Original Article

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Biochemistry Section

Elevated MDA Level Correlates with Insulin Resistance in Prediabetes

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ABSTRACT

Introduction: Various metabolic changes in obesity may increase oxidative stress, which if persists continuously may predispose to insulin resistance and diabetes mellitus. Oxidative stress will generate Reactive Oxygen species (ROS) which affects proteins, lipids, carbohydrates and cell membrane. Malon-Di-Aldehyde (MDA) is a lipid peroxidation product found to be elevated in diabetes. Measurement and reduction of oxidative stress in prediabetes may prevent the grave consequences of diabetes.

Aim: The present study was aimed at evaluating MDA levels in prediabetics and diabetics and correlating it with Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) of the same subjects.

Materials and Methods: Plasma MDA and serum insulin were measured in 96 prediabetics and 101 diabetics selected for study and their Body Mass Index (BMI) was calculated. Prediabetic and diabetic patients were divided into two groups, one with BMI>25 kg/m^2, and other with BMI<25 kg/m^2. Serum MDA was analysed by Thiobarbituric Acid Reactive Substances (TBARS) method. Serum insulin was analysed by Enzyme Linked Immunosorbent Assay (ELISA). HOMA-IR was evaluated from fasting plasma glucose and serum insulin levels.

Results: Insulin resistance was significantly increased in prediabetic (p=0.02) and diabetic (p<0.0001) patients when BMI was >25 kg/m^2. Statistically significant positive correlation was observed between MDA and insulin resistance in prediabetic group (p<0.0001).

Conclusion: Findings indicated that raised MDA level correlates with insulin resistance in obese prediabetic patients.

Keywords: Body mass index, Homeostatic model assessment of insulin resistance, Oxidative stress

INTRODUCTION

Recently Type 2 Diabetes Mellitus (T2DM) and obesity related metabolic disorders are commonly increasing worldwide. They appear like an epidemic [1]. Insulin resistance has a link with obesity [2]. Furthermore it has been observed that oxidative stress is strongly related with adiposity markers and insulin resistance [3]. A study reported that oxidative stress is responsible for insulin resistance in rodents [4]. Obesity is considered as one of the most significant factor for generation of oxidative stress [5]. Increased oxidative stress may be one of the pathways by which obesity and/or insulin resistance can establish T2DM in humans [6].

The association between increased oxidative stress and T2DM is already being established but there is a limited data available regarding oxidative stress and insulin resistance in prediabetics [7]. HOMA-IR is one of the frequently used indices of insulin resistance [8,9]. Once the oxidative damage sets in, a series of events further leads to cellular derangements which causes damage to cell membrane and membranes of cellular organelles by causing peroxidation of membrane lipids [10,11]. Malon-Di-Aldehyde (MDA), a biomarker of lipid peroxidation has been reported to be significantly raised in diabetes [12].

A limited data is available regarding oxidative stress and insulin resistance in prediabetic obese patients, henceforth present study was undertaken to assess the rationality of MDA as a marker of oxidative stress in obese prediabetics, so that the persistence of raised MDA level can slowly progress the prediabetes stage to T2DM.

Hence, the present study was done to analyse and compare the MDA, BMI and HOMA-IR in prediabetic and diabetic groups. To asses the correlation between HOMA-IR and MDA which is a marker of oxidative stress in these two groups for any significance which can guide us to control and reduce the grave consequences of this devastating disease.

MATERIALS AND METHODS

This cross-sectional, observational, hospital based study was carried out at Department of Biochemistry, National Medical College Kolkata, India. The study was carried out from January 2016 to June 2016.

Sample Size Estimation: Sample size was calculated according to the standard protocol based on data collection and sufficient statistical power.

Patient Selection: A total of 197 patients (96 prediabetics and 101 diabetics) were selected for the study (121 males and 76 females, age 30-65 years). All the patients were clinically diagnosed by physicians according to the biochemical investigations. Prediabetes was diagnosed as 2 hours plasma glucose between 140-200 mg/dL after ingestion of 75 g oral glucose load and has additional HbA1c value between 5.7% to 6.4%. Diabetes was diagnosed when 2 hours plasma glucose is more than 200 mg/dL and additional HbA1c value is more than 6.5% [13]. Written informed consent was taken from the study subjects. The study was approved by the Institutional Ethics Committee (IEC), according to the Helsinki declaration.

