Glucose Fluctuations and Activation of Oxidative Stress in Patients with Type II Diabetes Mellitus

ABSTRACT

Introduction: In recent years, the oxidative stress (OS)-induced free radicals have been implicated in the pathology of diabetes mellitus (DM). Persistently high glucose levels can lead to the generation of higher amounts of free radicals. The purpose of the present study was to evaluate the role of hyperglycemia [by measuring variables: Glycated hemoglobin (HbA1c), fasting plasma glucose (FPG)] in the induction of OS [(by analyzing the OS marker: Malondialdehyde (MDA))] in type II DM.

Materials and methods: This observational study was conducted among 50 type II DM patients without complications and 50 type II DM patients with complications in S.B.K.S. Medical Institute and Research Centre, Waghodiya, Gujarat, India. Correlations between variables were tested using the Pearson rho correlation test. Chi-squared ($\chi^2$) analysis was used for comparison of groups.

Results: The MDA values correlated significantly with HbA1c and FPG values in type II diabetic subjects with complications ($r = +0.29$, $p = 0.04$; $r = +0.47$, $p = 0.0006$).

Conclusion: Glucose fluctuations may activate OS in DM. Assessment of glycemic control marker HbA1c and lipid peroxidation marker MDA is useful in DM patients for detection of risk of diabetic complications at an early stage of the disease.

Keywords: HbA1c, Lipid peroxidation, Malondialdehyde, Oxidative stress.

INTRODUCTION

The term DM describes a metabolic disorder with heterogeneous etiologies, which is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The long-term complications of diabetes include development of retinopathy, nephropathy, and neuropathy and increased risks for cardiac, peripheral, arterial, and cerebrovascular diseases. Type II diabetes results from the body’s ineffective use of insulin.

Among the various markers of glycemic control, HbA1c has now been established as the most reliable. It is used as an ideal diagnostic test for screening of persons at high risk of diabetes. The human body is exposed to free radicals from outside the body (exogenous) and inside the body (endogenous). The cells utilize oxygen to produce the energy they need to work properly. Free radicals are produced in the process known as mitochondrial respiration, wherein the cells take in oxygen, burn it, and release energy. Oxidative stress occurs when free radical production exceeds the body’s ability to neutralize them. Abnormal HbA1c levels reflecting poor glycemic control have been suggested as a significant contributor of OS.

It has been suggested that fluctuating blood glucose concentrations may contribute significantly to OS. Increased glucose flux both enhances oxidant production and impairs antioxidant defenses by multiple interacting pathways. The DM can cause increased production of these endogenous free radicals and reduced antioxidant resistance. The OS functions on both sides, meaning that it helps in the progression and the development of DM and its complications.

Reactive oxygen species produced in the condition of OS degrades polyunsaturated lipids, forming MDA. This compound is a reactive aldehyde and forms advanced glycation endproducts (AGEs). The MDA is used as a biomarker to assess the level of OS in an organism. The purpose of the present study was to evaluate the role of hyperglycemia in the induction of OS and its effect in the pathogenesis of complications in type II DM.

MATERIALS AND METHODS

The study was conducted at the S.B.K.S. Medical Institute and Research Centre, Waghodiya, Gujarat, India. The subjects included in the study were:

- 100 healthy controls—group I (50 males and 50 females),
- 50 (25 males, 25 females) type II DM patients without complications—group II, and
• 50 (25 males, 25 females) type II DM patients with complications—group III attending the outpatient clinics or admitted in wards. The study subjects had DM for 5 to 16 years. All subjects were age; body mass index and sex matched.

Criteria for Selection of Diabetic Subjects

Type II diabetic subjects with complications were selected as known cases of various complications: Microvascular and macrovascular, such as retinopathy, nephropathy, peripheral vascular diseases, cardiovascular diseases, and cerebrovascular diseases. Type II DM subjects with complications were selected from the indoor/outdoor patients of Medicine/Cardiology Department.

