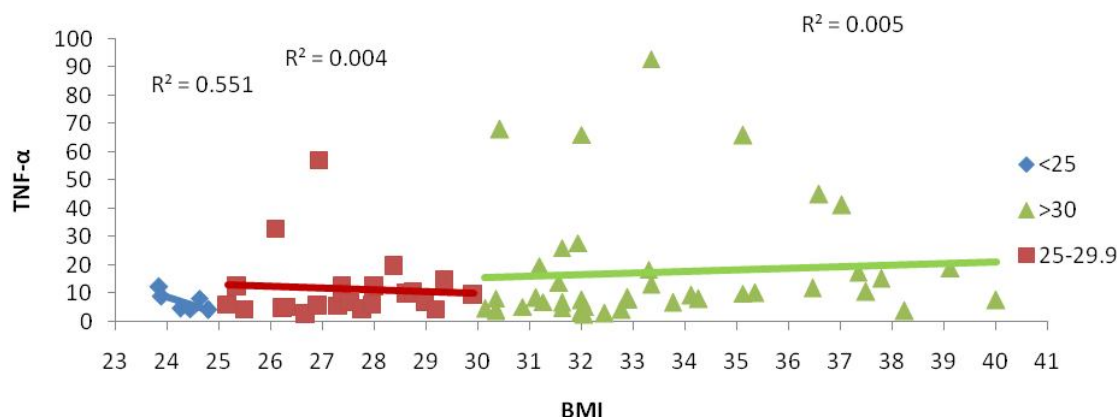


Fig. 3: Relationship of TNF- α levels with different levels of BMI in female diabetic study population

DISCUSSION

Sedentary life style results in obesity which is more common in diabetic people. Studies have shown that migrant Indians are more susceptible for insulin resistance, coronary heart disease than other ethnic group¹⁸⁻²⁰. Further, Asian Indians have more visceral fat for any given BMI¹ and for any given body fat they have greater insulin resistance².

World health organization data shows that, 1.6 billion adults worldwide were overweight in 2005 and 400 million were obese, it is estimated that by the year 2015 it will increase to 2.3 billion and 700 million, respectively. Our data showed that the percentage of diabetic in pre obese and obese were higher. This raise in number is mainly because of lack of fitness in diabetic population hence obesity sets in. A recent study has shown that 80% of individuals with type 2 diabetes are obese²¹. Obesity, particularly visceral obesity along with the physical inactivity is the more likely predictors of morbidity and mortality²². Research state that visceral obesity is believed to be a chronic low-grade inflammatory state linked with insulin resistance, type 2 diabetes, and cardiovascular disease^{23,24}. Similar results were also seen in a study by Centre for Disease Control .It is recorded that the proportion of obese adults has increased from 23 percent to 33.8 percent²⁵. An average increase of BMI by 5kg/m² is associated with an overall mortality by 30 percent²⁶.

Leptin and TNF- α levels were increased in our female diabetic study population in accordance with the BMI. This might be because major share of the study had no fitness activity, thus which increased their BMI level. Only prolonged

endurance exercise involving high energy expenditure induces a marked reduction in circulating serum leptin levels²⁷. Further it is shown that women have higher leptin concentrations than men for any given degree of fat mass²⁸. this might be a role for female sex hormones in the regulation of leptin secretion, hence women have higher levels than men in pre-pubertal¹⁶ and post menopausal¹⁷ signifying that testosterone in men may be the foremost determining factor for this sex based difference in leptin levels.

The raise in the inflammatory proteins in the circulation during obesity is mostly from the Adipose Tissue^{5,29} and studies have confirmed that weight loss reduces low-grade inflammation in Adipose Tissue and plasma³⁰.It was seen in our study that the inflammatory protein like TNF- α and the satiey protein like leptin was decreased in accordance with the decrease in their BMI.

Research has shown that exercise results in reduced fat mass which is associated with the lower leptin levels. Though leptin and BMI are related, we observed more linearity when leptin was plotted verses BMI, only when the BMI >30. The effects of TNF- α in accordance with the BMI were not that prominent in our study hence linearity is not observed. Research indicate that either in subcutaneous or in deep human adipose tissue depots, TNF- α is weakly expressed and this expression is not always modified in obesity³¹. Further the evidence from Mohamed et al shows that TNF- α production by subcutaneous abdominal adipose tissue was quantitatively negligible in lean and obese subjects³². This indicates that adipose tissue is not directly concerned in the increased

circulating TNF- α levels as observed in obese humans.

CONCLUSION

Though the adverse effects of obesity are not prominent, the biomarker of the study has provided an additional insight into the values in obesity. Hence these findings suggest that TNF- α shows a practical limitation in assessing the adverse effects in obesity. But leptin can be used to optimize the risk stratification in persons who have still not developed complication. Hence we conclude that leptin and TNF- α can be used to understand the interdependence in graded obesity.

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Declaration of interest

All authors declare that they have no conflicts of interest

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