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Oxidative stress in first degree relatives of type 2 diabetic patients

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Abstract
Epidemiological studies on twins and families have provided a strong correlation for genetic factors contributing to etiology of type 2 diabetes mellitus (T2DM). Diabetes mellitus is strongly associated with oxidative stress, which can be a consequence of either increased production of free radicals, reduced antioxidant defense or both. The present work was undertaken to study known markers of erythrocyte oxidative stress: malondialdehyde (MDA) and reduced glutathione (GSH) in first degree relatives of T2DM patients, and plasma antioxidant status in an effort to understand the role of oxidative stress in the etiology of type 2 diabetes. Our results show that the antioxidant potential of the plasma is 14% lower in first degree relatives, the intracellular GSH is lower by 9% and lipid peroxidation measured in terms of MDA is elevated by 20%, compared to normal controls. These findings show that an impaired redox balance may be a cause for disturbance of homeostasis in type 2 diabetic families even before the development of the disease. We hypothesize that that oxidative stress precedes the development of overt diabetic state.

Key words: *Type 2 diabetes mellitus, families, erythrocyte, oxidative stress*

Introduction

The past two decades have seen an explosive increase in the number of people diagnosed with diabetes worldwide. Epidemiological studies on twins and families have provided a strong correlation for genetic factors contributing to etiology of type 2 diabetes.[1] A strong familial aggregation has been observed among Asian Indians with high prevalence among first-degree relatives and vertical transmission through two or more generations.[2] Diabetes mellitus is strongly associated with oxidative stress, which can be a consequence of either increased production of free radicals, reduced antioxidant defense or both.[3] Despite large number of studies it is not clear whether oxidative stress is a factor contributing to the development of diabetic condition or it is a consequence of the disease. Erythrocyte oxidative stress is implicated in the pathogenesis of type 2 diabetes.[4] The present work was undertaken to study known markers of erythrocyte oxidative stress: malondialdehyde (MDA) and reduced glutathione (GSH) in first degree relatives of T2DM patients, in an effort to understand the role of oxidative stress in the etiology of type 2 diabetes.

Material and Methods

The study was conducted in normotensive subjects of age ranging between 30-45 with ≥ one parent diagnosed with type 2 diabetes .The activity was compared with age and sex-matched controls with negative family history of type 2 diabetes. Type 2 diabetic patients were also included in the study, the criteria for selecting type 2 diabetic patients was the same as described earlier.[5,6] All subjects gave informed consent for use of their blood samples for the study. The protocol for the study was in accordance with guidelines of institutional ethical committee. Blood samples were taken in the morning to avoid the confounding effect of diurnal variation of oxidative stress parameters.

Isolation of Erythrocytes: Venous blood was collected from normal, first degree relatives of type 2 diabetics and type 2 diabetic patients after an overnight fast using heparin as an anticoagulant. The blood was centrifuged at 1800 rpm for 10 minutes at 4°C. Plasma and buffy coat was removed and isolated erythrocytes were washed thrice with cold (PBS) phosphate buffered solution (0.9 % NaCl, 10 mM Na_2HPO_4, pH 7.4) and finally packed erythrocyte was obtained.

Determination of Total Antioxidant Capacity of the Plasma: The total antioxidant capacity of the plasma was determined in terms of Ferric Reducing ability of Plasma (FRAP) values following the method of Benzie and Strain.[7]

Determination of Malondialdehyde Content
Packed erythrocytes (0.2 ml) were suspended in 3 ml Krebs Ringer Phosphate buffer (KRP), pH 7.4. The lysate (1 ml) was added to 1 ml of 10% Trichloroacetic acid (TCA) and the mixture was centrifuged for 5 min at 1000g. MDA was determined in the supernatant according to the method of Esterbauer and Cheeseman.[8]

Determination of Reduced Glutathione Content
Erythrocyte GSH was measured following the method of Beutler.[9] The method was based on the activity of the –SH

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group to reduce 5,5´-dithiobis,2 nitro benzoic acid (DTNB) and form yellow colored anionic product whose optical density was measured at 412 nm. The concentration of GSH was expressed in mg/ml packed red blood cells and was determined by a standard plot.

Statistical analyses were performed using the software PRISM 4.

Results and Discussion

We observe a decreased antioxidant potential of the plasma in type 2 diabetic patients and also in first degree relatives (Fig 1). Other reports also show a decreased antioxidant potential of plasma in diabetes, however to the best of our knowledge this is the first report of a decreased plasma antioxidant capacity in first degree relatives of T2DM patients. Milani *et al*[10] has reported decreased total antioxidant capacity in diabetic rats.

