

CORRELATION OF ADIPOCYTOKINES AND OXIDATIVE STRESS IN TYPE 2 DIABETES MELLITUS AND THEIR ASSOCIATION WITH DEGREE OF INSULIN RESISTANCE.

Piyali Das¹, Subhradev Biswas², Subir Kumar Bandyopadhyay³, Madhumita Manna⁴, Gorachand Bhattacharya⁵.

1- Associate Professor, Physiology, Bankura Sammilani Medical College and Hospital, Bankura-722101. India

2- Assistant Professor, ENT, IPGMER, Kolkata-700007. India.

3- Professor, Medicine, KPC Medical College and Hospital, Kolkata -700032. India.

4- Associate Professor, zoology, Barasat Govt. College, Kolkata 700124. India.

5- Professor, Biochemistry, MGM Medical College and LSK Hospital, Kishanganj 855107. India.

Abstract: Background and Objectives: Adipocytokines and Oxidative Stress are often studied in relation to various morbidities but whether these two are interrelated in causing insulin resistance in type 2 diabetes mellitus, is controversial. This study explored their association in type 2 diabetes mellitus and correlation with insulin resistance. **Methods:** 69 male diabetics and 30 age-sex matched non-diabetic control were studied. Fasting blood glucose, serum insulin, leptin, adiponectin and plasma MDA (Malonaldehyde, marker for Oxidative Stress) were estimated and compared between two groups by unpaired Student's t – test. Insulin resistance score was calculated by HOMA-IR (Homeostatic Model Assessment- Insulin Resistance) method. Correlation among the parameters was assessed by Pearson's coefficient of correlation (r-value).

Results: Diabetics had higher levels of leptin, MDA, leptin- adiponectin ratio but lower adiponectin than non-diabetics. Correlation of MDA was positive with Leptin but inverse with adiponectin. Insulin resistance score was correlated positively with leptin, leptin- adiponectin ratio, MDA but negatively with adiponectin.

Interpretation: Study concludes that, Oxidative Stress may lead to over production of leptin and suppress that of adiponectin and thereby result in increased leptin- adiponectin ratio which is a necessary background for insulin resistance. This is how, oxidative stress may have a positive correlation with insulin resistance in type 2 diabetes mellitus.

Key words: oxidative stress, adipocytokines, insulin resistance.

corresponding author: Dr. Piyali Das, GolePark Cooperative Housing, flat no. 2-3-A1, 49 B Gobindopur Road, Kolkata-700045, phone: 9163440154, email id: dr.piyali@yahoo.com

Introduction:

Oxidative stress results from an imbalanced condition in which the generation of free radicals is greater than the capacity of the antioxidant defence system to detoxify them¹. When oxidative stress is chronic, it is thought to result in damage to DNA, lipids, proteins, and other molecules, which may contribute to the development and progression of chronic disease, including cardiovascular disease and cancer. Chronic oxidative stress is particularly dangerous for β -cells because pancreatic islets are among those tissues that have the lowest levels of antioxidant enzyme expression, and β -cells have high oxidative energy requirements². Traditionally, fat tissue is considered to be passive energy storage depot in the body. However, recent studies have shown that fat tissue exerts important endocrine functions, and their secretions are called adipocytokines. Adiponectin, leptin and resistin are some important molecules belonging to this class. Their role has been studied in relation to

deranged energy homeostasis, leukocyte migration, and polycystic ovary³. But adequate evidence is still lacking regarding the influence of above molecules in the pathogenesis of insulin resistance and often the findings of different studies in this regard are contradictory. When some of the studies showed positive correlation between leptin and insulin resistance⁴, others observed a reduced level of leptin in type 2 diabetes mellitus⁵. Moreover, whether oxidative injury has any implication in deviation of adipocytokine release has been inadequately explored and proved to be controversial in different studies. With this background, in the present study, attempt was made to explore whether oxidative stress has any association with production of adipocytokines like leptin, adiponectin and whether they at all have any relation with degree of insulin resistance in type 2 diabetes mellitus.

Material and Methods:

It was a hospital based cross sectional study done at Calcutta National Medical College and Hospital. Total 69 male patients with type 2 diabetes mellitus and 30 age sex matched non diabetic otherwise healthy control were included in the study. Subjects were randomly selected from patients attending the General Medicine and Diabetes OPD of Calcutta National Medical College and Hospital, and controls were pooled from community volunteers from surrounding neighbourhood. Detailed history was taken in a structured proforma from each subject and informed consent was taken. Entire study was conducted with due permission from Institutional Ethics committee.

