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Research Article

EFFECT OF ENALAPRIL AND METFORMIN ON OXIDATIVE STRESS IN NEWLY DIAGNOSED TYPE 2 DIABETES MELLITUS AND HYPERTENSIVE PATIENTS

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ABSTRACT

Objective: The present study was aimed to find out the impact of Enalapril and Metformin on oxidative stress (OS) in newly diagnosed diabetes mellitus and hypertensive patients.

Methods: A free radical production was measured by lipid peroxidation and antioxidants like catalase, reduced glutathione, glutathione s-transferase, and superoxide dismutase were measured using ultraviolet spectrophotometer.

Results: There was a significant decrease in free radical production and a significant increase in antioxidant enzymes in both the treatment groups.

Conclusion: Enalapril and metformin combination in diabetic patients has a more significant effect on OS than metformin alone. This combination also has a significant effect on OS in diabetes and hypertension coexisting patients.

Keywords: Oxidative stress, Enalapril, Metformin, Antioxidants.

INTRODUCTION

Diabetes mellitus (DM) is a clinical syndrome characterized by varying degrees of insulin hyposecretion and/or insulin insensitivity leading to hyperglycemia. Lack of insulin affects the metabolism of carbohydrates, protein and fat, which lead to metabolic alterations, and it causes a significant disturbance of water and electrolyte homeostasis [1]. DM is divided into two types based on their requirements for insulin: Insulin-dependent DM (IDDM) and Non-insulin-dependent DM (NIDDM). In 1997, The American Diabetes Association reclassified DM according to the etiology into (Type 1 DM) which is equivalent to IDDM, and (Type 2 DM) which is equivalent to NIDDM [2,3].

Type 1 DM most commonly affects juveniles characterized by an absolute deficiency of insulin caused by massive β-cell lesions or necrosis. As a result of the destruction of β-cells, the pancreas fails to respond to ingestion of glucose and Type 1 DM shows the classical symptoms of insulin deficiency (polydipsia, polyphagia, polyuria, and ketoacidosis) [4,5].

Type 2 DM (NIDDM) mature or adult onset DM is the most common form of DM. In contrast to Type 1 DM, the onset is slow and the metabolic alterations observed are less than those of Type 1 DM (for example, Type 2 diabetic patients typically are nonketotic [4,5]. On average, patients with Type 2 DM retain approximately 50% of their β-cell mass, resulting in variable insulin levels (normal or raised insulin level when compared with normal subjects), but are inappropriately low for the degree of hyperglycemia present [1,4]. Groups at higher risk to develop Type 2 DM include those with a family history of Type 2 DM, middle-aged to elderly (age older than 40 years), obese (especially visceral obesity), sedentary lifestyle, and those on high-fat or high-caloric diets [4].

Approximately, 30-60% of diabetics have systemic arterial hypertension (SAH), which shows the close relationship between such diseases [6]. SAH, in turn substantially contributes to morbidity in patients with diabetes [7], with oxidative stress (OS) configuring an important mechanism in the pathophysiology of DM and SAH [8-10]. Increased oxygen free radical activity, coupled with reduced protection against OS, could play a role in the etiology of neurovascular abnormalities in experimental DM [11]. Production of reactive oxygen species (ROS) is increased in diabetic patients, especially in those with poor glycemic control. ROS affect vascular smooth muscle cell growth and migration, endothelial function, including the abnormal endothelium-dependent relaxation and expression of a proinflammatory phenotype, and modification of the extracellular matrix. All of these events contribute to the development of diabetic macrovascular and microvascular complications. High blood glucose level determines over production of ROS by the mitochomdrial electron transport chain. The high reactivity of ROS determines chemical changes in virtually all cellular components, leading to DNA and protein modification and lipid peroxidation [12,13]. Thus, it is better to know the level of OS in newly diagnosed diabetes and hypertensive cases and delays the complications by using drugs having an effect on OS, i.e., antioxidant property. Since enalapril and metformin are the first line drugs in these disease conditions, we would like to study the impact of these drugs on the free radical injury. It can be estimated by the changes in the level of antioxidant enzymes by measuring the blood serum levels by ultraviolet (UV) spectrophotometer.

