Original Article

Elevated Level of Carbonyl Compounds Correlates with Insulin Resistance in Type 2 Diabetes

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Abstract

Introduction: Recent periodicals direct that reactive carbonyl compounds are formed due to existing oxidative stress in type 2 diabetes mellitus, which further nonenzymatically react with proteins and lipids to form irreversible advanced glycation end products (AGE) and advanced lipoxidation end products (ALE). In type 2 diabetes mellitus, insulin resistance plays a pivotal role in hyperglycaemia. In this study, we tried to find the relation between insulin resistance and carbonyl stress. Materials and Methods: Forty-seven patients of type 2 diabetes mellitus (age 51 ± 5.06 years) were selected and fasting plasma glucose, serum insulin, total carbonyl compounds, HbA1c, thiobarbituric acid reacting substances (TBARS) and Trolox equivalent antioxidant capacity (TEAC) were estimated using standard protocols. Homeostatic model assessement of insulin resistance (HOMA-IR) was evaluated from fasting plasma glucose and serum insulin levels. Results: We found highly significant correlations of carbonyl compounds with HOMA-IR, fasting plasma glucose and glycated haemoglobin (HbA1c). Correlations of lipid peroxidation end product, TBARS were not so significant. Conclusion: Findings from this study indicate that the level of carbonyl compounds can be a biomarker of insulin resistance in type 2 diabetes mellitus.

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Introduction

With rapid development of therapy, the mortality from acute complications of diabetes mellitus (DM) has decreased, but mortality from chronic complications like diabetic nephropathy has increased.[1] The incidence and prevalence of type 2 DM is increasing in developing countries.[2] Diabetes mellitus is a clinical syndrome characterised by chronic hyperglycaemia due to absolute or relative deficiency of insulin.[3] Insulin resistance is a prominent feature of type 2 DM and contributes significantly to hyperglycaemia. Chronic hyperglycaemia plays a causative role in the pathogenesis of diabetic microvascular complications.[4] Homeostatic model assessment of insulin resistance (HOMA-IR) is one of the frequently used indices of insulin resistance.[5,6] Intracellular hyperglycaemia in insulin-independent cells like erythrocyte, nerve, kidney and lens leads to the formation of advanced glycation end products (AGE) via nonenzymatic glycosylation of intra and extracellular proteins. Oxidative stress is a feature of type 2 diabetes.[7,8] This oxidative stress modifies body proteins, carbohydrates and lipids with generation of reactive carbonyl compounds. These compounds may also be formed by other mechanisms and may further modify proteins and , in turn, lead to development of AGE like carboxymethyl lysine and pentosidine.[7,9] AGE have been shown to cross-link proteins (i.e. collagen, extracellular matrix proteins), reduce nitric oxide synthesis, induce endothelial dysfunction, and accelerate atherosclerosis.[3,10] Studies have already shown the role of carbonyl stress in end-organ complications in type 2 diabetes including diabetic nephropathy.[7] Insulin resistance leads to hyperglycaemia and long-term hyperglycaemia is related directly or indirectly with chronic complications of diabetes.[1] In the present study, we tried to assess the correlation between carbonyl stress with insulin resistance.

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Materials and Methods

Patient Selection

The current study was done in the Department Bio-Chemistry, N R S Medical College Kolkata. It included 47 patients of clinically diagnosed type 2 diabetes mellitus attending the diabetic outpatient department (OPD) of the institution. Thirty-one were male and 14 female in the age group of mean 51 ± 5.06 years. Hypertensives patients with acute complications, severely ill, unconscious and disabled patients were not included in the study. According to the laboratory reports, liver and renal functions were within normal limits. The patients were not receiving insulin for at least 2 weeks before sample collection. There was no recent history of stroke or myocardial infarction. Informed consent was obtained from the patients. The study protocol was approved by the ethical committee of N R S Medical College.

Sample Collection

The amount of blood collected in absolute fasting condition with all aseptic precautions in 3 parts was 5 ml. The first part collected in ethylene diamine tetra-acetic acid (EDTA) vial for estimation of glycated haemoglobin (HbA1c) and thiobarbituric acid reacting substances (TBARS) while the second part in plain vial for determination of serum insulin, carbonyl and trolox equivalent antioxidant capacity (TEAC). The third part, kept in fluoride-oxalate vial was used to determine the level of fasting plasma glucose.

