HYPOMAGNESAEMIA IN TYPE-2 DIABETES MELLITUS PATIENTS: A STUDY ON THE STATUS OF OXIDATIVE AND NITROSATIVE STRESS

R D Ankush, A N Suryakar and N R Ankush.

Department of Biochemistry, Dr. V. M. Govt. Medical College, Solapur, Maharashtra, India.

ABSTRACT

This study was undertaken to evaluate the levels of plasma magnesium, lipid peroxides, nitric oxide end products, erythrocyte membrane lipid peroxides, erythrocyte reduced glutathione and erythrocyte superoxide dismutase activity in type-2 diabetes mellitus patients. 60 patients with type-2 diabetes mellitus and 30 healthy control subjects were included in this study. Among 60 type-2 diabetic patients, 30 patients were without complication and 30 patients were with various complications. Decreased levels of plasma magnesium, erythrocyte reduced glutathione and erythrocyte superoxide dismutase activity while increased levels of plasma lipid peroxides, nitric oxide end products and erythrocyte membrane lipid peroxides were observed in patients with type-2 diabetes mellitus. We propose that, under the shadow of hypomagnesaemia, there is excessive production of reactive oxygen species and reactive nitrogen species as reflected by elevated lipid peroxides and nitric oxide end products concomitant with dwindled antioxidants and suggest their association with late complications in type-2 diabetes mellitus.

KEY WORDS

Type-2 diabetes, Hypomagnesaemia, Oxidative stress, Nitrosative stress, Antioxidants.

INTRODUCTION

Type-2 diabetes mellitus is on track to become one of the major global public health challenges of the 21st century. It accounts for approximately 90 to 95% of all diagnosed cases of diabetes. Patients with type-2 diabetes may have complications like cardiovascular disease, nephropathy, retinopathy and polyneuropathy. According to WHO report, the prevalence of diabetes in adults worldwide has risen. Epidemiological data in India shows the same upward trend from 33 million diabetics in 2000 to 57 million in 2025. India has thus become the "Diabetic Capital of the World" (1, 2).

Magnesium (Mg) is the fourth most abundant cation in the body and second in the intracellular environment. It takes part

in more than 300 enzymatic reactions (2). Deficiency of Mg has been associated with wide variety of clinical conditions, including type-2 diabetes mellitus. One of the major pathogenic factors associated with most of these conditions is oxidative stress (3). Hypomagnesaemia may have negative impact on glucose homeostasis and insulin sensitivity in type-2 diabetes mellitus patients (4). Further, Mg deficiency has been proposed as a novel factor implicated in the pathogenesis of late diabetic complications (5).

Type-2 diabetes mellitus has been shown to be a state of increased free radical activity. The roles of surplus reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the diabetic complications of multiple organ systems were extensively documented, although the precise mechanisms of ROS/RNS-induced pathogenesis have not been fully understood (6). Recent studies have also indicated that, Mg deficiency may be associated with increased oxidative and nitrosative stress through reduction in antioxidants and increased lipid peroxidation (7).

The pathophysiological processes linking hypomagnesaemia and above evidences in type-2 diabetes mellitus are still not clearly explained. In order to focus on these issues, the

Address for Correspondence :

Dr. R. D. Ankush,
Department of Biochemistry,
Dr. V. M. Govt. Medical College, Solapur-413003.
(Maharashtra), India.
Tel. : (0217) 2749407.
E-mail: rajeshankush@sancharnet.in

relationship between plasma Mg levels and oxidative-nitrosative stress were investigated in type-2 diabetes mellitus patients with and without complications.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, Dr. V. M. Govt. Medical College, Solapur and Department of Biochemistry, S.R.T.R. Medical College and Hospital, Ambajogai. The study was conducted over a period of two years. Patients visited to the out-patient department of the hospital were included in the study. All the type-2 diabetic patients, with and without complications, were newly diagnosed through American Diabetes Association (ADA) diagnostic criteria (8).

Inclusion criteria: Newly diagnosed male and female type-2 diabetes mellitus patients with and without complications aged between 30 to 65 years. All these patients were not on any hypoglycemic supplementations, willing to participate in the study and provide informed consents, control group included healthy volunteers consistent with the patients according to age and body mass index (BMI) were included in the study.

