Research article

The ABC (HbA1c, blood pressure and LDL-cholesterol) of diabetes and oxidative stress: knowing the links

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ABSTRACT

Introduction and Aim: Large body of evidence suggests that oxidative stress has a crucial role to play in diabetes and its related complications. In the present research we have tried to determine potential risk factors for oxidative stress in type 2 diabetes by conducting a cross-sectional study.

Materials and Methods: We studied 238 patients with or without complications of diabetes, aged 40-75 years. Blood samples were analysed for fasting plasma glucose, glycated haemoglobin, malondialdehyde, total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol. Each patient's waist circumference, BMI, blood pressure was measured.

Results: Participants mean age was 57.5 ± 8.9 years. Logistic regression analysis identified glycated haemoglobin (95% CI: 1.02-1.92; p=0.04), hypertension (95% CI: 0.99-4.08; p=0.05) and LDL-cholesterol (95% CI: 1.12-5.87; p=0.03) as independent risk factors for oxidative stress. Other contributors were age and presence of complications like nephropathy, retinopathy, peripheral neuropathy and macrovascular disease in diabetes.

Conclusion: Our data demonstrate that 'ABCs of diabetes' namely HbA_1c, blood pressure and LDL-cholesterol are independent risk factors for oxidative stress. Oxidative stress aggravates with age and presence of complications. It appears important to achieve a good ABC goal to bring down oxidative stress in diabetes.

Keywords: HbA_1c; blood pressure; LDL-cholesterol; type 2 diabetes mellitus; oxidative stress.

INTRODUCTION

Oxidative stress is a state of imbalance between prooxidants and antioxidants in the living organism. In diabetes mellitus, glucose oxidation is the main source of free radical generation. Lipid peroxidation is a free radical mediated damage of polyunsaturated fatty acids (PUFA) in cell membranes to form hydroperoxides. Malondialdehyde (MDA) is one such by-product of lipid peroxidation which is increased in obesity, metabolic syndrome and type 2 diabetes mellitus (1). Hyperglycemia can induce oxidative stress by several mechanisms such as glucose oxidation, advanced glycation end product (AGE) formation, polyol pathway and protein Cβ 1/2 kinase (1,2). Protein kinase C is linked to the occurrence of pathologies (3) affecting large vessel (atherosclerosis, cardiomyopathy) and small vessel (retinopathy, nephropathy and neuropathy). In diabetes, hyperglycemia can lead to the accumulation of more oxidised LDL particles which are not recognized by LDL receptors. These are taken up by macrophages leading to foam cell formation and atherosclerotic plaques (4).

Metabolic abnormalities such as diabetes and dyslipidemia are often accompanied with hypertension. It is estimated that 30-60% of diabetes patients have associated hypertension (5). Superoxide anion, a free radical is a major determinant of nitric oxide (NO) bioavailability. In hypertension there is reduced NO levels and an increase in oxidative stress (6). Various enzymatic and non-enzymatic antioxidants are also compromised contributing to oxidative stress (7,8). Recent evidence indicate that LDL-cholesterol is a predictor of both oxidative stress and endothelial function (9). Possible explanation to this is that oxidized LDL-cholesterol may result in superoxide anion synthesis in endothelial cells, also via NADPH oxidase (10). Hypertension, hypercholesterolemia and diabetes have all been reported to upregulate NADPH oxidase (11). Yet, the role of oxidative stress in diabetes and its complications is not completely understood. With this

background, the current study was undertaken to see whether hypertension, hyperglycemia, hyperlipidemia and diabetes related complications are predictors of oxidative stress. The present study was undertaken with the aim to evaluate the relationship of various risk indices and types of complications in type 2 diabetes mellitus with oxidative stress.

MATERIALS AND METHODS

The present prospective study was performed on 238 diabetes patients who visited Kasturba Medical College Hospital. Prior to baseline visit, the severity of diabetes complications was noted down according to the details in medical records. Based on this, patients were stratified such that a minimum of n=50 were recruited from individual subgroups (diabetes without complications, macrovascular, microvascular, peripheral neuropathy groups). Inclusion criteria being type 2 diabetes mellitus with or without complications, aged 40-75 years. Exclusion criteria to be type 2 diabetes mellitus with acute macrovascular disease, and patients also suffering from cancer, rheumatoid arthritis, pulmonary tuberculosis, infections or any kind of severe illness which can affect oxidative stress levels. The study protocol was approved by Kasturba Medical College Review Board, Mangalore (IEC KMC MLR 10/05/321) and was registered in Clinical Trials Registry-India. All subjects gave a written informed consent.

