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Original Research Article

Assessment of serum nitrate-nitrite ratio vis-a-vis insulin sensitivity and resistance in type 2 diabetics in a tertiary hospital in Eastern India

Nayana Deb[1], Sharmistha Chatterjee[2]*, Mousumi Mukhopadhyay[3], Biswajit Majumder[4], Swati Bhattacharyya[5]

[1]Department of Biochemistry, Nalanda Medical College, Patna, Bihar, India
[2]Department of Biochemistry, College of Medicine and Sagore Dutta Hospital, Kamarhati, Kolkata, West Bengal, India
[3]Department of Biochemistry, IPGME& R, Kolkata, West Bengal, India
[4]Department of Cardiology, R.G. Kar Medical College, Kolkata, West Bengal, India
[5]Department of Biochemistry, North Bengal Medical College, Susrutnagar, West Bengal, India

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***Correspondence:**
Dr. Sharmistha Chatterjee,
E-mail: sharmisthacmajumder@yahoo.co.in

ABSTRACT

Background: Insulin Resistance is of paramount importance in the pathophysiology of Type 2 Diabetes Mellitus along with endothelial dysfunction is mediated by Nitric Oxide (NO). Central to this endothelial dysfunction is the action of Insulin on the Nitric oxide synthase enzyme. Since NO cannot be measured because of its short half-life, metabolites of NO (namely nitrite and nitrate) are measured towards assessing their relationship along with different direct and surrogate markers of insulin resistance in patients of Diabetes Mellitus attending a tertiary care hospital in Eastern India. Aim of the study was to assess the level of Insulin resistance with the direct and surrogate markers of insulin resistance in patients of Diabetes Mellitus attending a tertiary care hospital in Eastern India.
Methods: Blood samples from newly diagnosed Type 2 Diabetic patients were assayed for fasting and postprandial sugar and insulin, lipid profile and serum nitrate and nitrite and different anthropological parameters were measured. After that, HOMA-IR and QUICKI' index were measured.
Results: Values of anthropological parameters and the direct and surrogate markers of insulin resistance showed statistically significant difference between cases and controls. Bivariate analysis of post-prandial blood glucose showed strong co-relation with HOMA-IR while serum total nitrate-nitrite ratio showed a strong co-relation with QUICKI.
Conclusions: Serum nitrate-nitrite ratio showed a strong co-relation with HOMA-IR and QUICKI. The significance of this study lies in the fact that measurement of the serum nitrate-nitrite may give an idea of the level of insulin resistance of a diabetic patient.

Keywords: Insulin resistance, Insulin sensitivity, Serum nitrate-nitrite ratio, Type 2 diabetes mellitus

INTRODUCTION

Nitric oxide (NO), a free radical gas with a half-life in vivo of only a few seconds, is known to be involved in diverse physiological and pathological conditions like vasodilation, smooth muscle proliferation, immuno-regulation, anticoagulation and the antioxidant capacity of endothelial cells.[1-3] Endothelial dysfunction is the earliest abnormality in the pathogenesis of atherosclerosis, and along with dyslipidemia and hypertension, is associated with the insulin resistant state-which is the hallmark of type 2 diabetes.[4] Evidence

suggests that loss of nitric oxide generation is central to this endothelial dysfunction.[5] Insulin stimulates NO formation, whereas elevated glucose levels, as in diabetes, inhibits NO formation.[6,7] The major target for insulin action in man is the skeletal muscle, and studies indicate that blood flow in the skeletal muscles is NO dependent.[8,9] In this study, we wanted to assess the relationship of insulin sensitivity (as measured by the Quantitative Insulin Sensitivity Check Index or QUICKI) with the serum nitrate-nitrite ratio and the surrogate markers of insulin resistance in patients of Type 2 Diabetes Mellitus in the population attending a tertiary care hospital in Eastern India.

The aim of the study was to assess the relationship of Insulin resistance with the serum nitrate-nitrite ratio with the surrogate markers of insulin sensitivity in newly diagnosed patients of Diabetes Mellitus attending a tertiary care hospital in Eastern India.