Exclusion criteria: Patients who had diabetes other than type-2 diabetes mellitus, diabetic patients who had been treated with insulin, subjects who had acute complications such as severe infections, major operations, trauma, GI disorders, severe cardiovascular / respiratory disease, patients who were presenting with ketoacidosis, pregnant and breast feeding women, subjects on any concomitant medication such as antioxidant vitamins, minerals, herbal treatment which may interact with glycemic status and oxidative stress parameters, cigarette smokers and alcoholics were excluded from the study.

A total of 90 subjects were enrolled for this study. Among these 30 were taken as healthy control subjects. Remaining 60 patients were divided into two equal groups based on the absence or presence of diabetic complications at the time of their diagnosis. Group-wise distribution of the subjects was follows:

Group I : 30 Healthy control subjects; Group II : 30 Type-2 diabetes mellitus patients without complication; Group III : 30 Type-2 diabetes mellitus patients with complications. (7-Cardiovascular disease, 8-Nephropathy, 6-Neuropathy and 9-Retinopathy).

Ethical approval: The study protocol was planned with the approval of local ethical committee of the institute.

Study protocol: All the subjects, including controls, were fully informed for the purpose, procedures and hazards of the study. After taking voluntary informed consents, all these subjects were screened for inclusion / exclusion criteria. Their health status was defined as an absence of major medical or surgical illness during previous five years, no hospital admissions, no current medication and a subjective perception of good health. Information on age, lifestyle, dietary habits, height, weight, (body mass index (BMI) was calculated), smoking, medication and/or dietary supplements were obtained from careful personal interview.

Following enrollment, both patients and controls were instructed for: not to change their lifestyle or their dietary habits and not to take any dietary supplements. The diet was not monitored.

Fasting venous blood samples were collected in heparinized bulb. Plasma and erythrocytes were separated by centrifugation. Plasma glucose (Starchem Diagnostics) and glycated haemoglobin A_{1C} (HbA_{1C}) (Teco Diagnostics) were estimated by using commercial kits. Other quantitative analyses such as levels of plasma Mg (9), plasma malondialdehyde (MDA) and erythrocyte membrane malondialdehyde (10), plasma nitric oxide (NO*) end products (11), erythrocyte reduced glutathione (GSH) (12), and erythrocyte superoxide dismutase (SOD) activity (13) were done by well established methods. All the chemicals used through out the study were of analytical grade.

Statistical evaluation: For statistical analyses, the Statistical Package for the Social Sciences (SPSS, Version 10) was used. Quantitative data of biochemical parameters from each group were expressed as arithmetic mean $\pm$ SD. Differences between the groups were evaluated by using unpaired student's 't' test and a two-tailed P value < 0.05 was considered statistically significant. Further, to assess possible relationships between different variables, Pearson's correlation coefficient was used.

RESULTS

All the values obtained are given in Table 1 and Table 2 whereas, correlation analyses of different variables are depicted in Fig 1. As compared to Group I, significant decrease in plasma Mg ($p < 0.001$), erythrocyte GSH ($p < 0.001$) and erythrocyte SOD activity ($p < 0.001$) were found in Group II. In addition, we found significant lower levels of plasma Mg ($p < 0.001$), erythrocyte GSH ($p < 0.05$) and erythrocyte SOD activity ($p < 0.05$) in Group III as compared to Group II.

Fig. 1 : Correlation between plasma Mg and other variables in Type 2 diabetes mellitus

We also found significant higher levels of plasma glucose ($p < 0.001$), HbA_{1c} ($p < 0.001$), plasma NO^{*} end products ($p < 0.001$), plasma MDA ($p < 0.001$), erythrocyte membrane MDA ($p < 0.001$) in Group II as compared to Group I.

Table 1: Levels of plasma glucose, magnesium, MDA and NO^{*} end products

Variable	Group I	Group II	Group III
Plasma glucose (mg/dl)	87.61 ± 5.06	197.89 ± 39.44*	224.69 ± 37.04*
Plasma magnesium (mg/dl)	2.71 ± 0.26	1.98 ± 0.23*	1.16 ± 0.19*
Plasma MDA (nM/ml)	2.66 ± 0.68	5.86 ± 1.10*	10.81 ± 2.11*
Plasma NO [*] end products (μM/L)	38.49 ± 4.75	45.58 ± 3.78*	64.92 ± 6.49**

* P < 0.001, ** P < 0.05

Table 2: Levels of HbA_{1c}, erythrocyte GSH, erythrocyte SOD activity and erythrocyte membrane MDA

