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OXIDATIVE STRESS, SIALIC ACID AND TOTAL ANTIOXIDANT STATUS IN PATIENTS WITH TYPE2 DIABETES MELLITUS

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ABSTRACT

Oxidative stress plays a major role in the pathogenesis of diabetic mellitus. The present study was undertaken to assess lipid peroxidation by TBARS, antioxidant status using FRAP and inflammatory status using sialic acid in patients with Type2 Diabetes mellitus and healthy individuals in rural population. AnthropometricBiochemical, Fasting insulin, HOMA-IR were estimated in both groups. We observed a positive correlation among FBS,TBARS and Sialic acid and they are negatively correlated with FRAP. In this study, we observed that increased blood glucose may play a role in the pathogenesis of oxidative stress which could have lead to reduction of the total antioxidant power.

KEY WORDS: **Type2 diabetes mellitus,Oxidative stress, Thiobarbituric acid (TBARS),HOMA-IR.**

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INTRODUCTION

Diabetes mellitus is characterised by hyperglycemia together with biochemical alteration of glucose and lipid peroxidation (1).Oxidative stress plays a major role in the pathogenesis of diabetic mellitus which is caused by a relative over load of relative oxygen species(2,3). Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (4,5). When the generation of ROS exceeds cellular defense mechanism, the unstable molecules interact with biological macromolecules such as lipids, proteins and DNA and leads to structural changes as well as functional abnormalities(6). Inflammation could be a common antecedent for both diabetes and cardiovascular disease. Hyperglycemia and insulin resistance could also promote inflammation, and may be a factor of linking diabetes to the development of atherosclerosis. Elevated glucose levels could promote inflammation by increased oxidative stress (7). Circulating sialic acid concentration is found to be elevated in inflammatory conditions. (8).So the present study was undertaken to assess lipid peroxidation by TBARS, antioxidant status using FRAP and inflammatory status using sialic acid in patients with Type2 Diabetes mellitus and healthy individuals in rural population.

MATERIALSAND METHODS

Type2 diabetic patients (n=60) of both sexes aged 35-55 years with less than 5 years duration attending and on oral hypoglycemic drugs, diabetic out-patient department of Rajah Muthiah Medical College Hospital, Chidambaram were selected for our study. Healthy, age,, sex matched subjects (n=20) were selected as control. Patients on insulin, Smokers, Alcoholics, Tobacco chewers, Hypertension, and other systemic illness were excluded from this study. Institutional ethical committee of this medical college have approved the study and informed consent obtained from the patients.

Anthropometric measurement

Anthropometric data including height, weight, waist and hip circumferences, and BMI were measured using a standard technique. Body mass index (BMI) was calculated by dividing the weight in kilograms by height in meters squared. We defined BMI ≥ 23 kg/m2 as overweight individuals according to the revised guideline of WHO Western Pacific Region (WHO-WPR 2009).

Biochemical analysis

Fasting venous blood was collected immediately after enrollment in tubes containing EDTA. Blood samples were centrifuged at 2000×g for 10 mins. Samples were analyzed for Fasting Blood Glucose, Lipid Profile(Total Cholesterol, HDL, LDL, Triglycerides), Renal function Tests (Urea, Creatinine), HbA1C, Liver Function tests (Total protein, Albumin, Globulin, ALT, AST, ALP) by using Auto analyzer.

Hormones assay

Serum insulin levels were determined by using Enzyme immuno assay. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as fasting Insulin (mg/dl) x Fasting glucose (mg/dl) divided by405.

Determination of Total Antioxidant Capacity

The total antioxidant capacity of the plasma was determined in terms of Ferric Reducing Ability of Plasma (FRAP) values following the method of Benzie and Strain (9).

Thiobarbituric acid (TBARS)

TBARS levels were measured as an index of lipid peroxidation using the colorimetric method described by Satoh (10). After reaction of thiobarbituric acid with malondialdehyde (MDA), the reaction product was extracted in butanol. Separation of the organic phase was facilitated by centrifugation

at 3000 rpm for 10 mins. and its absorbance was determined spectrometrically at 530 nm.

Total sialic acid

Total plasma sialic acid was assayed using an enzymatic method (Boehringer Mannheim, Lewes, Sussex, UK.)

Statistical analysis

All results were shown as mean ±SD. Results were evaluated using Student's t-test. Simple correlations were determined by Pearson's correlations analysis. P-value <0.05 was considered statistically significant. Statistical analysis was performed using SPSS software.

RESULTS

Table 1
Biochemical Data of Patients and control subjects

PARAMETERS	Patients (n=60)	Control (n=20)	P VALUE
Age	46.4±6.2	41.4±5.4	0.373
Waist Hip Ratio	0.92±0.06	0.90±0.06	0.654
Body Mass Index	24.85±4.4	25.35±5.1	0.871
Serum Total protein (g/dl)	7.63±0.5	7.85±0.5	0.609
Albumin (g/dl)	4.1±0.2	4.2±0.2	0.387
Globulin (g/dl)	3.5±0.4	3.8±0.3	0.817
Blood Urea (mg/dl)	28.1±6.5	25.5±4.3	0.059
Serum Creatinine (mg/dl)	0.80±0.1	0.81±0.1	0.890
Serum Sodium (mmol/l)	136.2±2.9	138.2±1.2	0.000
Serum Potassium(mmol/l)	4.1±0.4	4.1±0.2	0.515
Serum Chloride(mmol/l)	100.3±1.9	100.14±2.7	0.211

Data are expressed as mean±SD, P<0.05 was considered statistically significant.