METHODS

The study was conducted after having obtained Ethical clearance from the Human Ethical Committee at Rajiv Gandhi Government General Hospital, Madras Medical College, Chennai. Informed consent in the prescribed form was obtained from all patients included in the study after explanation of the probable benefits.

Study design and participants

This was a randomized controlled study. The participants were 25 newly diagnosed diabetic patients (Group II) 25 newly diagnosed diabetes with hypertension (HT) patients (Group III). Group II and III patients were treated with enalapril (2.5 mg once daily) and metformin (500 mg twice daily). 25 healthy volunteers were included in Group I (controls).

Blood collection and laboratory methods

The blood sample was collected under aseptic precautions at the time of diagnosis and at 4 weeks interval for a period of 12-week. The biochemical profile was evaluated using standard laboratory methods. The samples were kept cool until the completion of blood collection and then centrifuged at 4000 RPM, at 4°C for 5 minutes. The serum was separated and analyzed. For measurement of catalase (method of Sinha, 1972), Reduced glutathione (method of Ellman, 1959), glutathione s-transferase (method of Habig *et al*., 1974) Superoxide dismutase (method of Marklund and Marklund, 1974) were performed. Free radical production was measured by lipid peroxidation (LPO) (method of Ohkawa *et al*., 1979). Protein content was measured by the (method of Lowry *et al*., (1951). All the parameters were determined using a UV spectrophotometer.

Statistical analysis

The results were publicized as mean±standard deviation. The statistical significance of the difference between controls, patient groups was evaluated using one-way ANOVA. All calculations were performed using the SPSS version 20.0 for windows.

RESULTS

When compared from 0 to 12 weeks, there is a significant increase in all antioxidant levels in Groups II and III ($p<0.001$), but less than Group I (healthy volunteers (Figs. 1-4). LPO shows a significant increase in both the Groups (II and III) when compared with controls (Group I) at 0 weeks ($p<0.05$) and showed significant decreases from 0 to 12 weeks (Fig. 5).