METHODS

76 newly diagnosed Type 2 Diabetic patients and 46 non-diabetic adults (both group's age and sex matched) were selected from the patients attending the outpatient department (OPD) of clinic of Department of Endocrinology and OPD of Clinical Biochemistry, Medical College, Kolkata, West Bengal, India. Newly diagnosed Type 2 Diabetics were treated as cases while non-diabetics were controls.

Cases of hyperglycemia other than Type 2 DM with known dyslipidemia, hypo or hyperthyroidism, pregnancy, females with history of gestational diabetes or with delivery of large-baby, fever, Cushings disease, coronary artery disease, cerebrovascular disease, known diabetic complications, history of medication intake or any active illnesses and those who denied of informed consent were excluded from the study. Approval for this study was obtained from the institutional ethics committee, Medical College, Kolkata, West Bengal, India.

First, the anthropological variables like weight, height, waist and hip circumference were measured for assessment of BMI and waist-hip ratio. Insulin sensitivity was measured by Quantitative Insulin Sensitivity Check Index (QUICKI) which has been defined as 1/[log(fasting insulin)+ log(fasting glucose)]. Insulin resistance was measured by the following equation:[10,11]

$$\mathbf{HOMA-IR} = \mathbf{Insulin\ (microUnit/ml)} \times \mathbf{Glucose\ (mmol)/22.5}$$

Where HOMA is homeostatic model of assessment insulin resistance

Subsequently, the desired biochemical parameters were assayed in the blood samples of cases and controls. The fasting and post-prandial plasma glucose was measured by the GOD-POD method. In lipid profile, where serum cholesterol was measured by the CHOD-PAP method, HDL by the PEG precipitation method, triglyceride by GPO-PAP method and LDL cholesterol by the Friedwald's formula. Fasting and Post prandial serum insulin were estimated by ELISA (ACCUBIND) and serum nitrate-nitrite ratios were estimated by CADMIUM REDUCTION METHOD in accordance with Cortas and Wakid.[12] The indicators of insulin resistance and sensitivity, HOMA-IR and QUICKI'S index (derived parameters) were calculated from plasma glucose and serum insulin. The data, thus accumulated, was statistically evaluated by the statistical package for social sciences, (S.P.S.S.16).

RESULTS

The present study included 2 groups of patients, 76 diabetic patients designated as cases and 64 non-diabetics designated as controls, both groups being age and sex matched. Among the anthropological parameters, height, weight, hip, waist, waist-hip ratio and BMI were noted. Fasting and post-prandial glucose, serum cholesterol, triglyceride, HDL cholesterol, VLDL and LDL cholesterol, serum nitrate-nitrite level and serum fasting and post-prandial insulin were among the biochemical parameters assessed.

The anthropological parameters were described in Table 1 while the biochemical parameters are described in Table 2.

Table 1: Descriptive statistics of anthropological data for cases and controls.

Parameters	Mean± SD n=76 (Cases)	Mean ±SD n=65 (Controls)	t	P value
Age (years)	51.61± 23.32	46.12± 28.34	2.52	0.01
Height(in cm)	154.91±17.88	156.15± 14.34	-0.9	0.37
Weight (in kg)	62.54± 18.06	55.33± 20.12	4.48	0
Waist (in cm)	90.61± 24.08	84.95± 23.49	2.82	0.01
Hip (in cm)	94.54±19.32	94.55± 19.76	0	0.1
Waist-Hip Ratio	0.96± 0.12	0.89± 0.14	5.15	0
BMI	26.4±5.84	22.63±7.96	22.63	0

Cases have a statistically significant increased mean value for age (p=0),weight (p=0.01), waist circumference (p=0.01) and waist-hip ratio(p==0) and consequently BMI (p=0) as calculated by the QUETLET''S Index as compared to controls in independent ' t' test as seen in Table 1.