Variable	Group I	Group II	Group III
HbA _{1c} (%)	5.77 ± 0.87	10.13 ± 1.87*	13.31 ± 1.95*
Erythrocyte membrane MDA (nM/ml)	1.20 ± 0.33	3.60 ± 1.05*	7.60 ± 1.77*
Erythrocyte SOD activity (U/mg protein)	25.47 ± 8.96	17.75 ± 7.04*	11.59 ± 3.85**
Erythrocyte GSH (μM/gm Hb)	6.92 ± 1.71	3.74 ± 0.99*	2.70 ± 0.97**

* P < 0.001, ** P < 0.05

Furthermore, the levels of plasma glucose ($p < 0.001$), HbA_{1c} ($p < 0.001$), plasma NO^{*} end products ($p < 0.05$), plasma MDA ($p < 0.001$), erythrocyte membrane MDA ($p < 0.001$) were significantly higher in Group III, as compared to Group II.

Besides above, plasma Mg levels were found to be positively correlated with erythrocyte GSH and erythrocyte SOD activity. Whereas, plasma Mg levels were negatively correlated with plasma MDA, erythrocyte membrane MDA, plasma NO^{*} end products and HbA_{1c}.

DISCUSSION

Plasma Mg concentrations were significantly lower in type-2 diabetics than in healthy controls. Further, hypomagnesaemia was more pronounced in patients with diabetic complications than in patients without complications. Numerous studies have reported indices of Mg depletion in diabetes by demonstrating reduced levels of Mg in plasma, serum and erythrocytes (2, 6). The precise mechanism behind hypomagnesaemia in type-2 diabetes mellitus is not completely known, but it is generally accepted that, increased urinary excretion of Mg as a result of osmotic diuresis is a major factor. Poor control of diabetes is often associated with low plasma Mg levels. Other factors, however, including diarrhea, vomiting and sodium intake may contribute to Mg deficiency in type-2 diabetes mellitus (14).

Earlier research suggests that subjects with type-2 diabetes have higher HbA_{1c} with lower serum Mg levels (15). We found a negative correlation between plasma Mg levels and HbA_{1c} in type-2 diabetic patients. Mg deficiency decreases insulin sensitivity via an alteration of the insulin-receptor associated tyrosine kinase in type-2 diabetes. Insulin-induced erythrocyte Mg accumulation was reported to be impaired in patients with type-2 diabetes mellitus and that such a defect possibly

Fig 2: Shows possible link between hypomagnesaemia in the development of late diabetic complications through oxidative-nitrosative stress.

NF-κB : Nuclear factor-κB; p38 MAPK: p38 Mitogen-activated protein kinase; JNK / SAPK :NH₂-terminal Jun kinases / stress activated protein Kinases; AGE: Advanced Glycation End Products; RAGE: Receptor for AGE; DAG: Diacylglycerol; PKC: Protein kinase C.

associated with impaired insulin-mediated glucose disposal in diabetic patients (16).

Studies also imply a link between hypomagnesaemia, augmentation of free radical production and its contribution to vascular injury and late complications of type-2 diabetes (5). Hypomagnesaemia can increase platelet reactivity, increase vascular and adrenal responses to angiotensin II, enhance thromboxane A₂ release and lead to organ damage from free radicals (17). Increased free radical formation and reduction in the antioxidant potential contributes to the development of oxidative stress in type-2 diabetes (14). Mechanisms contributing formation of free radicals in type-2 diabetes mellitus may include non-enzymatic and auto-oxidative glycosylation, the inflammatory mediators and status of antioxidant defense. Furthermore, Mg itself has been reported to possess antioxidant properties by scavenging oxygen radicals, probably by affecting the rate of spontaneous dismutation of superoxide anion (18).

MDA is a product formed as an end product of lipid peroxidation of cellular polyunsaturated fatty acids. Measurement of MDA helps to assess the degree of tissue damage (19). Lipid

peroxidation of cellular structures is thought to play an important role in development of late diabetic complications. Freedman et al (20) demonstrated that, erythrocytes from Mg-deficient animals are more susceptible to free radical injury. In our study, plasma MDA and erythrocyte membrane MDA were negatively correlated with plasma Mg, which are indicative of intricate involvement of hypomagnesaemia in generation of excessive free radicals and thereby causing lipid peroxidation. Plasma MDA and erythrocyte membrane MDA were also found to be positively correlated with HbA_{1c} which point toward connection of protein glycation in the formation of advanced glycation end products (AGEs) and generation of ROS.

