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ASSOCIATION OF ADIPONECTIN AND INTERLEUKIN-6 WITH INSULIN RESISTANCE IN PRE-DIABETES AND EARLY TYPE 2 DIABETES MELLITUS

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ABSTRACT

Aim: The objective of this study was to identify the association of Adiponectin and Interleukin-6 with insulin resistance in subjects with pre-diabetes and compare to it with the levels in newly diagnosed type 2 diabetes and healthy subjects. **Material & Methods:** It is a cross-sectional descriptive type of study. Total 900 subjects were distributed into three groups (300 pre-diabetic subjects, 300 type 2 diabetic subjects and 300 healthy subjects) as per ADA criteria. The biochemical parameters as FPG, 2-hr glucose (after 75 gm oral glucose intake), HbA1c and fasting insulin were analyzed. HOMA-IR was used to calculate insulin resistance mathematically. Anthropometric measurements were done. The quantitative sandwich enzyme immunoassay technique was used to analyse serum adiponectin and IL-6 by commercially available Human ELISA kits. **Results:** Adiponectin concentration was significantly reduced in patients with type 2 diabetes mellitus and pre-diabetes (3.98 ± 0.25; $5.47\pm0.31\mu g/mL$) in comparison to the control group ($6.9\pm0.30\mu g/mL$), p value<0.001.Similarly significant increase in case of IL-6 was observed in patients with type 2 diabetes mellitus and pre-diabetes (3.06 ± 0.35; 2.03 ± 0.13pg/mL) in comparison to healthy control group (1.74 ± 0.15pg/mL) p value<0.001. Adiponectin showed an inverse correlation and IL-6 showed a positive correlation with HOMA-IR. **Conclusion:** The findings of this study illustrates the significance of the impact of the adiponectin ($r= -0.7$), p value<0.01 and IL-6 ($r= 0.5$), p value<0.01 in the development of insulin resistance (HOMA-IR) in pre-diabetic patient. **Clinical Significance:** Screening for these biomarkers at an early stage might prove fruitful in the early detection of the development of insulin resistance and type 2 diabetes mellitus and positively delay the onset of this non communicable disease**.** Further, this will provide an opportunity for the research and invention of drugs to block this inflammatory pathway and in turn the development of type 2 diabetes.

KEYWORDS : Inflammation, Pre-diabetes, Adiponectin,, IL-6, Insulin resistance, ADA.

INTRODUCTION:

The number of diabetic patients has greatly increased in the past few decades in the world including Asia. Diabetes is a growing challenge in India with estimated 8.7% diabetic population in the age group of 20 and 70 years. (1) Obesity and overweight are the most important risk factors responsible for diabetes. Long term diabetes has been associated with complications as retinopathy, neuropathy and nephropathy. Diabetes also increases the risk of life-threatening diseases including cardiovascular disease and cancer. (2) The prevention of diabetes is thus one of the priority issues.

Pre-diabetes is the progeny of diabetes. It is also termed as Impaired Glucose Regulation (IGR) which consist of Impaired Fasting Glucose and/or Impaired Glucose Tolerance (IFG and/or IGT). It is a reversible condition that increases the risk for diabetes which is associated with insulin resistance or decline in insulin sensitivity. (3,4)

Inflammation plays an important role in development of several metabolic disorders as type 2 diabetes mellitus, obesity cardiovascular disease etc. Many previous researches have mentioned that the progression and severity of metabolic disorder are related with increased level of inflammatory parameters. (5,6) It may be hypothesized that in healthy population, change in pro-inflammatory markers is counterbalanced by altered anti-inflammatory markers. If there is deviation from this profile it will lead to systemic disorder. In this context the two important regulators of metabolic disorders are adiponectin and interleukin-6.

Adiponectin is a 30kDa protein which is mainly secreted by adipocytes. It has anti-inflammatory, antidiabetic and anti-atherogenic properties. (7,8) The major, hypothesized role of adiponectin against impairment of glucose metabolism is its effect on insulin sensitivity.(9) The other member is pro-inflammatory cytokine, IL-6 which is known to be elevated in diabetes.(10,11) Its irregular production and long-term exposure lead to the development of inflammation which induces insulin resistance and overt T2DM. It causes insulin resistance by impairing the phosphorylation of insulin receptor and insulin receptor substrate-1 (IRS-1) by inducing the expression of SOCS-3, a potential inhibitor of insulin signaling.(12)

In this study we have highlighted the role of balance of inflammatory regulators (adiponectin and IL-6) in progression of type 2 diabetes in subjects of Bhopal region. We also studied associations of adiponectin and IL-6 level with insulin resistance in all study subjects divided in three groups.