Outcome measures

At the baseline visit, venous blood was collected after an overnight fast. Parameters included- fasting plasma glucose, glycated haemoglobin, malondialdehyde, total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol concentrations. Blood glucose was assayed by GOD-PAP method. In whole blood glycated hemoglobin (HbA_1c) was measured by particle enhanced immunoturbidimetric method (Dia Sys diagnostic kits, Holzheim, Germany). Serum total cholesterol was determined by CHOD-PAP method, triglyceride by GPO-PAD method. HDL-cholesterol was estimated using homogeneous enzymatic colorimetric method and LDL-cholesterol by direct method. Assays were carried out in Hitachi 917 random access chemistry analyzer using reagent kits supplied by Roche diagnostics. Malondialdehyde was measured in RBCs by Stocks and Dormandy (12) method.

Waist circumference was taken in a standing position using a non-stretchable tape measured halfway between lowest rib and the top of right iliac crest. Blood pressure was measured in seated position when the subject was completely relaxed. Height and weight were recorded to determine body mass index (BMI) which is the ratio of weight (kg) to height squared (m^2). Blood pressure above 130/85 mmHg was considered hypertension. This group of diabetes subjects included patients consuming antihypertensive medicines, even though by treatment if they achieve a blood pressure level that is within target range.

Statistical methods

Statistical package SPSS version 16.0 was applied. Continuous variables are shown as mean ± standard deviation (SD). Analysis was done using chi-square test and F test as a univariate analysis. Logistic regression was applied to those factors which were significant in the univariate analysis. Oxidative stress was the dependent variable of interest. A p value < 0.05 was considered significant.

RESULTS

Total 238 diabetes patients were stratified into 4 groups; without complications (n=62), macrovascular disease (n=57), microvascular disease (n=57) and peripheral neuropathy (n=62). Basis for stratification was severity of complication for which they were been treated at the time of recruitment. Baseline characteristics of participants are given in Tables 1 and 2. The mean age of patients was 57.5 ± 8.9 years. More number of female patients were recruited in the study but was not significant. All patients in this study were on anti-diabetic / antihypertensive drugs.

Table 1: Baseline characteristics of diabetes subjects

Measurements	**Mean ± SD**
Age (years)	57.5 ± 8.9
Gender (male/female)	101/137
Duration of diabetes (years)	7.1 ± 5.8
Duration of hypertension (years)	3.5 ± 5.8
Family history of diabetes, yes	139 (58.4%)
no	77 (32.4%)
not known	22 (9.2%)
Smoking status, yes	4
no	234
Alcohol status, frequent	4
occasional	10
no	224

Table 2: Baseline parameters of diabetes subjects

Parameters	**Mean ± SD**
BMI (kg/m^2)	25.29 ± 3.85
Waist circumference (cm)	90.63 ± 9.74
Blood pressure (systolic) (mmHg)	139.89 ± 20.96
Blood pressure (diastolic) (mmHg)	84.32 ± 10.01
Fasting plasma glucose (mg/dL)	154.25 ± 55.19
Glycated haemoglobin (%)	8.02 ± 1.57
Malondialdehyde (µmol/L)	5.09 ± 0.91
Total cholesterol (mg/dL)	208.51 ± 47.84
Triglycerides (mg/dL)	144.64 ± 65.10
HDL-cholesterol (mg/dL)	41.44 ± 8.12
LDL-cholesterol (mg/dL)	140.25 ± 50.28

Logistic regression analysis using risk factors that could be related to oxidative stress was conducted in this population. Oxidative stress was significantly higher with increase in age, glycated haemoglobin and

Table 3: Logistic regression analysis showing the relationship between oxidative stress and other variables for the total group