Biochemical Analysis

Blood glucose was determined using reagent kit (Merck) by Glucose oxidase method.[11] Serum insulin was determined by ELISA with monoclonal antibody based reagent (Monobind).[12] Glycosylated haemoglobin, the index of long-term glycaemic control, was determined with Micromat II (Biorad) instrument based on boronate affinity chromatography.[13]

TBARS, the product of lipid peroxidation, was determined in plasma using the method of Okhawa.[14] It is based on the Spectrophotometric estimation at 532 nm of coloured products extracted in butanol. The colour developed when the sample reacted with thiobarbituric acid in presence of sodium dodecyl sulphate (SDS). Carbonyl compounds were determined by the method proposed by Cooper,[15] used reaction with DNPH in an acidic medium and determination of absorbance at 576 nm.

Trolox equivalent antioxidant capacity which signifies the total antioxidant status of our body was determined according to $ABTS^+$ decolourisation assay of Re R et al,[16] based on the inhibition of radical cation of ABTS, which has the characteristic of long wavelength absorbance maximum at 734 nm.

Statistical Analysis

Analysis of data was done after computing the data for graphical and statistical analysis in available statistical package.

Results of the present study have been summarised in the Tables 1 to 4. The mean and standard error of all parameters are shown in Table 1. The mean fasting plasma glucose is much above normal range (70 to 110 mg/dl). The mean HbA1c is also above reference range (4% to 6%). Using a median of 8.5% HbA1c, we divided the patient population into 2 groups: one group (n = 26) with HbA1c at and above the median and the other group (n = 21) below the median. The comparisons of the means of the different parameters between the two groups were made and were represented in Table 2. The rise in carbonyl compounds was more profound in the higher mean HbA1c level group (P <0.01). A similar pattern is observed for HOMA-IR, the index of insulin resistance. Table 3 shows the Pearson correlation coefficients among the different variables. Significant correlation has been observed between fasting plasma glucose with HbA1c, carbonyl compounds and HOMA-IR, although correlation with TBARS was not that significant. Serum insulin had no significant correlation at all. The correlations of carbonyl compounds were found to be highly significant (P <0.01) with fasting plasma glucose (r = 0.42), HbA1c (r = 0.50), and HOMA-IR (r = 0.30). TEAC was found to have no significant correlations.

Carbonyl stress is shown to be related directly with short- and long-term glycaemic control. The marker of lipid peroxidation, TBARS, had a very significant correlation with fasting glucose, but the other correlations were not significant. Finally, the regression analysis in Table 4 shows carbonyl compounds as the best predictor for HOMA-IR, over and above HbA1c and TBARS.

Table 1. Mean Concentration (± SE) of Different Parameters in the Patients of Type 2 DM (n = 47)

Parameters	Mean	SE
FPG (mg/dl)*	178.52	12.07
HbA1c (% of total)	8.97	0.33
Insulin (μIU/L)	10.94	1.55
TBARS (nmol/ml)	3.39	0.43
Carbonyl (nmol/ml)	158.37	4.76
HOMA-IR	6.82	0.58
TEAC(mmol/l)	2.26	0.07

*FPG: fasting plasma glucose

Table 2. Mean Concentration (± SE) and t Values of Different Parameters among the Higher and Lower HbA1c groups (Median = 8.5)

Parameters	(HbA1c > 8.5, n = 26)		(HbA1c < 8.5, n = 21)		
	Mean	SE	Mean	SE	Significance
FPG (mg/dl)	218.14	17.44	129.49	7.87	*P* <0.01
Insulin (μIU/L)	13.73	2.06	7.5	2.17	*P* <0.05
TBARS (nmol/ml)	3.93	0.69	2.74	0.39	not significant
Carbonyl (nmol/ml)	169.1	6.02	145.1	6.68	*P* <0.01
HOMA-IR	9.23	0.70	3.84	0.42	*P* <0.01
TEAC (mmol/l)	2.24	0.43	2.27	0.52	not significant

Table 3.Values of Pearson's Coefficient of Correlation among the Different Parameters of the Type 2 Diabetics (n = 47)

	Glucose	HbA1c	Insulin	TBARS	Carbonyl	HOMA-IR	
Glucose	-						
HbA1c	0.77†	-					
Insulin	0.12	0.09	-				
TBARS	0.31*	0.27	0.04	-			
Carbonyl	0.42†	0.5†	0.26	0.27	-		
HOMA-IR	0.46†	0.30*	0.82	0.13	0.47†	-	
TEAC	-0.03	0.02	-0.03	-0.17	0.02	0.005	-

*P <0.05; †P <0.01

Table 4. Multiple Regression Analysis Showing Contributing Variables to HOMA-IR in the Patients with Type 2 DM (n = 47)

Criterion variable	Predictor variable	R_2	β
Insulin resistance	carbonyl	0.117	0.342
(HOMA-IR)	HbA1c	0.031	0.177
	TBARS	0.001	0.039

Figure 1 shows the correlation between HOMA-IR and carbonyl compounds in type 2 diabetes mellitus (n = 47, r = 0.467, Y = 0.06X – 2.2).