Inclusion criteria for the subjects:

Male patients recently diagnosed to have type 2 diabetes mellitus (disease duration : less than 5 years), with 30- 65 years of age, given informed consent were included in the study. Criteria for diagnosing diabetes mellitus was as follows: (6)

1. Patients having cardinal symptoms of diabetes plus random blood glucose concentration ≥ 11.1 mmol/L (200 mg/dL) or
2. Fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dL) or
3. Two-hour plasma glucose ≥ 11.1 mmol/L (200 mg/dL) during an oral glucose tolerance test.

Exclusion criteria:

subjects with any other acute or chronic illness like any acute inflammatory state, tuberculosis, malignancy, hepatitis due to any cause, hormonal derangements other than diabetes like Cushing's syndrome, hypothyroidism etc, any acute or chronic complication related to diabetes, ongoing insulin therapy, oral hypoglycemics or steroid therapy were excluded.

study design:

after taking informed consent from each selected subject, they were assessed for physical parameters like Body Mass Index (BMI), abdominal circumference, height weight etc. Thereafter, they were asked to come after 12 hours of fasting for giving blood samples to the Department of Biochemistry. 8 ml of venous

blood was collected from the ante cubital vein of each subject and was separated in a fluoride-oxalate bulb (3ml) and a clot vial (5ml) for blood glucose, plasma malonaldehyde (MDA) and serum insulin, leptin, adiponectin, estimation, respectively.

From the findings degree of insulin resistance was calculated by HOMA-IR method (Homeostatic Model Assessment-Insulin Resistance method). HOMA-IR scores were derived by multiplying fasting blood glucose (in millimoles/liter) and fasting serum insulin (in microunits/milliliter) divided by 22.5⁷.

Laboratory techniques availed for estimating chemical parameters were as follows:

1. **Blood Glucose estimation:** This was done by Glucose Oxidase/ Peroxidase method using ECO GLUCO KIT from Crest Biosystems, Goa, India. In this method, glucose was oxidised to glucuronic acid and hydrogen peroxide in presence of glucose oxidase. Hydrogen peroxide further reacting with phenol and 4-aminoantipyrine by the catalytic action of peroxidase, formed a red coloured quinoneimine dye complex. Intensity of the colour which was proportional to the amount of glucose present in the sample was measured at 505 nm wave length spectrophotometrically.
2. **serum insulin estimation:** serum insulin was estimated using human ELISA kit from Monobind Inc., Lake Forest, USA following standard immunoenzymometric assay (type 3) technique.
3. **plasma MDA estimation:** This was done by Thiobarbituric acid method (modified method of Satoh K, 1978) (8). In this method, MDA in the sample reacted with thiobarbituric acid (TBA reagent) in acidic media at temperature 97°C and pH 2-3, to give a pink coloured substance which was extracted with butanol and the absorbance was measured at 535 nm spectrophotometrically.
4. **Serum leptin and adiponectin estimation:** These were done by ELISA method using

Human Leptin ELISA Kit and Human Adiponectin ELISA Kit from Ray Biotech, USA. Using prescribed techniques.

Statistical tools applied:

Data was analyzed in Microsoft Excel and SPSS (18) software. Value of individual parameter was expressed as mean and one standard deviation. Association among the parameters was tested by calculating Pearson's co-efficient of correlation (r) and significance of difference between the groups

was tested by unpaired Student's t- test. Every where $P < 0.05$ was considered to be significant.

Results:

Total 69 male patients of type 2 diabetes mellitus (<5 years disease duration) and 30 age sex matched non diabetic healthy controls were included. Mean Values of different parameters with one standard deviation found in two groups along with obtained p- values from unpaired Student's t test are summarized in table 1:

Table 1: values of different parameters in diabetic and non diabetic subjects

Parameters under study	Diabetic cases	Non - diabetic control
Age (years)	51.54 ± 9.75	50 ± 10.2
Total body weight (Kg)	65.79 ± 10.22	55.15 ± 0.73*
BMI (Weight in Kg/Height in Meter ²)	27.34 ± 5.24	25.88 ± 0.88*
Abdominal circumference (cm)	92.5 ± 11.24	89.28 ± 4.57*
Fasting Blood Glucose (mg/dL)	168.97 ± 77.21	83.33 ± 7.9*
HOMA -IR Score	2.64 ± 1.96	1.25 ± 0.48*
Leptin (ng/mL)	11.09 ± 10.9	10.27 ± 3.83*
Adiponectin (µg/mL)	6.04 ± 2.73	8.8 ± 3.96*
MDA (nmol/ml)	1.92 ± 1.24	1.17 ± 0.43*

