OXIDATIVE STRESS AND ANTIOXIDANT STATUS IN DIABETIC SUBJECTS TREATED WITH METFORMIN

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ABSTRACT

Background: Oxidative stress plays an important role in the pathogenesis of DM and its complications. However, antioxidant status and its contribution to type 2 DM are less explored in South Indian population. Metformin, is a biguanide anti hyperglycemic agent used for the management of type 2 diabetes. **Aim:** To study the alteration in oxidant and antioxidant status in type 2 diabetic subjects on treatment with Metformin and to evaluate the effect of metformin in improving the total antioxidant status. **Methodology:** All subjects were T2DM patients, on metformin monotherapy (500 mg, bd) and were grouped into Group 1 and Group 2 based on their HbA1c values with response to metformin. Baseline parameters (B.P, Waist Hip ratio, BMI, family history), glycemic status, lipid profile, Total antioxidant capacity (TAC), Malondialdehyde (MDA) and serum Metformin levels were assayed. **Results:** Fasting insulin (μIu/ml), TAC (μM), MDA (nmol/ml), Metformin (μg/ml) values in group 1 and group II are 22.38 ± 2.7, 14 ± 3.9, 268.71± 23.12, 355.75 ± 26.32, 3.37 ± 0.21, 1.68 ± 0.05, 0.17 ± 0.01, 0.08 ± 0.005 respectively. Oxidative stress was higher with reduced antioxidant status in Group I compared to Group II subjects. **Conclusion:** It may be concluded that total antioxidant status is lower in type 2 diabetic subjects of Group 1 category compared to diabetic subjects in the Group 2 and it may be related to the beneficial effects of the biguanide, Metformin.

Keywords: Type 2 diabetes; Antioxidants; Oxidative stress; Metformin; Biguanides.

INTRODUCTION

The incidence of type 2 diabetes mellitus (DM) is becoming as a serious public health concern worldwide. DM, particularly type 2 diabetes is now recognized as a major chronic public health problem. Globally, the prevalence of diabetes is ≈8%, and nearly 80% of patients with diabetes live in low- and middle-income countries [1]. According to the International Diabetes Federation, the prevalence will be 13% by 2030 [2]. ROS are a byproduct in type 2 DM, generated during protein glycation and as a consequence of advanced glycation end products-receptor binding; they impair insulin signaling pathways and induce cytotoxicity in pancreatic β (beta) cells [3]. Oxidative stress is now thought to be an important marker in the pathogenesis of type 2 DM and its complications through the impairment of pancreatic β-cells function [4]. The generation of reactive metabolites plays a central role in cell's life. These metabolites are continuously controlled by endogenous antioxidant enzyme systems and the balance is created between pro-oxidants and antioxidants. The impairment of antioxidant status, either by exogenous or endogenous sources, may disturb the cellular redox balance and the pathological conditions would be the main characteristics and forms oxidative stress in cells or tissues [5]. Oxidative stress is implicated in the pathophysiology of DM and its chronic complications [6] and may contribute to the pathogenesis of diabetes mellitus through impairment of insulin action, injury to pancreatic β-cells, increased lipid peroxidation, and vascular endothelial damage [6]. Lipid peroxidation (LPO) is a biochemical reaction due to ROS action on the cell membranes, which leads to serious structural damage, failure of metabolite exchange mechanisms, and under extreme conditions, cell death [7, 8]. The most common way to measure lipid peroxides is to estimate malondialdehyde (MDA) content which is measured as TBARS (thiobarbituric acid reactive substances). The clinical relevance of the reaction between MDA and protein is elevated in atherosclerosis, which is a major cause of coronary heart disease and stroke. The main goal of antidiabetic therapy is to prevent the complications of diabetes. The variation in the levels of antioxidant enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications [9, 10]. The assessment of oxidative /antioxidative status in patients with type 2 diabetes could be of help in the prediction of micro- and macro vascular complications [11]. Metformin, the primary drug for treating DM2 [12] is a biguanide anti hyperglycemic agent used for the management of type 2 diabetes, apparently acts on the mechanisms described above, thus having a pronounced antioxidant effect [13]. In the South Indian population, the role of oxidative stress in

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the pathogenesis of type 2 DM is less explored. The main aim of the work was to explore levels of lipid peroxide & antioxidants in type 2 diabetes mellitus and to assess the role of metformin in improving the same.

Aims and Objectives: To study the alteration in the oxidant and antioxidant status in type 2 diabetic subjects who are on treatment with Metformin.

MATERIALS AND METHODOLOGY

Study designs: An observational analytical study

Ethics approval: Institutional ethics committee of Rajah Muthiah Medical College Hospital, Annamalai Nagar have approved the study and written informed consent was obtained from all the participants of the study.

Research place: The research was conducted in the diabetic out-patient department of Rajah Muthiah Medical College Hospital, Annamalai Nagar, Tamil Nadu.

Inclusion criteria: Subjects of the study were randomly selected known type 2 diabetics from at least six months, aged between 35 and 55 years, of both gender and had HbA1c (glycated hemoglobin) values above 8%. All subjects included in the study, were put on Metformin (500 mg bd) therapy throughout the period of study (4 months).