MATERIALS & METHODS:

This cross sectional study was carried out in the Department of Biochemistry, People's College of Medical Sciences & Research Centre and Centre for Scientific Research & Development (CSRD), People's University, Bhopal during July 2017 to July 2019. The blood sample was collected from the outpatient department (OPD) and inpatient department (IPD) of People's Hospital. The study was designed taking 300 human subjects in each arm, in which, 300 age matched healthy subjects were considered as control group, 300 as pre-diabetic subjects and 300 as type 2 diabetic subjects. Ethical principles such as respect for the persons, beneficence and justice were adhered. Ethical clearance was obtained from the research committee and the Institutional Review Board of People's University. Written informed consent was taken from all the subjects. The evaluation involved a full medical history and anthropometric measurements (weight, height, BMI, waist and hip circumferences, waist hip ratio) and arterial blood pressure.

Inclusion criteria:

- Patients diagnosed with pre-diabetes according to the ADA (American Diabetes of Association) values of FPG (100-125mg/dl), 2 hr glucose (140-199mg/dl) and HbA1c (5.7-6.4%) were taken into consideration for selection of patient.
- Patients newly diagnosed with type 2 diabetes mellitus as per ADA criteria (FBG ≥126 mg/dl , 2-hr glucose ≥ 200 mg/dl, HbA1c ≥ 6.5%) and
- Patients aged between 30-60 years were taken up into the study.

Exclusion criteria:

- Patients with diagnosis of any other disease other than pre-diabetes & type 2 diabetes mellitus (based on their medical history and physical examination) were excluded.
- Patients on antidiabetic drugs, insulin and corticosteroids were excluded from the study.
- Patients below 30 years and above 60 years were excluded from the study.

All the biochemical parameters as FBG, 2-hr Glucose and HbA1c were estimated by Standard Kit method by using Cobas c311 fully automated analyzer (Roche diagnostics). Serum Insulin was assayed on Cobas c411 fully automated immunoassay analyzer (Roche diagnostics) by using Cobas kits. Adiponectin and interleukin-6 were estimated by using Human Elisa kits. Serum Insulin resistance was estimated by the **Homeostasis model assessment (HOMA-IR)** and calculated as Fasting Insulin (microU/L) x Fasting glucose (mg/dl)/405.

Calculation and Statistical analysis:

The data was entered into Microsoft Excel software package. The entered data were transferred to SPSS 24.0 software (SPSS Inc., Chicago, Illinois, USA) package for analysis. ANOVA test was applied to proportions to test the level of significance. Pearson's correlation was used to study the strength of association. The level of significance was fixed at 0.05. Confidence interval (CI) was set at 95%.

RESULTS:

In our study we have compared anthropometric and biochemical parameters in pre-diabetic, type 2 diabetic and healthy control groups. Anthropometric parameters (BMI, waist circumference, waist to hip ratio) as well as systolic and diastolic blood pressure are statistically significantly differed in pre-diabetic and diabetic groups. (Table 1)

Table 1: Comparison between the three groups selected for the study:

Parameter	Control	Pre-diabetes	T2DM	p-value
WC (cm)	74.87±7.4	79.95±5.7	84.2±5.4	<0.001
WHR	0.82±0.09	0.87±0.	0.98±0.	<0.001
BMI(Kg/m2)	22.22±2.79	24.89±2.4	29.25±3.06	<0.001
SBP(mmHg)	120±8.03	131±6.4	145.5±15.45	<0.001
DBP(mmHg)	76.28±6.9	83.04±7.5	96.33±9.4	<0.001
FBG(mg/dl)	83.62±7.7	114.58±7.3	149.78±30.27	<0.001
2-hr Glucose	120.72±10.05	163.2±14.77	255.58±40.07	<0.001
HbA1c(%)	4.5±0.63	6.10±0.25	8.85±1.39	<0.001
Fasting Insulin (μU/mL)	6.09±2.13	7.19±3.	29.006±5.06	<0.001
HOMA-IR	1.48±0.80	2.04±0.98	10.67±2.7	<0.001
Adiponectin (ng/mL)	6.9±0.30	5.47±0.31	3.98±0.25	<0.001
Interleukin-6 (pg/ml)	1.74±0.15	2.03±0.13	3.06±0.35	<0.001

