Original Article

Insulin Resistance & Inflammatory Markers in Obesity

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ABSTRACT

Objective: To estimate the insulin resistance and its correlation to gender and inflammatory cytokines IL6 & TNFα in obese Pakistani patients.

Methodology: In one fifty obese patients of both sex, weight, height and Waist circumference was noted and BMI was calculated. Fasting blood was drawn to check for blood sugar and insulin levels. IL6 and TNFα were checked via ELISA. Insulin resistance was calculated by HOMA IR.

Results: Mean BMI in males was 29.566±4.4322 kg/m^{2}and 33.96± 5.5609 kg/m^2 in females. Sixty one (40.7%) had normal insulin resistance (1.704±0.647) and 89 (59.3%) had increased insulin resistance (9.678±10.143). Mean TNFα and Mean IL6 were not statistically different in both the groups (p value 0.891 and 0.386 respectively). Mean HOMA IR was 5.649±6.03 in males and 6.8005±9.76 in females. Mean IL6 was 46.166±117.67pg/ml and mean TNF was 22.492±89.99pg/ml. IL6 and TNF was more in males as compared to females (p-value 0.001). TNFα and IL6 significantly correlated to each other (p value 0.001).

Conclusion: Insulin resistance was increased in sixty percent of obese subjects but showed no difference in mean inflammatory cytokine levels from those with normal HOMA IR. TNF and IL6 levels were more in males. These markers significantly correlated to each other but not to obesity parameters.

KEY WORDS: Insulin resistance, Obesity, Inflammatory markers, IL6, TNFα.

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INTRODUCTION

Throughout the world obesity is increasing at a very fast rate and has very important health implications like type 2 diabetes mellitus, hypertension, coronary heart disease, sleep apnea, gall stones, osteoarthritis.[1]This epidemic is because of fast urbanization, resulting in decrease physical activity and energy expenditure so excess of fat deposition in the body resulting in obesity. According to Nanan DJ, there is an extreme increase in the rate of non-communicable diseases like diabetes and coronary heart disease in South Asia. In his study Pakistan ranked 8th worldwide in diabetes case load and he predicts it to rank 4th by 2025, if no desirable actions are taken.[2] Type 2 diabetes which ultimately develops in most of the overweight and obese patients is the result of an insulin resistant state.[3]

According to Keifer FW et al insulin resistant state in obesity is the result of chronic inflammatory state as demonstrated by the increase in plasma levels of C-reactive protein, inflammatory cytokines like TNFα, IL6, MCP-1 and IL8 and multifunctional protein like leptin and osteopontin.[4,5] Adipose tissue which was previously known to be a deposit of fat tissue is now considered as the largest endocrine gland secreting, proteo hormones, leptin,

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Table-I: Physical and metabolic parameters of subjects.

Parameters	*Mean±Standard Deviation*
Age (years)	40.127±11.7429
BMI (kg/m2)	32.560±5.609
BMI Males(kg/m2)	29.566±4.4322
BMI Females (kg/m2)	33.96±5.5609
Abdominal Circumference (cm)	102.059±10.7421
Abdominal Circumference Males (cm)	101.88±11.29
Abdominal Circumference Females (cm)	102.148±10.52
Fasting Insulin (uU/ml)	23.7316±29.832
Fasting Insulin Males(uU/ml)	21.046±17.84
Fasting Insulin Females (uU/ml)	25.0074±34.09
Mean HOMA IR	6.432±8.7425
HOMA IR Males	5.649±6.03
HOMA IR Females	6.8005±9.76
Mean IL6 (pg/ml)	46.166±117.67
Mean TNFα (pg/ml)	22.492±89.99

adiponectin and visfatin.[6] In addition it also secretes inflammatory adipokines TNFα, IL6, IL1 that not only cause local steatonecrosis, but are distributed throughout the body by a vascular system resulting in a chronic inflammatory state.[7]

The aim of this study was to estimate the insulin resistant state in Pakistani obese patients and the level of inflammatory cytokines in them. This will help us to know the status of insulin resistance in Asian Pakistani subjects and also in developing more research in the field of obesity and ultimately in the methods for reducing these inflammatory cytokines and thus the insulin resistant state.

METHODOLOGY

Approval from institutional review board was taken for this study. For data collection banners and brochures for research participation were distributed around the vicinity of Civil Hospital Karachi. Only obese patients were asked to participate in the study and come in the morning at 9 a.m. after 12 hours fasting. After informed consent demographic data was filled in preformed Performa. BMI was calculated by using the formula weight/height in m^2 and waist circumference was measured in centimeters. Eight cc of blood was drawn after aseptic measures, 3 cc of blood was taken for fasting blood sugar (hexokinase method) and fasting insulin levels (chemiluminiscence method) and 5 cc of blood was centrifuged and serum was saved at -4°C for inflammatory markers (IL6 and TNF) which were checked with ELISA method (Invitrogen Immunoassay kit). Insulin resistance was calculated by the formula of HOMA IR (fasting blood sugar x fasting insulin / constant 22.5), and values above 2.5 was considered as insulin resistance.

