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Archives of Medical Research 38 (2007) 822–826

Archives of Medical Research

ORIGINAL ARTICLE

Enhanced Glycation of Hemoglobin and Plasma Proteins Is Associated with Increased Lipid Peroxide Levels in Non-Diabetic Hypertensive Subjects

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Received for publication March 5, 2007; accepted May 2, 2007 (ARCMED-D-07-00097).

Background. Accumulating evidences indicate that lipid peroxidation and protein glycation play a vital role in the pathogenesis of cardiovascular disease. The purpose of the present study was to evaluate the levels of lipid peroxides and glycated proteins in non-diabetic hypertensive patients and to assess the possible nexus between them, among these subjects.

Methods. Thirty hypertensive patients and 25 normotensive subjects were enrolled in the present study. Lipid peroxides, glycated hemoglobin, and fructosamine levels were estimated in both groups.

Results. Lipid peroxides, glycated hemoglobin, and fructosamine levels were significantly increased in hypertensive subjects in comparison with normotensive subjects. When partial correlation analysis was performed, malondialdehyde was significantly associated with glycated hemoglobin and fructosamine.

Conclusions. An increased glycation of proteins was found in non-diabetic hypertensive subjects. These data also support the premise that lipid peroxidation per se plays a role in glycation of hemoglobin and plasma proteins. © 2007 IMSS. Published by Elsevier Inc.

Key Words: Hypertension, Lipid peroxides, Glycated hemoglobin, Fructosamine.

Introduction

Oxidative stress has been recognized as a general mechanism relevant in the pathogenesis of several human diseases (1). It is triggered by exposure to exogenous factors or by chemicals producing reactive oxygen species and is associated with an overproduction of reactive oxygen species, as well as an impairment of antioxidant defense capacity (2). Hypertension has been shown to be one of the conditions associated with decreased antioxidant capacity (3–5).

Oxidative damage to unsaturated lipids is a well-established general mechanism for oxidant-mediated cellular injury (6). In addition to extensive experimental studies, increased lipid peroxidation has been reported in a wide variety of clinical and toxicological conditions (7). Among the various pathological aberrations linked to lipid peroxidation in chronic disorders, the role of lipid peroxides on glycation of protein has recently gathered significant attention (8,9). An *in vitro* study by Jain et al. has indicated that the process of protein glycation can be promoted by malondialdehyde (MDA) per se (8). In accordance with this report, we have also recently reported that MDA per se can enhance the glycation of hemoglobin (9).

Glycation is a common post-translational modification of proteins in which reducing sugars bind covalently to the free amino groups (10). This non-enzymatic modification of proteins alters not only the structure but also the biological properties of proteins (10). This can lead to a variety of chemical entities and induce structural changes in enzymes starting

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0188-4409/07 $–see front matter.
doi: 10.1016/j.arcmed.2007.05.008

from conformational alterations, progressing to thiol oxidation, aggregation, formation of disulfide, and other covalent cross-links, and inactivation of enzymes (11). The two classical factors known to incite the glycation of proteins *in vivo* are glucose concentration and half-life of proteins (10). However, there is convincing evidence that non-enzymatically glycated protein concentrations are also higher in many non-diabetic pathological states (12−14). Increased glycated hemoglobin has also been previously reported in both non-diabetic essential hypertensive and gestational hypertensive subjects (15,16). Recently, we have observed an increased glycated hemoglobin and fructosamine in prehypertensive subjects (17).

Even though scattered reports are available on abnormal nonenzymatic glycation of proteins in hypertensive subjects, little is known about the relationship between lipid peroxides as measured by MDA with glycation of hemoglobin and plasma proteins as measured by fructosamine in hypertensive patients. As glycation can cause deleterious effect on biological processes, it was of interest to investigate the levels of glycated proteins in non-diabetic essential hypertensive patients.

The objectives of this study were 1) to determine whether glycated hemoglobin (HbA_{1C}), fructosamine, and MDA concentrations were increased in non-diabetic, hypertensive non-obese individuals and, if so 2) to determine the relationship between MDA concentrations with fructosamine and glycated hemoglobin levels.

Materials and Methods

The current study was conducted in the Department of Biochemistry and the Department of Physiology, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry. Our subjects included the nonteaching staff affiliated with our institute as well as outpatients who visited our laboratory for blood pressure check-up. Blood pressure was measured using a mercury sphygmomanometer (Diamond, Mumbai, India) with the patients in the sitting position, legs uncrossed. After 5 min of rest in the sitting position, BP was measured on both arms and the higher of the two was taken into consideration. If the systolic and diastolic blood pressure were in different categories, the higher of the two was used in the classification. Blood pressure was measured three times over a 3-week period. At each visit, an average of three readings was recorded to stage the patients as having normal blood pressure or being hypertensive. They were classified as normotensive and hypertensive as per the recommendation of the JNC 7 report.

Subjects

Thirty newly diagnosed hypertensive subjects (systolic blood pressure [SBP] ≥140 mmHg and/or diastolic blood pressure [DBP] ≥90 mmHg), and 25 normotensive (SBP <120 mmHg and DBP <80 mmHg) men with BMI <25 kg /m^2 in the age group of 25−55 years were enrolled in the study. Subjects with history of diabetes, cardiovascular diseases, infection, endocrine disorders, smoking, alcoholism, and those who were on any kind of medications were excluded.

Sample Collection

After an 8-h overnight fast, blood (5 mL) was drawn and collected in EDTA bottles. Whole blood was used for the estimation of glycated hemoglobin. Plasma was collected from the rest of the sample by centrifuging at 5000 × g for 5 min at 4°C. Plasma fasting glucose levels were estimated immediately and the remaining samples were stored at −70°C for the estimation of MDA and fructosamine.

Measurement of Glycated Hemoglobin (HbA_{1C})

Glycated hemoglobin was measured by using hemoglobin A_{1C} microcolumns (Biocon, Vöhl-Marienhagen, Germany) and expressed as the percent of total hemoglobin.

Estimation of Fructosamine

Plasma fructosamine was measured by *p*-indonitrotetrazolium violet kinetic method using Raichem kits (Haemagen Diagnostics, San Diego, CA) adapted to 550 express plus analyzer (Ciba Corning Diagnostics, Oberlin, OH).

Determination of MDA

MDA was measured using the established thiobarbituric acid (TBARS) method (18). This assay is based on the formation of red adduct in acidic medium between thiobarbituric acid and MDA, a colorless product of lipid peroxidation, measured at 532 nm. MDA values are calculated using the extinction coefficient of MDA-thiobarbituric acid complex 1.56×10^5 L × mol^{-1} × cm^{-1} at 532 nm and expressed as nmol/mL.

Estimation of Plasma Glucose

Fasting plasma glucose was measured by using commercially available kit (Agappe Diagnostics, Kerala, India) adapted to Ciba Corning 550 express plus.

Statistics

All results are shown as mean ± SD. The statistical significance of between-group differences was evaluated using Student's *t*-test. Simple correlations were determined by Pearson's correlation analysis. MDA and fasting plasma glucose were considered to act as independent variables and their relation to HbA_{1C} and fructosamine was assessed by the partial correlation analysis; *p* value of 0.05 was selected as the point of minimal statistical significance.