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Original Research Paper

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"RELATIONSHIP BETWEEN IR, BMI, LEUCINE AND OTHER VARIABLES AND THE RISK OF TYPE 2DIABETES MELLITUS IN PRE-DIABETES"

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ABSTRACT

Background: Pre-diabetes is the progeny of diabetes. There were about 77.2 million people in India with pre-diabetes as of 2013. Here the problem is how to reduce the future risk of T2DM. Now recent research interest is the role of branched chain amino acids specifically leucine is significantly correlated with insulin resistance and BMI in the future risk of diabetes and this is first study in Bhopal M.P. **Objectives:** To investigate the changing levels of Branched chain amino acid(Leucine),insulin resistance, glycosylated hemoglobin, body mass index and other variables in pre-diabetic and diabetic patients and to analyze the co-relation of leucine with insulin resistance ,body mass index and other variables. **Material and Methods:** It was a hospital based study conducted in the department of biochemistry, People's College of Medical Science and Research Center (PCMS and RC), Centre for Scientific Research and Development (CSRD), People's University, Bhopal. Patients with complication of diabetes like diabetes neuropathy, nephropathy, retinopathy etc and on any medication were excluded. Epi-info software was used for statistical analysis. **Results:** There is a significant difference in leucine, insulin resistance, glycosylated hemoglobin and body mass index. Elevated branched chain amino acids have been shown to be significantly correlated with insulin resistance and BMI and the future risk of diabetes. **Conclusion:** Branched chain amino acid (leucine) may be involved in the etiology of insulin resistance in type II diabetes mellitus with obesity and with the help of BMI we can identify the risk.

KEYWORDS : T2DM (Type2 diabetes mellitus), BMI(body mass index),IR(insulin resistance), Pre-diabetes, mTORC1(mammalian target of rapamycin complex1), Leucine.

INTRODUCTION

Diabetes is a growing challenge in India with estimated 8.7% diabetic population in the age group of 20 and 70 years.(1) Pre-diabetes is the progeny of diabetes. It is a condition in which individuals have blood glucose levels higher than normal but not as high as 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).(2) It is a reversible condition that increases the risk for diabetes which is associated with insulin resistance or decline in insulin sensitivity.

The action of insulin is to lower the glucose levels in the blood and to stimulate the uptake of glucose principally in muscle and liver cells, thus involved in promoting glucose oxidation and glycogenesis [3].

Increase amount of branched chain amino acids have been shown to be significantly correlation with insulin resistance and the future risk of diabetes. The hallmark feature of type 2 diabetes is that it stimulates β cell proliferation via nutrient signaling. This activates Rag guanosine triphosphatases (GTPases), activates mammalian target of rapamycin complex 1(mTORC1) and increases translational protein activity. It can also trigger triglyceride synthesis in the liver. It is caused by activating sterol regulatory element binding protein (SREBP) and over-activating folding of newly synthesized protein in the endoplasmic reticulum (ER) causing β cell dysfunction and death, which can lead to diabetes. (4)

The aim of this study was to investigate the changing levels of Leucine, IR, $Hb1A_c$, BMI, and other variables in pre-diabetic and diabetic patients and to analyze the co-relation of BCAA with insulin resistance, BMI and other variables.

MATERIAL AND METHODS

The study was conducted in the department of biochemistry, People's College of Medical Science and Research Center (PCMS and RC), Centre for Scientific Research and Development (CSRD), People's University, Bhopal. For the purpose of study 900 cases were selected as per inclusion and exclusion criteria. with the help of physicians during the period January 2017 to July 2019 from people's hospital. Out of which age and sex matched 300 as 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).

Inclusion criteria

1. Patients newly diagnosed with type 2 diabetes mellitus as per ADA criteria.
2. 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%) are taken into consideration for selection of patient.
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 pre-diabetes & type 2 diabetes mellitus (based on their medical history and physical examination) are excluded.
2. Patients below 30 years and above 60 years are excluded from the study.
3. Subjects those who are currently not on any oral hypoglycemic agents (OHA).
4. Patient on smoking, alcohol and high protein BCAA powder for exercise are excluded.

Anthropometric measurements: Height was noted using a measuring tape (to the nearest 0.1 cm), with the subjects wearing light clothes and no shoes. Weight was measured to the nearest 0.1 kg using a mechanical weighing machine. BMI, defined as mass in kilograms divided by the square of height in meters, was calculated. Homeostatic Model Assessment (HOMA) method, which has been validated as a reliable measure of insulin sensitivity in humans was used to estimate insulin resistance (HOMA IR).

Biochemical parameters: After overnight fasting blood samples were obtained by vein puncture. The serum was separated and stored at -20°C. Serum BCAA (Leucine) was quantified using thin layer chromatography(4) in which readymade silica gel plate is used for estimation. Fasting glucose(5), 2-hr glucose(6), HbA1c(7) were analyzed by standard kit method by Cobas c-311 and insulin(8) by cobas c-411 fully automated auto analyzer (Roche diagnostics) (Table-4).

Statistical analysis

The analysis was done using statistical package SPSS 24 and Microsoft Excel 2010. Independent *t* test was used for comparison. Pearson's correlation was used to estimate the association between the variables.The p-value < 0.001 were considered as significant.