Table-II: Parameters in subjects with normal and increased insulin resistance.

Parameters	*Normal HOMA IR (n=61)*	*Increased HOMA IR (n= 89)*	*p-value*
Age (years)	39.80± 12.39	40.34± 11.33	0.781
BMI (kg/m2)	31.08± 5.53	33.57± 5.45	0.007
Abdominal circumference (cm)	99.83± 10.40	103.58± 10.75	0.036
TNFα(pg/ml)	21.26± 87.90	23.33± 91.88	0.891
IL6(pg/ml)	56.26± 122.96	39.24± 114.09	0.386

Statistical Analysis: Data was analyzed by SPSS 19. Mean and standard deviation were calculated for all parameters. For comparing the means student 't test' was used and for correlation among the risk factors Pearson's correlation was used.

RESULTS

Table-I describes the physical and metabolic parameters of the study population. In 150 obese patients, 61 (40.7%) had normal insulin resistance (1.704±0.647) and 89 (59.3%) had increased insulin resistance (9.678±10.143). Mean parameters in both the groups are shown in Table-II. Mean TNFα and Mean IL6 were not stastically different in both the groups (p value 0.891 and 0.386 respectively). On comparing the means on gender basis, with student t test there was a significant difference in the mean levels of IL6 and TNFα in males and females. IL6 was 90.625±186.73pg/ml in males and 25.244±53.099pg/ml in females with a significant p-value 0.001.Similarly TNFα was 50.34±152.86pg/ml in males as compared to 9.38±23.06pg/ml in females with a significant p-value of 0.001. Fasting insulin and HOMA IR were not statistically different in the two groups as shown in Table-III.

A Pearson's correlation coefficient was computed to assess the relationship between TNFα and IL6 to age, BMI, abdominal circumference, fasting insulin level and insulin resistance. There was a negative correlation of inflammatory cytokines to BMI, abdominal circumference and fasting insulin and a positive correlation to age and insulin resistance but the correlation was not statistically significant. Although TNF and IL6 significantly correlated to each other (p value 0.001). Table-IV

Table-III: Metabolic parameters with gender difference.

Parameters	*Male (n =48)*	*Female (n=102)*	*p-value*
Fasting Insulin (Uu/ml)	21.046±17.84	25.0074±34.09	0.45
HOMA IR	5.649±6.03	6.8005±9.76	0.454
IL6 (pg/ml)	90.6258±186.73	25.244±53.099	0.001
TNFα(pg/ml)	50.34±152.86	9.38±23.06	0.001

Table-IV: Pearson's correlation between different parameters and inflammatory cytokines.

	TNFα		*IL6*	
	r value	*p-value*	*r value*	*p-value*
Age	0.137	0.095	0.107	0.194
BMI	-0.113	0.169	-0.132	0.108
Abdominal Circumference	-0.020	0.808	-0.087	0.291
HOMA IR	0.022	0.729	0.012	0.882
Fasting Insulin	-0.023	0.785	-0.012	0.886
IL6	0.625	0.001		
TNFα			0.625	0.001

DISCUSSION

This study was conducted on 150 obese subjects. Mean fasting insulin and mean insulin resistance calculated by HOMA IR was increased but there was no significant difference with sex. Mean TNFα was increased in all obese subjects and showed a significant difference in both sex. We found TNFα to be significantly high in males as compared to females with p-value of 0.001. On Pearson's correlation TNFα showed no significant relation to BMI, abdominal circumference and insulin resistance. Burtin et al detected a correlation between TNFα and BMI with indexes of intra-abdominal fat tissue and not with glycemia or the total amount of fatty mass in the body.[8] Similarly M. Maachi et al found a significant correlation between CRP and circulating levels of IL6 and TNFα which they related to increased fat amount. They also measured adipocytokines content directly in adipose tissue and reported a positive association between the adipose tissue content of IL6, TNFα and leptin and the level of circulating inflammation proteins in non-diabetic obese women.[9]