Exclusion criteria: Patients on insulin, smokers, alcoholics, tobacco chewers, hypertension and other systemic illness were excluded from this study.

Sample size: 150 subjects were included according to inclusion-exclusion criteria.

Grouping: For the convenience of the study, subjects were divided into two groups. **Group 1:** responded poorly towards Metformin (n=120) **Group II**: responded well to metformin therapy (n=30)

Anthropometric measurement: Anthropometric data including height, weight, blood pressure and BMI were measured. Body mass index (BMI) was calculated by dividing the weight in kilograms by height in meters squared [14]. BP was measured with a standard mercury sphygmomanometer [15].

Biochemical analysis: Fasting venous blood was collected immediately after enrolment in tubes containing EDTA. Blood samples were centrifuged at 2000 x g for 10 min. Samples were analyzed for Lipid Profile (Total Cholesterol, HDL-c, LDL-c, Triglycerides) by auto analyzer using kits. Serum insulin levels were determined by ELISA kit. The fasting venous plasma glucose (FPG), was determined with the glucose oxidase method. HbA1c was measured with a high-performance liquid chromatography [16]. Estimation of lipid peroxidation was done by TBARS and antioxidant status was studied by FRAP assay.

Hormones assay: Serum insulin levels were determined by using Immunoenzymometric assay [17]. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as fasting Insulin (mg/dl) x Fasting glucose (mg/dl) divided by 405 [18].

Thiobarbituric acid reactive substances (TBARS): TBARS levels were measured as an index of lipid peroxidation using the colorimetric method described by Satoh [19]. After reaction of thiobarbituric acid with malondialdehyde (MDA), the reaction product was extracted in butanol. Separation of the organic phase was facilitated by centrifugation at 3000 rpm for 10 mins. and its absorbance was determined spectrometrically at 530 nm.

Total antioxidant activity-FRAP Assay: FRAP assay [20] uses antioxidants as reductants in a redox linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess.

Serum Metformin Analysis: Serum Metformin levels were measured with a high-performance liquid chromatography (HPLC) [21]. Chromatograms were recorded at 241 nm using a detector SPD-20AVShimadzu UV visible detector. The retention time for Metformin was 3.78 minute. The optimum wavelength selected for determination of Metformin was 241nm.

RESULTS

Table 1: Baseline parameters in Group I & II

Variables	Group I	Group II
Age (years)	46.4 ± 6.2	41.4 ± 5.4
Systolic B.P (mmHg)	129± 24.47	119.7±13.6*
Diastolic B.P (mmHg)	82 ± 9.51	76 ± 9.91
Waist Hip Ratio (%)	0.92 ± 0.06	0.90 ± 0.06
Body Mass Index (kg/ m^2)	27.1 ± 4.5	21.1 ± 2.1*
Family history (n)	27	14

P values below 0.05 (<0.05) is considered significant.

Table 1, shows Baseline parameters like Diastolic B.P and Waist –Hip ratio did not show significant difference between Group I and Group II. The Systolic B.P and BMI were elevated in Group I and significantly high when compared to the Group II (p<0.05).

Table 2: Glycemic status and lipid profile in Group I & II patients

Variables	Group I	Group II
Fasting Plasma Glucose (mg/dL)	170.50 ± 42.7	77.5±13.48**
HbA1c (%)	12.4 ± 1.08	7.2 ± 0.15*
Serum Cholesterol(mg/dL)	179.5 ± 11.8	145± 14.2*
Triglycerides (mg/dL)	150.90 ± 63.1	161.35±73.8
HDL Cholesterol(mg/dL)	43.60 ± 2.89	44.15±3.2
LDL Cholesterol (mg/dL)	118 ± 14.2	86.61 ± 12.8*
fasting insulin (μIu/ml)	22.38 ± 2.7	14 ± 3.9 *
HOMA-IR (Mass Units)	7.6 ± 1.1	5.6 ± 1.0 *

*Significant P=<0.05

Table 3: Oxidant, anti-oxidant parameters and serum metformin levels in Group I & II

Variables	Group I	Group II
Total antioxidant activity (µM)	268.7±23.1	355.75± 26.32*
MDA (nmol/ml)	3.37 ± 0.21	1.68±0.05*
Metformin (µg/ml)	0.17 ± 0.01	0.08±0.005*

*Significant P=<0.05

Glycemic parameters like fasting blood glucose, HbA1c, HOMA-IR and fasting serum Insulin levels (FINS) were elevated in Group I and showed significant difference when compared to Group II. Among the Lipid profile parameters, Total Cholesterol and LDL -Cholesterol were elevated significantly in Group I while HDL-Cholesterol and Triglycerides did not show any significant difference (Table 2).

Table 3 shows oxidant and antioxidant levels with serum metformin levels in Group I and Group II subjects. Serum metformin was significantly raised in Group I and TBARS was significantly lower with higher FRAP assay values, in Group II. The presence of cell injury due to free radicals is characterized by the formation of lipid peroxide. This process of fat degradation will produce malondialdehyde (MDA), found in the blood, and is often used as an indicator of the presence of cellular and tissue damage due to free radicals [22-24].

