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RESEARCH ARTICLE

ASSOCIATION OF SERUM TNF-A WITH INSULIN RESISTANCE IN PRE-DIABETES AND TYPE 2 DIABETES MELLITUS

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Key words:-
TNF-α, Tumor Necrosis Factor Alpha, IR- Insulin Resistance, T2DM- Type 2 Diabetes Mellitus, PD- Pre-Diabetes

Abstract

Background- First adipocyte derived biomarker is tumor necrosis factor alpha which connect the link between inflammation and diabetes. It expresses m-RNA which is contributing to the development of insulin resistance. However, the relationship between tumor necrosis factor alpha, pre-diabetes and Type2diabetes mellitus is still contradictory and restricted.

Material & Methods: This study was hospital based analytical cross-sectional 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 Fasting Blood Glucose, 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. Tumor necrosis factor alpha was done by ELISA method.

Results: Tumor necrosis factor alpha concentration was significantly increased in patients with type 2 diabetes mellitus and pre-diabetes in comparison to the control group at p value < 0.001 and showed a positive correlation with HOMA -IR.

In conclusion, our findings suggested that systemic inflammation has an important role in the pathogenesis of pre diabetic condition. There is a progressive raises value of tumor necrosis factor alpha in pre diabetic and diabetic patients. Therefore, inflammation reflects the severity of the disease and signifies the presence of ongoing disease process..

Introduction:-

Diabetes, which is defined as a fasting blood glucose level of greater than 6.9 mmol/L**[1]** and this is the most widespread complex metabolic disorder among the world's population currently affecting around 250 million people globally **[2,3].** 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 consists 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. **[4,5]**

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Elevated levels of inflammatory proteins are found in chronic low-grade inflammation and the presence of these proteins has been found in the development of T2DM **[6]**. TNF-α is an adipocytokine involved in systemic in inflammation and stimulates the acute phase reaction **[7].** It is primarily secreted by macrophages, variety of cells including adipocytes and inhibits insulin transduction which effect on glucose metabolism **[8,9]**

TNF-α is the first adipocyte derived biomarker to pinpoint the link among obesity, inflammation and diabetes. It expresses m-RNA which is contributing to the development of insulin resistance. It can impair insulin signaling in hepatocytes and in adipose tissue **[10].**

The aim of this study is to investigate the changing levels of TNF-α, IR, HbA1c , BMI, in pre-diabetic, type 2 diabetic patients and to analyze the correlation of TNF-α with insulin resistance and with other variables ie. WC, WHR, BMI .

Material & 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 w**ere** considered as control group**, 300 as** prediabetic 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 (Body Mass Index, waist circumferences, waist hip ratio) and arterial blood pressure

Inclusion criteria:

1. Patients diagnosed with prediabetes **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%) are taken into consideration for selection of patient**.**
2. 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
3. Patients aged between 30-60 years are taken **up into the study**

Exclusion criteria:

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

All the biochemical parameters as Fasting blood glucose **[11]**, 2-hr Glucose**[12]** and HbA1c**[13]** were estimated by Standard Kit method by using Cobas c311 fully automated analyzer (Roche diagnostics). Serum Insulin **[14]** was assayed on Cobas c411 fully automated immunoassay analyzer (Roche diagnostics) by using cobas kits. TNF-α were estimated by using Human Elisa kits **[15].** Insulin resistance was estimated by the **Homeostasis model assessment (HOMA-IR)** and calculated as Fasting Insulin (microU/L) x Fasting glucose (mg/dl)/405.

Statistical analysis-

Epi-info software was used and results or continuous variables are given as mean and SD. Pearson's correlation was used to estimate the association between the variables.

Observations:-

A total of 900 subjects enrolled during the period july 2017 to july 2019. The ratio of prediabetes: diabetes: control is 1:1:1. and out of these 300 were type 2 diabetes patients, 300 were pre-diabetes, and 300 were controls in the present study.

Table 1:- The correlation among the variables and BCAA chosen for study among controls and pre diabetes.

Variables		WHR	BMI	FBG	2hr-glucose	HbA1c	HOMA IR

	WC						
TNF-α(r)	-.012	.019	.022	.122	.086	.192**	.470**
Sig(2 tailed)	.867	.785	.753	.086	.227	.006	.000

Table 1 depicts correlation of TNF-α with other variables in which TNF-α is showing weak correlation with WC, WHR, BMI, ,FPG,2hr-glucose at p<0.05.and significant correlation with HbA1c , HOMA IR at p < 0.01.

Table 2:- The correlation among the variables and BCAA chosen for study among controls and DM Type2.

Variables	**WC**	WHR	BMI	FPG	2hr-glucose	HbA1c	HOMA IR
TNF-α(r)	.096	-.036	.460**	.515**	.603**	.667**	.566**
Sig(2 tailed)	.175	.611	.000	.000	.000	.000	.000

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 2 depicts correlation of TNF-α with other variables in which TNF-α is showing significant correlation with ,FPG,2hr-glucose, BMI,HOMA IR and HbA1c at p<0.01 and weak correlation with WC,WHR, at p<0.05 .

Table 3:- Level of changes among different biochemical parameters in three groups.