Inclusion criteria: Newly diagnosed prediabetic and diabetic patients, aged between 30-65 years, attending outdoor patient department of Endocrinology Clinic of National Medical College were selected for the study.

Exclusion criteria: Patients suffering from any acute illness, or other endocrine disorders, hypertension, recent history of stroke and myocardial infarction, any other disease which may alter insulin resistance and BMI were excluded from the study. Patients who were on antidiabetic drugs or any drugs which can alter the

oxidative stress were also excluded from the study. Antenatal mothers, psychiatry patients, smokers and tobacco chewers were not selected for the study.

Methods for Analysis of Test Parameters: Height in meters, weight in kg was measured for all participants. BMI was calculated by the formula, as weight in kilograms divided by the square of height in meters [14].

Sample collection: 5 mL of venous (antecubital vein) blood collected in overnight fasting with all aseptic precautions in three parts. The first part was collected in Ethylene Diamine Tetra-Acetate (EDTA) vial for estimation of Thiobarbituric Acid Reactive Substances (TBARS). The second part was collected in plain vial for determination of serum insulin. The third part, kept in fluoride-oxalate vial which was used to determine the level of fasting plasma glucose.

Blood Glucose Estimation: Blood glucose was analysed using reagent kit (Merck) by Glucose oxidase and peroxidase method [15].

Brief methodology: Hydrogen peroxide formed by the oxidation of glucose reacts with phenol and 4-aminoantipyrine to form red coloured dye. Intensity of the colour formed is directly proportional to the amount of the glucose present in the sample.

Serum Insulin Estimation: Serum insulin was estimated by ELISA with monoclonal antibody based reagent (Monobind) [16].

Brief methodology: Enzyme linked two-site immunoassay for quantitation of intact insulin in human serum and plasma. The method uses two murine monoclonal antibodies that bind to two different epitopes on the insulin molecule. The immunoassay is specific.

HOMA-IR was calculated by the formula using fasting insulin × fasting glucose/405 [17].

MDA (TBARS) estimation: MDA, the product of lipid peroxidation, was determined in plasma [18]. Protein in the sample was precipitated by Trichloroacetic Acid (TCA), and then the sample was reacted with Thiobarbituric acid (TBA) reagent, followed by measurement of coloured product spectrophotometrically at 532 nm after extraction in butanol.

STATISTICAL ANALYSIS

Data was analysed using Statistical Package for the Social Sciences (SPSS) 17 version used for chi square and t-test. Correlation of biochemical parameters was done by Pearson's correlation analysis. The p <0.05 was considered as statistically significant.

RESULTS

In [Table/Fig-1], Demographic profile of groups are showing age and biochemical parameters (mean±SD) of prediabetic and diabetic patients.

In [Table/Fig-2], main results about markers, are showing that, among prediabetics the HOMA-IR was significantly altered in patients whose BMI>25kg/ m^2 as compared to the patients whose BMI was <25 kg/m^2 (chi-square= 7.09, p=0.02). The similar trend was also observed in diabetics (chi-square=19.42, p<0.0001).

In [Table/Fig-3], statistical analysis shows that there was no significant difference between prediabetic and diabetic groups with respect to HOMA-IR.

	Prediabetic (mean±SD)	Diabetic (mean±SD)
Age (years)	50.25±5.681	48.04±6.638
BMI (kg/m^2)	27.101250±3.0835326	26.6265±2.91570
FBS (mg/dL)	119.34±3.560	166.68±38.522
HOMA-IR	5.296±0.87	6.429±4.18
MDA (µmol/L)	2.760±0.63	3.409±0.921

[Table/Fig-1]: Demographic profile of prediabetes and diabetes group.

	Prediabetic (n=96)		Diabetic (n=101)	
	BM1(<25)	BM1(>25)	BM1(<25)	BM1(>25)
HOMA-IR(<3)	12	21	22	21
HOMA-IR(3-5)	10	15	1	21
HOMA-IR(>5)	6	32	6	30
Test results	Chi-square=7.09, df=2, p=0.0288		Chi-square=19.42, df=2, p=0.00006074	

[Table/Fig-2]: Statistical analysis of different parameters in between groups.

	T-test value
HOMA –IR (Prediabetic)	t-test (unpaired)=1.270, p=0.207
HOMA –IR (Diabetic)	

[Table/Fig-3]: Comparison of HOMA-IR between two groups.