Inclusion Criteria for Type II Diabetic Subjects

Diabetes was diagnosed according to the American Diabetes Association revised criteria (2008). Fasting is defined as no caloric intake for at least 8 hours (in the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day) or symptoms of hyperglycemia and a random plasma glucose >200 mg/dL. The classic symptoms of hyperglycemia include polyuria, polydipsia, and unexplained weight loss or 2-hour plasma glucose >200 mg/dL during an oral glucose tolerance test. In this study, we considered the absence or presence of diabetic complications and not the severity of complications. Guidelines given by the National Cholesterol Education Programme (NCEP) Adult Treatment Plan (ATP) III were followed to diagnose dyslipidemia in the type II DM subjects. The diabetic subjects included in the study were nonhypertensive.

Exclusion Criteria for Type II Diabetic Subjects

The diabetic subjects included in the study were nonhypertensives. Patients with active inflammatory diseases, nutritional deficiencies, estrogen therapy, malignancy, and active immunological diseases were not considered for the study. Patients with these diseases were excluded from the study. Also, smokers and alcohol consumers as well as patients with hypertension (According to NCEP III criteria >130/85 mm Hg) were excluded from the study.

Approval of the local ethics committee was taken to conduct the study. Each patient provided written informed consent for study participation and the use of their data for research purposes. Blood samples were drawn from patients and controls, after overnight fast. Aliquots of serum samples were prepared by transferring them into separate plain vials. These were labeled properly and stored at −20°C until assayed. For estimation of MDA, plain vacutainers were used. The MDA was analyzed by colorimetric method as given by Satoh using thiobarbituric acid reactive substances assay. Kit employed was from Merck Diagnostics (MAK085 SIGMA Lipid Peroxidation). For plasma glucose estimation, fluoride vacutainers were used. Plasma glucose was estimated by glucose oxidase–peroxidase method, and for estimation of HbA1c, ethylenediaminetetraacetic acid vacutainers were employed. The HbA1c was estimated by cation exchange resin method, respectively. Commercially available kits were used for testing purposes (manufacturer Diasys Diagnostic Systems GmbH). Instrument used was ERBA XL-300. The analysis of various parameters was performed on ERBA Mannheim’s Erba XL300. Differences in the parameters between the groups were analyzed by means of Student’s t-test. Variables were presented as mean ± standard deviation (SD). Correlations between variables were tested using the Pearson rho (r: Correlation coefficient) correlation test. Chi-squared (χ²) analysis was used for comparison of groups. Data were compared in the groups using Statistical Package for the Social Sciences for Windows (version 16; SPSS Inc., Chicago, Illinois, USA); p < 0.05 was considered as a statistically significant level.

RESULTS AND DISCUSSION

The mean FPG values in type II diabetic subjects were significantly higher (p < 0.0001) than the values obtained in healthy controls (Table 1). Also, in the study, Table 1 shows that the mean HbA1c levels were significantly high (p < 0.001) in type II diabetic subjects with complications [mean HbA1c: 11.1%; 95% confidence interval (CI) 10.8, 11.3] compared with type II diabetic subjects without complications (mean HbA1c: 9.57%; 95% CI 9.1, 9.7). The results indicate that sustained hyperglycemia may be a major factor of diabetic complications. Reactive oxygen species and OS are increased under hyperglycemia. Oxidative stress is widely regarded as the mechanism by which glycemic variations may induce diabetic complications. Several pathways may be initiated due to hyperglycemia-induced OS: (1) The polyol pathway; (2) hexosamine pathway; (3) protein kinase C activation; and (4) formation of AGEs. Any period of disease can be considered a period of stress, and therefore, some degree of hyperglycemia is normal during these times, and can be seen as initially protective and part of the adaptive response for survival. However, in acute and severe diseases, the resulting hyperglycemia can be much too high and require glycemic control therapy to manage. Elevated plasma MDA levels were observed in type II diabetic subjects. This is considered as a marker of OS. Mean values of MDA in type II diabetic subjects with complications and without complications were significantly higher (p < 0.001; p = 0.003 respectively)
It is believed that the resulting hyperglycemia is due to insufficient insulin secretion. It is also believed that insulin resistance plays a factor in chronic disease with significant amounts of tissue injury.\textsuperscript{11,12} The role of HbA1c and FPG in causing complications of DM can be further elucidated by its positive correlation with MDA in type II diabetic subjects with complications. A significant positive correlation was observed between MDA and HbA1c as well as MDA and FPG values in type II diabetic subjects with complications (Table 2).