Parameters	**Healthy controls**	**Pre-diabetes**	**Diabetes**	**ANOVA**
WC (cm)	74.87± 7.4	79.95± 5.7	84.2 ± 5.4	0.001*
WHR	0.82± 0.09	0.87± 0.06	0.98 ± 0.23	0.001*
BMI (kg/m2)	22.22± 2.79	24.89± 2.4	29.25 ± 3.06	0.001*
FBG (mg/dl)	83.62± 7.7	114.58± 7.3	149.78±30.27	0.001*
2-hr Glucose (mg/dl)	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	6.09± 2.13	7.19± 3.63	29.006 ± 5.06	0.001*
HOMA-IR	1.48± 0.80	2.04± 0.98	10.67 ± 2.7	0.001*
Hs-CRP (mg/L)	0.29±0.11	0.37±0.05	0.53±0.17	0.001*
TNF-α (pg/ml)	255.74± 29.07	265.21± 34.20	296.74 ± 33.33	0.001*

*p value significant < 0.001

Table 3 depicts level of changes of different biochemical parameters in three groups; there is a significant difference in TNF-α, IR, HbA1c, FPG, 2hr-glucose, WC, WHR and BMI.

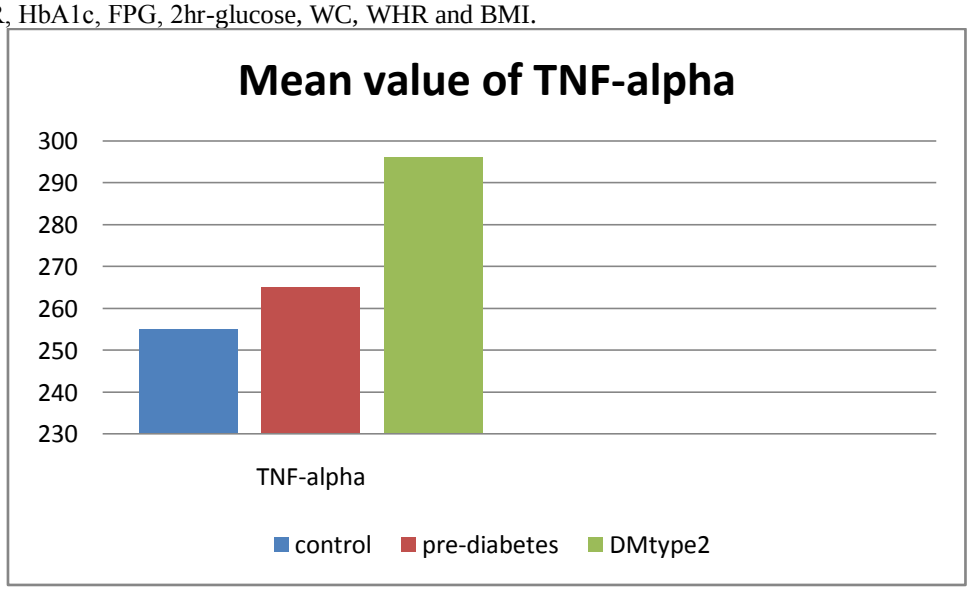

Fig 1:- Mean score of TNF-α.

Discussion:-

People's College of medical Science & Research Center Bhopal, shows a good patient output. Since there is dearth in the literature as no other study was conducted before in this region to show the expression of TNF-α with variables specially with BMI, IR in pre-diabetic and type 2 diabetic patient.

In the present study fasting serum TNF-α shows a statistically significant rising trend among controls, pre-diabetes, and Type 2diabetes mellitus (Fig-1) and I found significant difference of WC, WHR, BMI and IR found in pre-diabetes, and Type 2diabetes mellitus when compare with healthy subjects (Table-3). In pre-diabetes and control conditions, no positive correlation found between TNF-α, BMI, WHR, and WC (Table-1) but with BMI and IR found positive correlation in type 2 diabetes mellitus and control (Table-2). These results support the hypothesis that in obese persons, the cytokine TNF alpha is produced by adipose tissue in higher amounts compared to lean individuals as much as 7.5 folds **[16]**. In obesity, chronically elevated TNF-a affects the glucose metabolism **[17]** and also alters the insulin sensitivity by different ways i.e., disrupting the insulin receptor signaling pathways **[18]** lowering glucose transporter-4 in adipocytes **[17],**and suppressing adiponectin.

TNF-α is an adipocytokine produced by adipose tissue, known to play a major role in the inflammatory process. When secreted in excess, may promote the production of interleukins such as IL-8. Several studies have indicated the raised TNF-α levels and its involvement in the onset of metabolic disorders such as obesity and IR. The elevated levels of proinflammatory molecule, i.e., TNF-α has been critically involved in inducing IR by distorting serine phosphorylation in insulin signaling in adipocytes and peripheral tissues which eventually leads to the development of T2DM.**[19,20]**

Dandona **[21]** proposed various mechanisms for the progression of T2DM from PD. They strongly confirm that the biomarker TNF-α is increased causing a pro-inflammatory condition which leads to the development of diabetes. We also show a similar finding that TNF-α is positively correlated with HOMA-IR significantly. This confirms the link proposed by the author. Another interesting factor to be discussed here is the increased insulin levels in PD due to resistance to insulin. Our pancreatic beta cells detect hyperglycemia and in turn release insulin. Due to insulin resistance, it is not able to act on this condition and achieve glucose homeostasis. We show that TNF α is positively correlated with fasting insulin significantly and this proves the above-mentioned fact that inflammation is the cause for the development of diabetes and TNF-α can help in early diagnosis.

Conclusion:-

In our study we conclude that there is a significant change in TNF-α , IR and BMI levels in healthy, pre-diabetic, and diabetic population of Bhopal region The early diagnosis of **pre-**diabetes is important in order to avoid long-term micro- and macro vascular complications in individuals at high risk of diabetes. So the identification of biomarkers that accurately predict incident diabetes is of great interest .

Screening of 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.

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