Comparing the mean TNF and mean IL6 between patient groups with normal and increased insulin resistance, there was no significant difference of these cytokines in both the groups. A study in Bangladeshi patients for the association of TNF and IL6 in three groups of patients, normal glucose tolerant, impaired fasting group and impaired fasting and impaired glucose tolerant group found no difference in the serum levels of both these cytokines[10], also in Korean population, subjects with normal and impaired glucose tolerance there was no difference in the levels of these cytokines and they were not correlated with components of metabolic syndrome.[11] Similar to our results, Sujaita et al found that serum TNFα was associated neither with insulin, HOMA IR nor with obesity parameter. They also found significant correlation of TNFα levels to IL6in all the study population, but in contrast to our study they found no gender based difference. Serum IL6 were also not associated with HOMA IR and obesity parameter and no gender difference was found, in contrast in our study male patients had significantly high levels of IL6.[12]Simona et al studied the TNFα in the development of insulin resistance and other disorders in metabolic syndrome, they made two groups of people, one with normal lipids and other with increased lipids. In hyperlipidemic women, the mean serum TNFα was no different from the control group, in hyperlipidemic men there was a decrease in TNFα as compared to those with normal lipids. Multiple regression analysis demonstrated the important role of TNFα in the regulation of both the insulin resistance and in the secretion of insulin in women and in men BMI and HDL cholesterol played a dominant role, while the role of TNFα seemed to be minimal.[13] In contrast to our study Corica et al found more increased TNFα concentration in women than in men and this was found both in control group and in obese persons.[14] In Canadian population with a higher prevalence of type 2 diabetes no difference between men and women regarding the serum TNFα concentration were found. Correlations between TNFα and fasting plasma concentration of insulin and triacylglycerol, HOMA index, waist and blood pressure were more distinct in women than in men.[15]

IL6 is a major pro inflammatory cytokine and is produced in a variety of tissues including activated leucocytes, adipocytes and endothelial cells. IL6 is primarily involved in hepatic bio synthesis of CRP which is involved in the development of obesity, insulin resistance and ultimately diabetes. Elevated levels of CRP and IL6 predict the development of type 2 diabetes.[16]Circulating IL6 are elevated years before the onset of type 2 diabetes but its involvement in precipitating diabetes is still debatable. There is a role of IL6 in causing impaired insulin signaling in adipocytes in vitro but not demonstrated in vivo. Long term IL6 stimulation does not cause insulin resistance in skeletal muscle but this needs further investigation to confirm it, so Kristiansen OP, concluded that IL6 may contribute to but is probably not sufficient for development of type 1 and 2 diabetes.[17] In Pima Indians, fasting plasma IL6 were positively related to adiposity and negatively related to the insulin action.[18] According to M.Fasshner et al IL6 concentration are high and adiponectin level is low in patients who are at high risk of developing diabetes and insulin resistant state.[19] IL6 was among the first cytokine which acts as a predictor or pathogenic marker of insulin

resistance and cardiovascular disease. Visceral fat contains three times more IL6 as compared to subcutaneous adipose tissue and thus results in more insulin resistance.[20] In our study also patients of both sex had mean abdominal circumference 100 cm which is a marker of visceral fat. They also had increased IL6 levels which were more profound in males as compared to females.

Inflammatory markers IL6 and TNFα work locally at adipose tissue or systemically have been studied. Philip A Karen et al measured TNFα and IL6 gene expression at several levels from adipose tissue of lean and obese subjects and related it to be reliable measure of insulin sensitivity. Both IL6 and TNFα were expressed and secreted by human adipose tissue, although IL6 levels were much higher in both adipose tissue and plasma. They found that obesity related insulin resistance involved, increase TNFα secretion from adipose tissue and increase plasma IL6 levels.[21]

Limitation of the study: In our study there are some limitations as we have measured only plasma level of TNFα and IL6 and not the adipose tissue expression which showed no significant correlation to insulin resistance. Also the total study population was obese who had increased TNFα and IL6 levels which significantly correlated to each other but not the obesity parameters. Moreover the plasma levels of inflammatory markers may not reflect the cytokine biological effects at the tissue level.

CONCLUSION

Insulin resistance is increased in obesity with no significant gender difference. Plasma levels of inflammatory cytokines IL6 and TNFα are increased in males as compared to females. These inflammatory cytokines significantly correlate to each other but not to the obesity parameters. Also these cytokines show no difference in subjects with normal and increased insulin resistance. More studies particularly measuring adipose tissues as well as plasma levels of IL6 and TNFα are needed in our population to reach to a better understanding of it and relation to insulin resistance as we have seen in literature, their diverse relation in different population groups. Such studies will ultimately help us in developing therapeutic targets for prevention as well as treatment of insulin resistant states.

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Contributions of Co-Authors:

Dr. Sina Aziz is my supervisor for my PhD thesis, Prof. Niaz A Shaikh and Prof. Tahir Hussain being co supervisors for my thesis, have contributed in planning out the study, being subject specialist have guided me in statistical results interpretation and discussion.