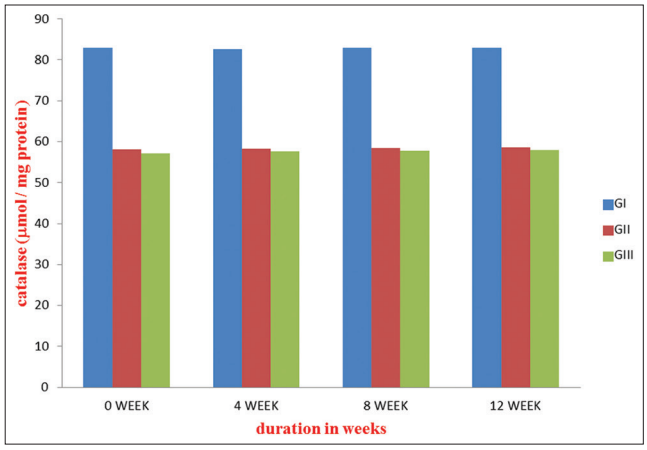

Fig. 1: Comparison of serum catalase in Groups from 0 to 12 weeks

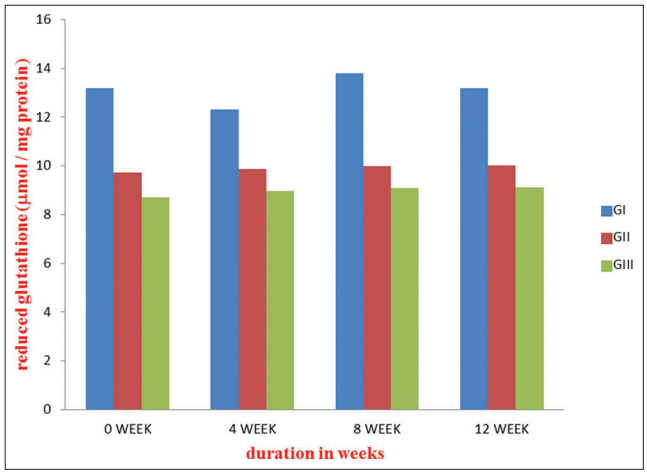

Fig. 2: Comparison of serum reduced glutathione in Groups from 0 to 12 weeks

Group II shows a more significant improvement in all antioxidant levels and decrease in LPO levels than Group III ($p<0.01$). This shows the effect of enalapril and metformin in combating OS in DM and HT.

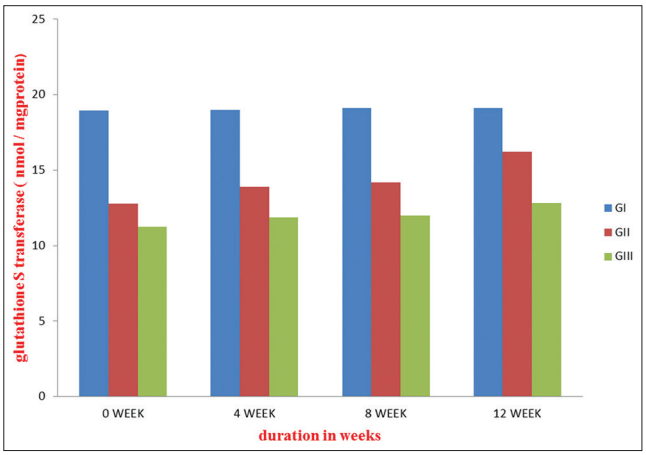

Fig. 3: Comparison of serum glutathione s-transferase in Groups from 0 to 12 weeks

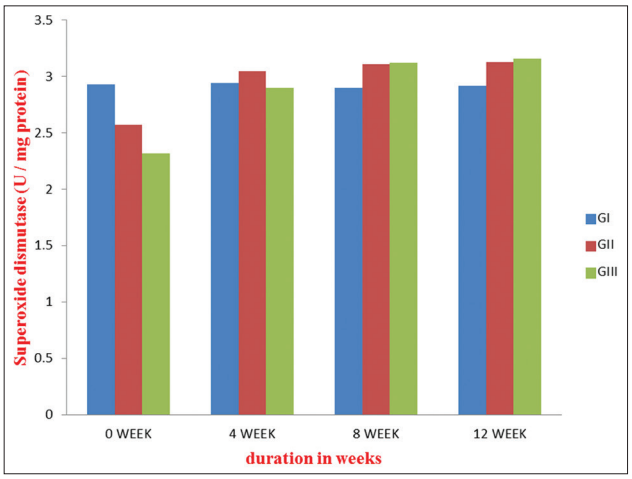

Fig. 4: Comparison of serum superoxide dismutase in Groups from 0 to 12 weeks

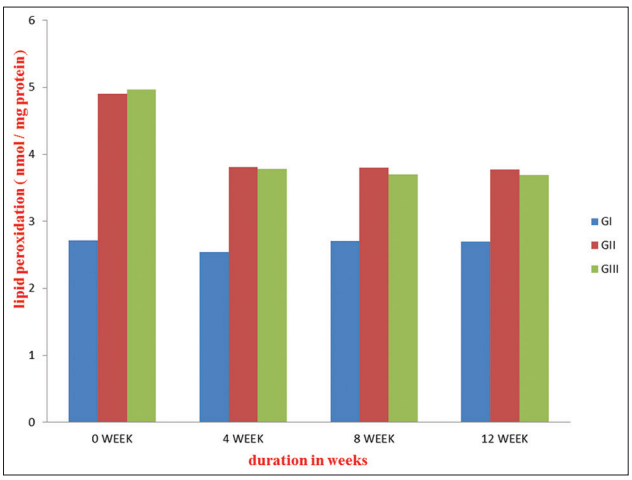

Fig. 5: Comparison of serum lipid peroxidation in Groups from 0 to 12 weeks. n=25 in each group, GI: Controls, GII: Diabetes mellitus treated with enalapril and metformin, GIII: Diabetes mellitus and hypertension treated with enalapril and metformin