Risk factors	Wald	p value	Odds ratio	95% confidence interval	
				Lower	Upper
Age (years)	1.33	0.25	1.42	0.79	2.55
Hypertension	3.73	0.05*	2.01	0.99	4.08
Macrovascular disease	0.32	0.57	1.24	0.59	2.64
Nephropathy	2.44	0.12	2.00	0.84	4.79
Retinopathy	1.24	0.27	1.57	0.71	3.44
Peripheral neuropathy	0.50	0.48	1.27	0.65	2.48
Glycated haemoglobin (%)	4.24	0.04*	1.40	1.02	1.92
Total cholesterol (mg/dL)	0.04	0.83	1.10	0.48	2.47
Triglycerides (mg/dL)	0.47	0.49	1.07	0.64	2.51
HDL-cholesterol (mg/dL)	0.00	0.99	0.99	0.47	2.12
LDL-cholesterol (mg/dL)	4.96	0.03*	2.56	1.12	5.87

Oxidative stress is dependent variable. Independent parameters are age, hypertension, glycated haemoglobin, total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, presence of macrovascular disease, nephropathy, retinopathy, peripheral neuropathy. Values are mean ± SD, $p<0.05$ considered significant (*)

LDL-cholesterol (Table 3). Presence of hypertension and diabetes related complications like nephropathy, retinopathy, peripheral neuropathy and macrovascular disease aggravated oxidative stress. We observed that the ratio of odds of outcome of interest i.e., oxidative stress among people with hypertension as a confounder was 2.01 (95% CI: 0.99-4.08; p=0.05) times the odds of OST in people without hypertension. Other risk factors were HbA_1c, odds ratio 1.40 (95% CI 1.02-1.92; p=0.04) and LDL-cholesterol, odds ratio 2.56 (95% CI 1.12-5.87; p=0.03). The analysis identified hypertension, HbA_1c and LDL-cholesterol as highly significant predictors of elevated oxidative stress in type 2 diabetes patients.

DISCUSSION

The foremost finding of the current analysis is that hypertension, HbA_1c and LDL-cholesterol are independent risk factors for oxidative stress in type 2 diabetes mellitus. Glycated hemoglobin, hypertension and LDL-cholesterol form the 'ABC of diabetes'. The current study is to our knowledge, the first to look into the predominant role of ABC goal in determining oxidative stress in type 2 diabetes patients. Oxidative stress is also elevated with increase in age and presence of complications.

Studies in the past have shown that oxidative stress is associated with ageing (13). In our previous study conducted in elderly and younger population of type 2 diabetes patients we observed that oxidative stress parameters are elevated in the elderly group (14). Several studies in the past have shown that oxidative stress play a central role in the occurrence and progression of diabetes complications (15-17). Hypertension can also induce endothelial dysfunction through oxidative stress (18). Erciyas *et al.,* (19) in their study showed that MDA and MDA - LDL cholesterol levels were significantly higher in poorly controlled diabetes patients and there is a positive association between lipid profile and oxidative stress. In diabetes, variability in blood glucose is channelled to increased amounts of circulating AGE's and thereby oxidative stress (17, 20). Tan *et al.,* (21) reported by multiple regression model that there is an independent correlation between AGE levels and insulin resistance.

The cause and effect of oxidative stress in diabetes is least understood. Results of our study are in accordance with earlier data. All these results point in the same direction i.e., oxidative stress is affected by age, hypertension, hyperglycemia, LDL-cholesterol and complications of diabetes mellitus. The current study further adds to the existing data that glycated haemoglobin, hypertension, and LDL-cholesterol are the three independent risk factors for elevating oxidative stress in diabetes mellitus. 'ABC' variability in diabetes can be the root cause for altered prooxidant/antioxidant balance which further can lead to the development of complications. Future studies are needed to find better approaches to achieve the 'ABC' goal with a focus on nutritional and lifestyle interventions for oxidative stress prevention.

CONCLUSION

In conclusion, the present study demonstrates that glycated haemoglobin, hypertension, and LDL-cholesterol are the independent risk factors for oxidative stress in type 2 diabetes mellitus. These form the ABC goal for a diabetes patient. Oxidative stress is elevated with higher age and presence of complications. The results presented herein suggest that any strategies targeted at achieving ABC goal is likely to keep oxidative stress under check.

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CONFLICT OF INTEREST

None declared

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