There were no significant difference in age,waist, hip ratio, BMI,Total protein, Albumin, Globulin, Blood Urea, Creatinine, Potassium, chloride except sodium between Type2 diabetics and control subjects.

Table 2
Glycemic status and lipid profile of patients and control subjects.

PARAMETERS	Patients (n=60)	Control (n=20)	P VALUE
Fasting plasma glucose (mg/dl)	150.50±42.07	77.50±13.48	0.001*
HbA_1C	8.5±0.81	6.5±0.50	0.010*
Serum cholesterol (mg/dl)	165.32±32.8	181.35±25.4	0.177
Serum Triglycerides (mg/dl)	150.90±63.1	161.35±73.8	0.462
HDL cholesterol (mg/dl)	43.60±2.89	44.15±3.2	0.290
LDL cholesterol (mg/dl)	91.48±30.59	108±20.93	0.080
Serum Insulin (U/ml)	14.77±9.43	39.05±14.41	0.006*
HOMA-IR	5.48±4.34	7.41±3.19	0.297
FRAP(μmol/l)	268.71±59.31	355.75±43.55	0.032*
TBARS(mmol/l)	3.37±0.88	1.68±0.46	0.004*
Sialic Acid(mmol/1)	2.28±0.39	1.93±0.19	0.04*

Data are expressed as mean±SD, P<0.05 was considered statistically significant.

According to Table2 all analyses were statistically significant in patients, compared to control subjects (Fasting plasma glucose, HbA_1C, Insulin, FRAP, TBARS, Sialic Acid) except Lipid Profile (Total Cholesterol, HDL, LDL, Triglycerides) and HOMA-IR.

Table 3
Significant Correlation in the Patients Group

PARAMETERS	Correlation Coefficient(r=)	P VALUE
FBS with FRAP	-.797	0.001*
FBS with TBARS	.898	0.001*
FBS with Sialic Acid	.882	0.001*
TBARS with Sialic Acid	.767	0.001*
Sialic Acid with FRAP	-.757	0.001*
FRAP with TBARS	-.790	0.001*

P<0.05 was considered statistically significant.

Table 3 showed that Fasting blood glucose were significantly correlated with TBARS and Sialic Acid (r=0.898,p=0.000 and r=0.882,p=0.000) and TBARS with Sialic Acid further FRAP were found to have a significant negative correlation with FBS and TBARS (r=-0.797,p=0.000).

DISCUSSION

The pathogenesis of microand macro vascular complication of type2 diabetes is partly mediated by oxidative stress (11). The hypothesis that peroxidative tissue damage may be an important factor in the pathogenesis of complications of diabetes has supported by several studies (12-16).These complication seem to be related to excessive production of reactive oxygen species or to a deficiency in antioxidant defence as a result of reduced micronutrient levels or radical induced inactivation of enzyme (13,15). We found that, plasma FRAP levels of diabetes patients were decreased significantly as compared with controls. Decreased FRAP levels attributed to increased oxidative stress in diabetes.

Oxygen free radicals react with all biological substance, however, the most susceptible ones are polyunsaturated fatty acids. Reactions with these cell membrane constituents lead to lipid peroxidation (17,18). Membrane phospholipids, specifically esterified with polyunsaturated fattyacid are converted by peroxidation to MDA, which can be analysed by its reactivity to thiobarbituric acid (19).In our case control study, we have observed that increase in accumulation of lipid peroxidation (plasma MDA levels) and also significant correlation with FBS. Excess nourishment, combined with a sedentary lifestyle, results in over abundance of glucose and fatty acid accumulation within muscle, adipose tissue and pancreatic cells. This might have lead to the generation of excess reactive oxygen species (ROS) and have contributed significantly to the oxidative stress – perhaps even more than chronically elevated blood glucose (20,21).

Chronic inflammation has been postulated to play a role in the pathogenesis of Type2 diabetes (26). Inflammation may be an important modulator of the relationship between obesity and the metabolic syndrome (22). Total sialic acid is a marker of the acute- phase response (23) and also a predictor of cardiovascular events (24) in diabetes (25). Chronic activation of innate immune system which produces the acute-phase response has been postulated to lead insulin resistance and abnormalities in glucose tolerance

and lipid metabolism (26).Sialic acid is a terminal component of the non-reducing end of carbohydrate chain of glycoproteins and glycolipids (27). In an earlier study, elevated plasma lipid status and plasma sialic acid concentrations were observed among individuals who were genetically at high risk for developing diabetes and they were without clinical diabetes (28).Previous studies showed that serum sialic acid concentration were significantly elevated in NIDDM patients compared with nondiabetic subjects and insulin dependent diabetes mellitus (IDDM) patients with miroalbuminuria and clinical proteinuria(29,30). Our findings indicate a significant increase in sialic acid concentration in diabetic patients in comparison with controls and also had a significant positive correlation with FBS and TBARS. Increased glucose metabolism can lead to a rise in mitochondrial production of ROS. ROS production is elevated in obesity, which causes enhanced activation of inflammatory pathways (31, 32).

CONCLUSION

In our study, we observed a positive correlation among FBS,TBARS and sialic acid and they are negatively correlating with FRAP. This suggests that increased blood glucose may play a role in the pathogenesis of oxidative stress leading to reduction of the total antioxidant power as observed in our study. This would cause the inflammation in diabetes which is reflected by increased lipid peroxidation and sialic acid. So maintaining blood glucose level at euglycemic status would help in reduction of inflammatory changes and their consequences.

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