RESULTS AND OBSERVATIONS

We have equal numbers in each age group and sex. Total 900 subjects included in study and 300 in each group like control, pre-diabetes and T2DM. We had significant differences among all the three groups with respect to all the anthropometric like waist circumference, waist hip ratio, body mass index and biochemical parameters used in our study namely fasting blood glucose, 2hr- Glucose ,HbA1c, fasting insulin and Leucine. Rise in biomarker among the three groups have p value less than 0.001 and hence statistically significant.

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

Variables	WC	WHR	BMI	SBP	DBP	FPG	2hr-glucose	HbA1c	HOMA IR
BCAA(r)	.670**	.493**	.687**	.292**	.157*	.435**	.440**	.473**	-.080
Sig(2 tailed)	.000	.000	.000	.000	.026	.000	.000	.000	.260

Table 1 depicts correlation of BCAA(leucine) with other variables in which BCAA is showing significant correlation with WC, WHR, BMI, ,FPG,2hr-glucose and HbA1c at p<0.001. With SBP, DBP at p < 0.05 and negative correlation with HOMA IR.

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

Variables	WC	WHR	BMI	SBP	DBP	FPG	2hr-glucose	HbA1c	HOMA IR
BCAA(r)	.630**	.540*	.702**	-.024	-.006	.476**	.522**	.534**	.516**
Sig(2 tailed)	.000	.000	.000	.735	.938	.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 BCAA(leucine) with other variables in which BCAA is showing significant correlation with WC, WHR, BMI, SBP,DBP ,FPG,2hr-glucose, HOMA IR and HbA1c at p<0.001.

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

Parameters	Healthy controls	Pre-diabetes	Diabetes	ANOVA
WC	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	22.22 ± 2.79	24.89 ± 2.4	29.25 ± 3.06	0.001*
SBP	120 ± 8.03	131 ± 6.4	145.5 ± 15.45	0.001*
DBP	76.28 ± 6.9	83.04 ± 7.5	96.33 ± 9.4	0.001*
FPG	83.62 ± 7.7	114.58 ± 7.3	149.78 ± 30.27	0.001*
2hr-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	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*
BCAA (leucine)	0.17 ± 0.06	0.23 ± 0.04	0.25 ± 0.02	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 BCAA (Leucine), IR, HbA1c, FPG, 2hr-glucose, WC, WHR and BMI.

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 Leucine levels were significantly increased in the pre-diabetes and type II diabetes compared with the control group and expression of Leucine with variables specially with BMI, IR in pre-diabetic and diabetic patient. Same as our study George Jency et al(9)highlighted regarding insulin resistance and branched chain amino acids. BCAA's improved metabolic parameters including body composition, glycemia levels and hunger satiety. As proposed in this paper, we have quoted a moderately positive correlation between insulin resistance and BCAA's when compared between controls and diabetes (table-2).Same as our study the systematic review by Zhao X et al(10)reveal significant alterations of branched chain amino acids in obese adults and the association between branched chain amino acids and insulin resistance. They also put forth in their review that obese individuals had 2.3 times higher insulin resistance compared to lean controls. We also documented a significant positive correlation between HOMA_IR and BCAA (r = 0.52) when equated with controls and diabetes (table-2). We establish a significant positive correlation between BMI and BCAA in both pre-diabetes (r= 0.69) and diabetes (r = 0.70)(table-1,2), though we did not classify them based on BMI as lean, normal and obese. We also present a significant positive correlation between of WHR, waist circumference, BMI with BCAA in both pre-diabetes and type 2 diabetes (table-1,2). Yoon MS et al (11) explained the activation of mammalian target tissue by BCAA to cause insulin resistance. Insulin is required for maintenance of glucose homeostasis. Leucine is a well-known allosteric activator of insulin secretion which is secrete from the pancreatic -cell (12) and others BCAAs are also a powerful insulin-secretion stimulators(13).

Kolanu B et al(4) explained in his recent study, positive association between increased excretion of BCAA and insulin resistance (IR) in obese or diabetic patients same as my study (table-2) but BCAA concentration is not positively correlate with IR and significantly correlate with BMI when compare pre-diabetes and healthy subjects (table-1). An elevated branched chain amino acid level is associated with a high risk of metabolic disorder and future resistance to insulin or T2DM via mammalian target of rapamycin (mTOR) signalling. In our study we used serum but he used urine as a sample. Probably

this study showed first time the comparison of excretion of leucine in urine between control and T2DM and same for my study in M.P. region. Correlation between T2D and BCAA especially leucine can help in managing the disorder [14]

BCAA may play a potentially important pathophysiological role in the development of insulin resistance with high BMI in diabetes mellitus type2. On the other hand, high level of circulating BCAA may be a result of impaired catabolism or clearance, that is, a consequence of a disease process rather than an upstream cause. (15,16)

CONCLUSIONS

Recorded observations in our study support the hypothesis that BCAA may be involved in the etiology of insulin resistance and significant association of BCAA with BMI and HOMA IR reduce the risk of type II diabetes mellitus.

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