**CONCLUSION**

The estimation of lipid peroxide (MDA) along with HbA1c in DM would serve as a useful monitor to judge the prognosis of the patient.

**REFERENCES**


**(Table 1)** than values observed in healthy controls (Table 1). It is believed that the resulting hyperglycemia is due to insufficient insulin secretion. It is also believed that insulin resistance plays a factor in chronic disease with significant amounts of tissue injury.\textsuperscript{11,12} The role of HbA1c and FPG in causing complications of DM can be further elucidated by its positive correlation with MDA in type II diabetic subjects with complications. A significant positive correlation was observed between MDA and HbA1c as well as MDA and FPG values in type II diabetic subjects with complications (Table 2). Glucose fluctuation has been shown to cause overproduction of superoxide and OS generation. Additionally, glucose fluctuation has also been shown to cause loss of pancreatic β-cells due to increased apoptotic cell death. The loss of pancreatic β-cells may result in deterioration of glycemic control and subsequent progression of vascular complications or poor prognosis.\textsuperscript{13}

**CONCLUSION**

The estimation of lipid peroxide (MDA) along with HbA1c in DM would serve as a useful monitor to judge the prognosis of the patient.

**REFERENCES**


**(Table 1)** than values observed in healthy controls (Table 1). It is believed that the resulting hyperglycemia is due to insufficient insulin secretion. It is also believed that insulin resistance plays a factor in chronic disease with significant amounts of tissue injury.\textsuperscript{11,12} The role of HbA1c and FPG in causing complications of DM can be further elucidated by its positive correlation with MDA in type II diabetic subjects with complications. A significant positive correlation was observed between MDA and HbA1c as well as MDA and FPG values in type II diabetic subjects with complications (Table 2). Glucose fluctuation has been shown to cause overproduction of superoxide and OS generation. Additionally, glucose fluctuation has also been shown to cause loss of pancreatic β-cells due to increased apoptotic cell death. The loss of pancreatic β-cells may result in deterioration of glycemic control and subsequent progression of vascular complications or poor prognosis.\textsuperscript{13}

**CONCLUSION**

The estimation of lipid peroxide (MDA) along with HbA1c in DM would serve as a useful monitor to judge the prognosis of the patient.

**REFERENCES**


**(Table 1)** than values observed in healthy controls (Table 1). It is believed that the resulting hyperglycemia is due to insufficient insulin secretion. It is also believed that insulin resistance plays a factor in chronic disease with significant amounts of tissue injury.\textsuperscript{11,12} The role of HbA1c and FPG in causing complications of DM can be further elucidated by its positive correlation with MDA in type II diabetic subjects with complications. A significant positive correlation was observed between MDA and HbA1c as well as MDA and FPG values in type II diabetic subjects with complications (Table 2). Glucose fluctuation has been shown to cause overproduction of superoxide and OS generation. Additionally, glucose fluctuation has also been shown to cause loss of pancreatic β-cells due to increased apoptotic cell death. The loss of pancreatic β-cells may result in deterioration of glycemic control and subsequent progression of vascular complications or poor prognosis.\textsuperscript{13}

**CONCLUSION**

The estimation of lipid peroxide (MDA) along with HbA1c in DM would serve as a useful monitor to judge the prognosis of the patient.

**REFERENCES**