*- $P < 0.05$ (statistically significant)

Between two adipocytokines, leptin was having positive but adiponectin inverse correlation with insulin resistance score. Oxidative stress marker, MDA showed positive correlations with insulin resistance score, leptin and leptin adiponectin ratio, though it was found to have an inverse correlation with adiponectin. Values of co- efficient of correlations among different parameters with their level of significance at specific degree of freedom, are summarized in table 2.

Table 2: Values of co- efficient of correlations among different parameters

Parameters for correlation	Co-efficient for correlation (r- value)
Leptin and HOMA- IR score	0.51, $P < 0.05$ *
Adiponectin and HOMA-IR score	-0.34, $P < 0.05$ *
leptin-adiponectin ratio and HOMA- IR score	0.64, $P < 0.05$ *
MDA and serum leptin	0.74, $P < 0.05$ *
MDA and adiponectin	-0.44, $P < 0.05$ *
MDA and leptin- adiponectin ratio	0.45, $P < 0.05$ *
MDA and HOMA-IR score	0.50, $P < 0.05$ *

*- statistically significant

Discussion:

Increased adiposity and type 2 diabetes mellitus are two major health problems running globally in recent times. It has been addressed in many previous studies and proposed to be interrelated⁶. But, what actually leads to development of insulin resistance, the major metabolic derangement in type 2 diabetes mellitus, is grossly controversial. In the present study, we found a significant positive correlation of MDA, the detrimental end product of lipid peroxidation with insulin resistance, which is corroborative with the findings of some other studies^{9,10}. Regarding, pathogenesis of insulin resistance in oxidative stress, different possible mechanisms are put forward. It has been suggested that, there may occur excessive free radical production and oxidative stress in the accumulated fat in type 2 diabetes mellitus which may further lead to dysregulated production of adipocytokines such as resistin, leptin and adiponectin as well as inflammatory mediators like plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor α (TNF- α), IL-6 etc. Deranged activity of the adipocytokines as well as the inflammatory mediators may ultimately impair glucose uptake in the muscle and the fat and decreases insulin secretion from pancreatic β cells. This is how, oxidative stress may contribute to insulin resistance by mediation of adipocytokines.¹¹ Being in agreement with such mechanism, we also found a positive correlation of oxidative stress with serum leptin and leptin adiponectin ratio and negative correlation with adiponectin. Our finding is also supported by Nakanisi et al.¹² Though their marker for oxidative stress was different from us (urinary isoprostane), they also concluded that leptin, adiponectin both are associated with oxidative stress but leptin modulates it positively and adiponectin inversely. Thus, leptin is promotive and adiponectin is protective for oxidative stress in diabetes mellitus. We also found a positive correlation of leptin and negative correlation of adiponectin with insulin resistance. Our finding is also supported by some other workers^{13,14}. Corroborative with our study finding, recently some studies have shown that leptin prevents insulin secretion and antagonises

insulin effects on liver and adipose tissue. Similarly, Reduction in adiponectin gene expression in adipose tissue has been found to be associated with obesity and insulin resistance in some animal models.^{15,16} Remarkably, we found a significant positive correlation of leptin adiponectin ratio with insulin resistance which is in agreement with some previous workers.^{17,18} This may suggest that, it is actually the interplay between these two adipocytokines that regulate insulin sensitivity. Correlation coefficient was higher in case of leptin –adiponectin ratio than leptin or adiponectin alone with insulin resistance score. This may suggest that, leptin adiponectin ratio can be a better predictor of insulin resistance than leptin or adiponectin alone.

Conclusion:

Study concludes that, leptin may promote but adiponectin prevent generation of insulin resistance. Leptin –adiponectin ratio may have a direct association with insulin resistance. Oxidative Stress may lead to over production of leptin and suppress that of adiponectin and thereby result in increased leptin- adiponectin ratio which is contributory to insulin resistance. This is how, Oxidative Stress may have a positive correlation with insulin resistance in type 2 diabetes mellitus.

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