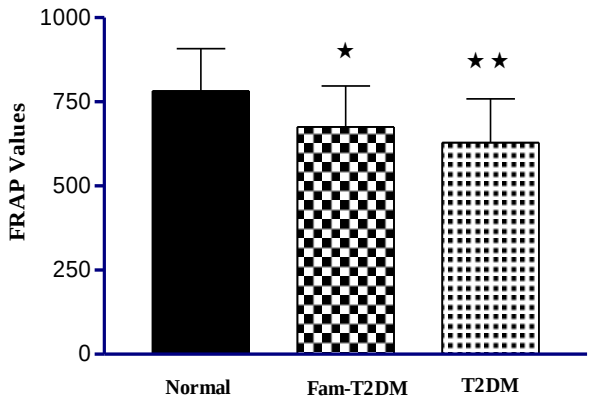

Figure 1: Antioxidant Potential of the Plasma determined in terms of FRAP values in normal, first degree relatives of type 2 diabetic patients (Fam-T2DM) and T2DM patients. . FRAP values expressed as µmol Fe (II) per liter of plasma. * P < 0.05 and ** P < 0.01 compared to normal control

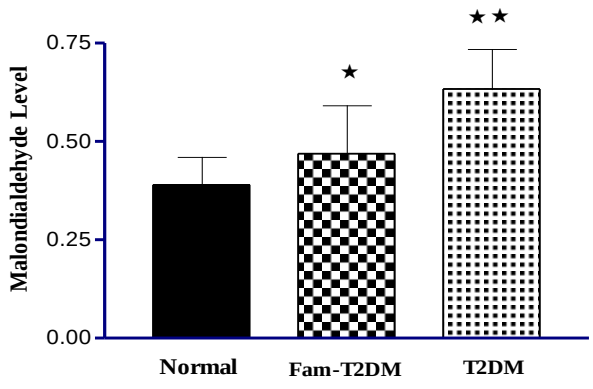

Figure 2: Erythrocyte malondialdehyde in normal, first degree relatives of type 2 diabetic patients (Fam-T2DM) and T2DM patients. MDA is expressed as nmol/mL of packed erythrocytes. * P < 0.01 and ** P < 0.001 compared to normal control.

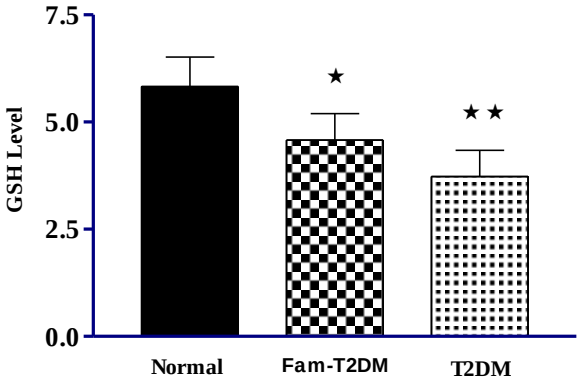

Figure 3: Reduced glutathione level in erythrocyte in normal, first degree relatives of type 2 diabetic patients (Fam-T2DM) and T2DM patients. GSH is expressed in mg per mL packed erythrocytes. * P < 0.01 and ** P < 0.001 compared to normal control

Figures 2 and 3 show the levels of MDA and GSH in erythrocytes in the three groups of patients. We show an increased MDA and decreased GSH in T2DM patients, these findings are consistent with our already published results. Significantly we observe an increased erythrocyte MDA and decreased intracellular GSH in first degree relatives of T2DM patients.

The erythrocyte membrane is prone to lipid peroxidation under oxidative stress that involves cleavage of polyunsaturated fatty acids at their double bonds leading to the formation of MDA. The elevation of erythrocyte MDA in first-degree relatives of T2DM patients is also corroborated with a recent report.[11] Reduced glutathione is a major intracellular non-protein SH compound and is accepted as the most important intracellular hydrophytic antioxidant. Our results showing substantially reduced level of GSH in prediabetic group indicate the prevalence of oxidative stress in erythrocytes of first degree relatives of T2DM patients.

These findings show that an impaired redox balance may be a cause for disturbance of homeostasis in type 2 diabetic families even before the development of the disease. We hypothesize that that oxidative stress precedes the development of overt diabetic state.

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