	Correlation value
Pre Diabetic-MDA and Pre Diabetic-HOMA -IR	Pearson correlation value{r}=0.865, p<0.0001
Diabetic-MDA and Diabetic HOMA -IR	Pearson correlation value(r)=0.101, p=0.317

[Table/Fig-4]: Correlation coefficient of MDA and HOMA-IR in two groups.

Furthermore in [Table/Fig-4], significant positive correlation between MDA and HOMA-IR was observed among prediabetics (r=0.5941, p<0.0001). No such correlation was found among diabetics.

DISCUSSION

From the results of the present study, it is evident that insulin resistance (HOMA-IR) was significantly increased among the patients in whom BMI is> 25kg/ m^2 in prediabetics and diabetics, suggesting that BMI plays an important role to develop insulin resistance. Results showed no significant alteration of HOMA-IR between two groups. Furthermore, significant positive correlation observed between MDA and HOMA-IR in prediabetics but the same trend was not observed among diabetics. This observation indicated that oxidative stress (MDA) may be responsible for the development of insulin resistance initially as evidenced by the fact that MDA is correlated with HOMA-IR in prediabetics. However, once the diabetes sets in this correlation was not found significant.

Few authors suggested that insulin resistance correlates with obesity [19]. Insulin resistance, expressed by HOMA-IR, was also associated with obesity in poorly controlled T2DM patients after insulin therapy, suggested by some authors [20]. Compared to them, present study excludes prediabetic and diabetic patients who are on antidiabetic drugs and insulin therapy.

Obesity can be associated with increased insulin secretion. Insulin can increase obesity since it is a key hormone in adipogenesis [21]. This phenomenon can be supported by the fact that cell function is modulated by insulin sensitivity which is mostly decreased in obesity [22]. Furthermore, it has been suggested that adipose tissue modulates metabolism by releasing Non-Esterified Fatty Acids (NEFA) and glycerol [23]. In non-diabetic subjects raised insulin resistance enhances insulin secretion to maintain plasma glucose level within normal range. Increased insulin secretion or hyperinsulinemia may be a cause of increased adiposity, which enhances further development of insulin resistance [24]. Furthermore, release of hormones like leptin, adiponectin, and proinflammatory cytokines might also have a role in the development of insulin resistance [25]. Diabetic subjects do not have adequate insulin secretion capacity to keep blood glucose level within the normal range, but have enough insulin secretion capacity to enhance fat cell growth and body composition [2]. The present study observed that in prediabetic and diabetic groups the patients having BMI>25kg/m^2, suffered from increased insulin resistance expressed by HOMA-IR.

Oxidative stress has found to be increased in T2DM [26], but the data are scarce on oxidative stress and insulin resistance in prediabetes stages [7]. Present study tried to correlate the insulin resistance and oxidative stress in prediabetics and diabetics. Few authors has found correlations between HOMA-IR and carbonyl stress in T2DM but no association was found between MDA and HOMA-IR in their study. Furthermore they didn't select the prediabetes group [27].

Oxidative stress may be a key pathway leading to insulin resistance, furthermore, oxidative stress may be a risk factor to develop the T2DM in humans observed by some authors [4], and their findings supported the present study.

However, few authors observed that the systemic oxidative stress is associated with insulin resistance among individuals without diabetes in the community. Furthermore, this association was statistically independent of BMI and was similar in obesity, metabolic syndrome, and impaired glucose tolerance–defined pre-diabetes [6].

The present study observed that HOMA-IR is significantly correlated with MDA in prediabetics, but no such correlation was observed in diabetics. The mean age of prediabetics was around 50 years, with BMI>25kg/m^2. Measurement and reduction of MDA in prediabetics can prevent the risk of grave consequences of T2DM.

LIMITATION

Small sample size and duration may interfere the results of the study. Furthermore, the study caters the scattered population attending a tertiary care hospital. Patient selection didn't cater a particular geographical area. Study population may have different food habit. Larger sample size may be beneficial.

CONCLUSION

The study parameter MDA, a product of lipid peroxidation was selected as a marker of oxidative stress. The results have shown increasing trend of mean MDA levels in prediabetics (2.7 µmol/L) and diabetics (3.4 µmol/L). Furthermore, raised level of MDA correlated positively with insulin resistance (HOMA-IR) among prediabetics. The study recommends that, MDA can be an useful marker in prediabetes stage, the reduction of raised level of MDA may be beneficial.

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