DISCUSSION

The prevalence of diabetes is rapidly increasing due to lifestyle, eating habit, population growth, aging, obesity and physical inactivity [25] and the rising rate is higher in developing countries. Oxidative stress may play a major role in the development and progression of short-term and long-term complications of DM. For the management of DM, several approaches are taken to achieve glycemic goal. The pharmacological agents, i.e., Biguanides like Metformin are frequently used for the management of DM due to potential benefit on glycemic status [26]. In this study, a significantly increased plasma TAS was observed in type 2 diabetic subjects treated with Metformin. This finding is consistent with findings of Abdulkadir et al. [27] who reported a significant rise in total antioxidant status (TAS) after a 2 months biguanide monotherapy. This may be due to increase in endogenous antioxidants (Cu-, Zn- superoxide dismutase, catalase and glutathione reductase levels) [28, 29] or exogenous antioxidants [28] in type 2 diabetic subjects treated with biguanide. In this study, two groups were matched for age, sex, waist-hip ratio, blood pressure and chronic glycemic status (HbA_{1c}). There are several evidences that hyperglycemia enhances oxidative stress. Among these autoxidation of glucose [30], activation of the polyol pathway [31, 32] is critically important. Since "biguanides" are effective antihyperglycemic agents, a decreased glucose autoxidation and deactivation of the polyol pathway may drastically improve the antioxidants by controlling blood glucose levels and this fact support our results as Metformin is an effective biguanide to control chronic glycemic status. Again, in this study, TAS (Total Antioxidant Status) in type 2 diabetic subjects treated with Metformin are significantly higher compared to the other group. Several methods for evaluating insulin resistance in humans have been reported [33]. Among these indexes, fasting plasma insulin and the insulin resistance index (IR) by HOMA, calculated from fasting plasma insulin and fasting plasma glucose (FPG) levels, are likely to be the most simple and repeatable indexes in diabetic outpatient clinics. HOMA-IR is used as an index of insulin resistance in type 2 diabetic patients [23, 34]. The study showed that HOMA-IR was significantly different among Group I and Group II. MDA is a product of lipid peroxidation and it is considered a significant biomarker for OS [35].

There is a clear link between lipid peroxidation and glucose concentration, which may also play a role in increased lipid peroxidation in diabetes [36]. Researchers have also reported elevated lipid peroxidation products in type1 and 2 diabetic patients [37, 38]. Oxidative stress can be evaluated as TBARS. We also found a positive correlation between HbA1c and TBARS. Plasma MDA concentrations are increased in diabetes mellitus and MDA can be found in the atherosclerotic lesions [39, 40]. MDA levels are significantly higher in Group I diabetic patients compared to that of Group II and there is significant positive correlation with FBS and HOMA-IR.

TBARS (thiobarbituric acid reducing substances) assay is an indirect measure of lipid peroxidation by indirectly measuring free radicals in the blood. In diabetics, TBARS levels were increased because of increased superoxide ions and reduced activity of S. O. D. In this study, the TBARS level of Group I diabetic patients are relatively higher compared to Group II subjects. FRAP was suggested as a useful marker to measure antioxidant capacity in cells [41]. Studies showed that FRAP level was significantly lower in diabetic subjects with poor glycemic control compared to patients with good glycemic control [42].

In this study, we found FRAP levels significantly lower in Group I subjects compared to Group II subjects and significantly negative correlation with TBARS. This result supports the concept that, among the mechanisms involved in the increase of oxidative stress in diabetic patients, hyperglycaemia induced glucose auto oxidation and non-enzymatic glycation of proteins play an important role [41, 43]. In this study significant negative correlation was found with HOMA-IR, FBS, and FRAP in Group I subjects.

Also, serum metformin levels were elevated in Group I subjects compared to Group II. The fasting blood glucose, HbA1c and serum Insulin levels of Group I subjects were increased compared to Group II. Our findings were in parallel with that of the study done by Bonora E et al and Caroll M et al. [44, 45]. It was also identified that total cholesterol and LDL-cholesterol were high in Group I- a possible explanation for the same could be the beneficiary effects of the drug metformin which effectively controlled blood sugar and lipid levels in Group II. Metformin efficacy may be affected by gene polymorphism, which may be a the probable explanation for the poor glycemic control and low total antioxidant capacity of Group I subjects.

CONCLUSION

The antioxidant status was apparently higher in type 2 diabetic subjects belonging to Group II, who responded well with Metformin therapy. This could be probably explained as the benefits of Metformin which was well taken in their body compared with the subjects belonging to the Group I.

Limitations of the study: The major limitation of this study includes cross-sectional design with small sample size, lack of follow up, lack of other confounding variables like eating habit, lifestyle and physical activity. Intense prospective studies including other confounding factors are required to be done in south Indian population to clarify the issues further.

Conflict of Interest: Declared none

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