*** p value significant < 0.001**

The mean adiponectin biomarker value is least for diabetes 3.98 ± 0.25 as compared to pre-diabetes 5.47 ± 0.31 and healthy controls 6.9 ± 0.30. The difference is statistically significant. There is a statistically significant decrease in the IL-6 mean value among diabetes, pre-diabetes and controls which are 3.06 ± 0.35, 2.03 ± 0.13 and 1.74 ± 0.15 respectively.

Correlations among variables in control and pre-diabetic group:

HOMA-IR is strongly negatively correlated with adiponectin (r = - 0.7; p value < 0.01). HOMA-IR is moderately positively correlated with interleukin-6 (r = 0.54; p value < 0.01) which is statistically significant.

Correlations among variables in control and diabetic group:

HOMA-IR is strongly negatively correlated with adiponectin (r = - 0.9; p value < 0.01). HOMA-IR is strongly positively correlated with interleukin-6 (r = 0.85; p value <0.01 which is statistically significant.

DISCUSSION:

Obesity is a condition in which the number and size of adipocytes increases with further increase of the total fat mass. (13) Visceral fat compartments are largely responsible for the secretion of adiponectin, when compared to subcutaneous deposits. This is of particular interest because of the close association between visceral obesity and metabolic disease; however, in vitro and in vivo studies performed in humans have shown that adipocytes with exhausted lipid storage, filled with fatty acids, and located in the intra-abdominal region can inhibit transcription of the adiponectin gene by secreting inflammatory and angiogenic factors, reducing its plasma levels. (14)

This condition may also play an important role in the development of a chronic low-grade pro-inflammatory state associated with adipose tissue dysfunction and diabetes. Enlarged adipocytes leads to an imbalance between pro- and anti-inflammatory adipokines.(15,16) The secretions of pro-inflammatory cytokines IL-6, IL-8 have been positively correlated with adipocyte size.(17) Significant correlation with insulin resistance makes adiponectin a powerful prognostic marker for diabetic risk in patients who do not yet manifest T2DM.

Interleukin-6 (IL-6), another biomarker of importance in our study, is a proinflammatory cytokine that causes the development of insulin resistance. This is done by the generation of inflammation by controlling differentiation, migration, proliferation and cell apoptosis. In vitro studies have shown that IL-6 treatment downregulates adiponectin mRNA suggesting a negative role of IL-6 in adiponectin regulation.(18) Experimental studies and cross-sectional analyses have shown that circulating IL-6 is associated with hyperglycemia and insulin resistance. It has also been shown that circulating IL-6 increases with the degree of insulin resistance.(19,20). In our study also we have got a positive coorelation of IL-6 with HOMA-IR and negative coorelation of adiponectin with HOMA-IR in both pre-diabetic and type 2 diabetic condition.

CONCLUSION:

Adiponectin is a key adipokine, which participates in many metabolic activities and its levels are reduced in obese people. This increases the risk of developing insulin resistance and type 2 diabetes as well as cardiovascular disease.

From our data it can be summarized that there is a significant change in both adiponectin and IL-6 levels in healthy, prediabetic, and diabetic population of Bhopal region. There

is a significant but gradual change during the progression of healthy towards diabetic population via pre-diabetic condition.

Clinical Significance: Screening for these biomarkers at an early stage might prove fruitful in the early detection of the development of insulin resistance and type 2 diabetes mellitus and positively delay the onset of this non communicable disease. Further, this will provide an opportunity for the research and invention of drugs to block this inflammatory pathway and in turn the development of type 2 diabetes.

Strength and Limitations:
Our study is done on a representative large sample. We matched the age and sex to prevent their confounding. Pearson's correlation was done to assess the strength of association.

The use of a cross sectional study limits the interpretation of the causal pathway. Hence, further longitudinal studies in a larger sample can be taken up to ascertain the cause-effect relationship. The same needs evaluation in a larger cohort to establish validity and confirm the correlation factors.

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