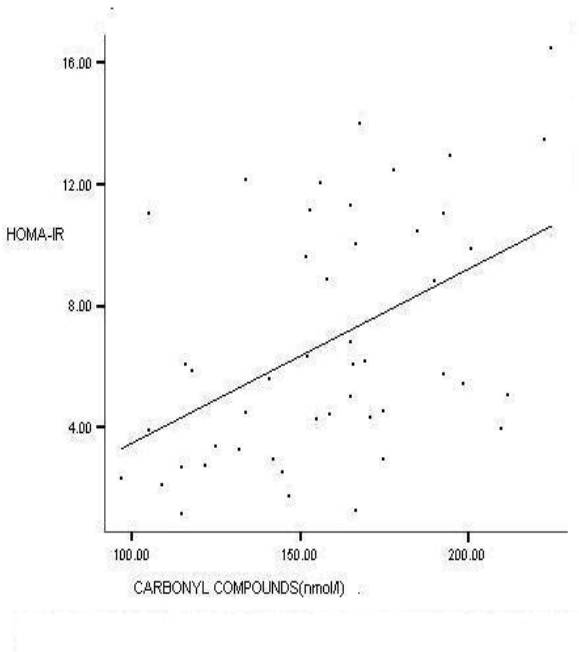

Fig. 1. Correlation between HOMA-IR and carbonyl compounds in type 2 diabetes mellitus (n = 47, r = 0.467, Y = 0.06X – 2.2).

Discussion

Although genetic and environmental factors are responsible for type 2 DM, it is insulin resistance, which is a prominent feature of the disease. Homeostatic Model of assessment of insulin resistance (HOMA-IR) is based on fasting glucose and insulin levels. Several workers are currently using it as a standard index of insulin resistance.[17] Insulin resistance is relative and it is only evident when the patient presents with hyperglycaemia. However, this may happen much later in the course of disease. Type 2 DM is associated with chronic complications including microvascular such as eye disease, nephropathy and neuropathy and macrovascular such as cerebrovascular and coronary artery disease. As many as 50% of individuals

with Type 2 DM have one or more diabetes specific complications at the time of their diagnosis. It has been reported that strong electrophilic carbonyl compounds which are generated slowly in the disease process can glycate several proteins containing basic amino acids and alter their functional properties.[18,19] Studies from our laboratory have shown that in type 2 DM, increased formation of carbonyl compounds like methylglyoxal were significantly correlated negatively with reduced glutathione and positively with TBARS.[20] Thus 'carbonyl stress' may be a causative factor for lipid peroxidation and chronic complications of type 2 DM. It has been recently reported that methylglyoxal, an important carbonyl compound causes an inhibition of insulin stimulated phosphorylation of protein kinase B without affecting insulin receptor tyrosine kinase. It is also reported that this deleterious effect appeared to be a direct consequence of impairment in insulin induced phosphorylation of substrates.[21] Insulin hormone with its small secondary structure contains one arginine residue in 22nd position of the B chain. The adjacent 23 to 25 part of the B chain is responsible for binding to insulin receptor. Any change in this region will affect the biological activity.[22,23] It can be hypothesized that elevated level of carbonyl compounds like methylglyoxal may nonenzymatically glycate arginine (B22) and inhibit receptor binding of the peptide.

Conclusions

The findings of our study have clearly shown a significant positive correlation between the level of carbonyl compounds and HOMA-IR. Our study also shows that HOMA-IR is the best predictor of insulin resistance. The above observations may help us to conclude that carbonyl stress can be used as a most valuable tool to assess insulin resistance in type 2 DM. Suzuki et al[1] had shown the relationship between diabetic nephropathy and carbonyl stress. Thus therapeutic measures to control carbonyl stress may be a serious consideration in treating type 2 diabetes mellitus.

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