Table 2: Descriptive statistics of biochemical data for cases and controls.

Parameters	Mean ±SD n=76 (Cases)	Mean±SD, n=65 (Controls)	t	P value
Fasting plasma glucose (mg/dl)	166.47±106.52	95±20.38	10.58	0
Post prandial plasma glucose	273.67±169.04	109±35.7	15.27	0
Serum cholesterol (mg/dl)	179.46±85.62	161.32±51	2.65	0.01
Serum triglyceride (mg/dl)	162.94±140.98	110.3247±.76	4.73	0
Serum HDL cholesterol (mg/dl)	44.35±5.9	45.49±5.82	0.66	0.51
Serum VLDL cholesterol (mg/dl)	32.54±28	45.49±5.82	3.19	0
Serum LDL cholesterol (mg/dl)	101.61±78.9	93.92±48.54	-0.88	0.41
Serum total nitrite (micromole/l)	46.5±24	82.5±34.5	14.74	0
Serum endogenous nitrite (micromole/l)	16.5±7.5	18.75±15	-2.22	0.03
Serum nitrate (micromole/l)	30±16.5	66.45±4.5	-17.15	0
Serum nitrate-nitrite ratio	1.78±1.16	3.52±2.08	12.51	0
Serum fasting insulin (microunit/ml)	21.22±22.9	5.65±4.14	10.8	0
Serum post prandial insulin	44.82±53.5	7.36±6.68	11.2	0

Comparison of the above data shows, that fasting and postprandial glucose (p=0), serum cholesterol (p=0.03), triglyceride (p=0), VLDL cholesterol (p=0), and fasting and postprandial serum insulin (p=0.01), values are significantly higher in diabetics as expected, but the serum total and endogenous nitrite, (p=0 and 0.03 respectively), nitrate and nitrate-nitrite ratio (p=0) was higher in controls. The following table shows the calculated indices, i.e. the derived parameters.

Table 3: Calculated indices of insulin resistance & sensitivity (cases and Controls).

Calculated indices	cases	Controls	t	P value
HOMA-IR (micromole/ml)	8.7±10.42	1.31±1	11.39	0
QUICKI'S INDEX	0.29±0.04	0.37±0.06	19.15	0

A HOMA-IR value more than 2.5 indicates insulin resistance and QUICK'S INDEX less than 0.3 is taken to be indicative of subnormal insulin sensitivity. In the present study, comparison of this data in cases and control population shows that the diabetic patients (cases) are all insulin resistant and less insulin sensitive. Further, bivariate correlations were calculated between different anthropological and biochemical parameters along with calculated indices of IR and IS.

Post prandial plasma glucose of cases showed medium correlation (r=0.358, p=0) with HOMA-IR. Post prandial plasma glucose (r=0.284, p=0.01), serum total nitrite (r=0.24, p=0.04) and serum nitrate (r=0.16, p=0.04) level of cases showed good correlation with QUICKI'S index.

Subsequent multivariate analysis among the indices of insulin resistance (HOMA-IR) and insulin sensitivity (QUICKI), the serum nitrate- nitrite ratio, and the surrogate markers of insulin resistance (namely anthropological markers like waist circumference, hip waist ratio and BMI) failed to yield any significant correlation.

We wanted to see whether the serum nitrate-nitrite ratio and the surrogate markers of insulin resistance could use as predictors of insulin resistance and sensitivity in the study population, but unfortunately, they failed to yield any significant statistical correlation when analysed in multivariate. In other words, while, the post prandial blood glucose and the serum nitrate-nitrite ratio show a good co-relation with HOMA-IR and QUICKI individually, multivariate analysis involving the post prandial blood glucose, the serum nitrate-nitrite ratio, the anthropological markers and biochemical parameters (the surrogate markers of insulin resistance), HOMA-IR and QUICKI could not demonstrate any statistically significant relationship.