DISCUSSION

OS is caused by imbalance between the production of reactive oxygen and biological systems inability to detoxify or repair the resulting damage [14,15]. Free radicals produced in excess may lead to oxidative damage of molecules, membranes, and tissues. Lipids, proteins, and nucleic acids are important targets of free radicals. Oxidation of membrane lipids resulting in LPO is the primary event of cellular damage [16,17]. Our body has a natural antioxidant defense system to protect from free radicals. ROS which cause cellular damage by the oxidation ability have been implicated in the pathogenesis of DM [18]. In diabetes, free radicals are produced through glucose oxidation [19]. Many experimental studies in animals and humans have proved the role of OS in diabetes [20]. One of these studies using streptozotocin-induced diabetic rats showed increased levels of lipid peroxidation as measured by thiobarbituric acid reactive substances, one of the OS markers [21]. This enhanced ROS production is one of the determinants of diabetic complications [22]. In HT, there is oxidative damage due to the free radicals, studies have shown increased LPO in plasma of patients with HT [23-25]. Enhanced superoxide anion formation, p22phox mRNA expression and NADH/NADPH oxidase activity have been demonstrated in spontaneously hypertensive and experimentally induced hypertensive rats [26-28]. In this study, influence of an anti-diabetic metformin and antihypertensive drug enalapril was studied for their effect on OS in patients with diabetes alone and in patients with both DM and HT. The study results showed increased LPO in both patient groups, which proves the presence of OS in diabetes and HT. Group III (DM + HT) showed more OS than Group II (DM alone) (Fig. 5). After 12 weeks treatment in Groups II, III with enalapril and metformin there is a significant increase in all antioxidant levels from 0 to 12 weeks (Figs. 1-4) and decrease in lipid peroxidation (Fig. 5). Increase in antioxidant levels in treatment groups suggests that both the drugs possess antioxidant property. Enalapril, an angiotensin-converting-enzyme inhibitor represents an antioxidant strategy by preventing the increased superoxide flux associated with activation of the renin-angiotensin system in HT thereby limits the stimulation of NADPH oxidase. Enalapril also inhibits LPO through reduced formation of peroxynitrite; this notion is consistent with observations that angiotensin II induces LPO in experimental animals [28,29]. Metformin, a biguanide decreases OS and increases all antioxidant levels by causing inhibition of mitochondrial complex I which decreases ATP levels and activates AMPK-dependent catabolic pathways, increasing lipolysis and ß-oxidation in white adipose tissue and reducing neoglucogenesis. The resultant reduction in triglycerides and glucose levels could decrease methylglyoxal (MG) production through lipoxidation and glycoxidation, respectively [30]. Group II shows more significant improvement in all antioxidant levels and decrease in LPO levels than Group III ($p<0.01$). This implies that low-dose enalapril can decrease OS, thereby delays complications in patients and combination of both the drugs decrease free radical injury and delays the disease progression in newly diagnosed hypertensive and diabetic patients.

CONCLUSION

OS has been demonstrated to have a major impact in the progression of diabetes and HT, including impairment of insulin action, and elevation of the complication incidence. Antioxidants have already shown to be prospective in the treatment of Type 2 diabetes and also in HT. The results of this study showed both enalapril and metformin combination possess a significant effect on combating OS. Further studies are required to see the long-term effect of these drugs in combating OS in correlation with glycemic control and blood pressure reduction.

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