The endothelium derived NO^{*} is a potent endogenous nitrovasodilator and plays an important role in regulation of vascular tone. It has been hypothesized that, oxygen derived free radicals may interfere with NO^{*} action and attenuates its vasodilatory activity (21). Howard et al (22) demonstrated inhibitory effects of Mg on nitric oxide synthase (NOS) activity in endothelial cells. Therefore, it is plausible that, during Mg deficiency, superoxide overproduction may accompany increased NO^{*} generation; caused by endothelial NO^{*} synthase (eNOS) and inducible NO^{*} synthase (iNOS) uncoupled state, a phenomenon favoring the formation of the strong oxidant peroxynitrite (23) that causes nitrosative stress in the organ system (6). Nitration of proteins is also postulated to be involved in the disruption of protein assembly and functions with possible pathological consequences and results in oxidation of protein sulfhydryl groups. Peroxynitrite may directly oxidize erythrocyte glutathione and initiate membrane lipid peroxidation in type-2 diabetes mellitus (24).

GSH is a tripeptide and its unique structure holds the key to its diverse functional activities. In the reduced state, GSH has antioxidant property and is present in human plasma as well as intracellularly. It participates in reactions that destroy hydrogen peroxide, free radicals and certain foreign compounds (18). At the expense of reduced GSH, the antioxidant enzyme glutathione peroxidase catalyzes the elimination of peroxides. GSH also helps in regeneration of other antioxidants like ascorbate and tocopherol (25). Thus, depletion of GSH may impair the activity of antioxidant enzymes as well chain breaking aqueous and lipid phase antioxidants in the cell and renders it susceptible to oxidative injury. Mg is essential in the maintenance of glutathione concentration to protect against oxidative damage in the erythrocyte membrane. Therefore, loss of erythrocyte GSH in type-2 diabetic patients may be owing to decreased Mg dependent synthesis, increased oxidative degradation and/or

overproduction of NO^{*} (26). Positive correlation between plasma Mg and erythrocyte GSH levels while, negative correlation between plasma Mg with plasma NO^{*} end products found in our study are suggestive of involvement of hypomagnesaemia in loss of erythrocyte GSH and increased production of NO^{*}.

SOD is one of the most important endogenous antioxidant enzymes and neutralizes toxic effects of superoxide radicals. Decreased SOD activity may lead to insufficient neutralization of free radicals which causes oxidation of cellular lipids, proteins, nucleic acids, glycolipids and glycoproteins (26). This oxidative effect can also damage the vascular endothelial cells and other tissues, as evident from increased MDA levels observed in type-2 diabetic patients in our study. The increased levels of lipid peroxides may have inhibitory effects on SOD activity which may further lead to accumulation of superoxide radicals and thus maximum lipid peroxidation and tissue damage in type-2 diabetes mellitus patients (27). On the other hand, glycation of the SOD is also reported to inhibit SOD activity in type-2 diabetes. Erythrocyte SOD activity was positively correlated with plasma Mg levels, which points out that, hypomagnesaemia may possibly have an effect on erythrocyte antioxidant defense system through excessive ROS production (28).

Our results strikingly show that, under the shadow of hypomagnesaemia, there is excessive production of ROS and RNS as reflected by elevated MDA and NO^{*} end products in newly diagnosed type-2 diabetes mellitus patients and moreover in the patients with complications. Vital antioxidants such as GSH and SOD were also found to be decreased in these patients, which is not affordable because both these antioxidants help to mop up superfluous free radicals. In addition, overwhelmed oxidative stress may lead to activation of stress-sensitive intracellular signaling pathways thereby producing gene products that cause cellular damage which are ultimately responsible for the late complications (Fig 2) (29).

To conclude, it is obvious from the presented data that, an apparent relationship exists between hypomagnesaemia, oxidative stress and nitrosative stress in type-2 diabetes mellitus. This next of kin perhaps is linked to various ensuing complications in type-2 diabetic patients. Consequently, we put forward that, a better understanding of Mg metabolism and efforts to minimize hypomagnesaemia, oxidative and nitrosative stress in the management of type-2 diabetes mellitus are warranted.

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