DISCUSSION

Insulin resistance is a condition caused by the inability of insulin target tissues to respond properly to insulin. Insulin is the key regulatory hormone of several metabolic functions of the body. In addition insulin plays a very important role in NO mediated vasodilatory action in different body organs. The state of IR precedes the development of IGT and predates the onset of DM by ten to twenty years and is the best clinical predictor of subsequent development of Type II DM.[13-15] IR can be measured by direct markers (like HOMA-IR QUICKI) or by indirect or surrogate markers (i.e. anthropological or biochemical markers).

Insulin resistance is also an integral component of metabolic syndrome, so anthropometric measures of body fat content have been included in the present study. Among them mean value of weight, waist circumference, and waist-hip ratio and BMI show statistically significant increase in cases compared to controls (Table 1). Altered lipid profile in cases as against controls as in Table 2 serves as another surrogate marker of insulin resistance.

Insulin resistance is also associated with accelerated atherosclerosis, hypertension and abnormal vasomotor responses to insulin.[16,17] Endothelial dysfunction represents the earliest abnormality in pathogenesis of atherosclerosis and a central feature of generation.[18] The half-life of NO in blood is very short because of its rapid oxidation into nitrites and nitrates by oxyhaemoglobin, its binding to various cell structures, and the scavenging effects of reactive oxygen species.[19]

Therefore nitrates and nitrites provide alternative tools to estimate NO concentration. In the present study, the value of total nitrite is 46.5±24 micromole/l in diabetics and 82.5±34.5 micromole/l in non-diabetes. Kawakatsu and Ishihara measured the serum total nitrite level in diabetics without complication to be 35.4±16 micromole/l whereas it is 51±29 micromole/l in nondiabetics.[20] NO synthase is classified into three subtypes: neuronal NOS (nNOS) mainly formed in brain, endothelial NOS (eNOS) found in vascular endothelium and inducible NOS (iNOS) mainly by mediators of inflammation and obesity.[21,22] Under physiologic condition, vasodilatory effects of NO (which is eNOS dependent) contributes to insulin mediated glucose uptake in muscle cells.

Stimulation of skeletal muscle blood flow by insulin has been inferred to be NO dependent based on studies employing inhibitors of NOS .Conversely mice with gene disruption of eNOS exhibit insulin resistance, hypertension and dyslipidaemia. Skeletal muscle is a major target for insulin action in man and impaired insulin-stimulated glucose transport is a characteristic defect in type-II diabetes. Serum nitrate and nitrites level might decline in insulin resistance due to reduced NO production or an increase in the NO consumption level.

Bivariate analysis showed post-prandial blood glucose had a strong correlation with HOMA-IR and post-prandial blood glucose, serum total nitrite and nitrate showed strong correlation with QUICKI.

Multivariate analysis could not establish any linear significant correlation between dependent and independent variables. The attempt might not have been fruitful due to small sample size which is not true representative of the population. Also presence of the confounding effects of unforeseen variables (heterogenicity of diet, habit, physical activity, hypertension etc.) which may have influenced dependent or independent variables were not taken into consideration.

Nevertheless, this study gives us an indication that assessment of the NO status in newly diagnosed diabetes mellitus patients could give us an idea about the level of insulin resistance and may help us in predicting the time of manifestation of diabetic complications.

CONCLUSION

In this study, bivariate analysis revealed a strong co-relation among HOMA-IR and post-prandial blood glucose, while QUICKI showed strong correlation with post-prandial blood glucose and the serum nitrates. Both the anthropological (weight, hip-waist ratio and BMI) and biochemical parameters (fasting and post-prandial plasma glucose, lipid profile and serum nitrates) showed a statistically significant difference among cases and controls.

This study may have strong clinical implication in the preventive aspects of long-term follow up of patients of diabetes mellitus. If nitric oxide status could be assessed in insulin resistant states-(by measuring the total nitrites or the nitrate-nitrite ratio) suitable measures (e.g. lifestyle modification, therapeutic intervention, etc.) could be initiated in time to delay the onset and the progression of the dreaded vascular complications that are so characteristic of diabetes.

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