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Original Article

Role of Serum Oxidants and Antioxidants as Biomarkers in Maturity Onset Diabetes of the Young (MODY)

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Abstract

Background: Maturity onset diabetes of the young (MODY) is a heterogeneous familial diabetes mellitus characterized by early age of onset, autosomal dominant inheritance and primary defects of insulin secretion. Studies have shown increased oxidative stress levels in diabetes patients which has the potential to be used as biomarkers for detection and management. This study was undertaken on patients presenting with MODY in a medical college hospital to asses the oxidative stress levels. **Materials and Methods:** Serum levels of protein carbonyls, malondialdehyde (MDA), catalase and superoxide dismutase (SOD) were estimated in 31 patients categorized as MODY. The serum levels of the oxidants and antioxidants were also measured in age and sex matched controls. Mann-Whitney test was used to assess the statistical significance of differences in the serum assay values of the oxidative stress parameters between the groups. **Results:** The catalase and SOD levels were 44.4 ± 11.8 and 47.3 ± 16.8 U/L respectively and protein carbonyls and MDA were 33.2 ± 8.5 and 0.84 ± 0.29 µ mol/L respectively. **Conclusions:** Hence it is concluded that antioxidants levels are increased and the oxidants levels are decreased in patients with MODY suggesting their possible role as biomarkers.

Keywords: MODY, oxidants, antioxidants, biomarkers.

Introduction

Maturity onset diabetes of the young (MODY) is a genetically and clinically heterogeneous subtype of familial diabetes mellitus. The characteristics of MODY are its early age of onset, autosomal dominant inheritance and primary defects of insulin secretion.[1] Around 2-5% of type-II diabetes are estimated to be of MODY type. Detection of MODY is a challenge and is largely underdiagnosed, yet diagnosis of MODY has important implications for the individual patient. It allows individualized case to be tailored to the underlying genetic cause and provides information about the natural history of the disease. Awareness of this uncommon disease, the use of additional clinical clues and biomarkers can improve its detection rates and hence its management.[2]

Though MODY has an autosomal dominant inheritance it is also seen among those exposed to greater oxidative stress levels which can be estimated by serum oxidant and antioxidant levels.[4] Animal experiments show association of increased oxidative stress with diabetes mellitus and its complications. Studies on humans with diabetes have shown increased serum levels of oxidative by-products and decreased levels of antioxidants.[6] This study aims to determine the levels of oxidative stress among patients presenting with MODY.

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Materials and Methods

The study was conducted on a series of young patients diagnosed with diabetes in a medical college hospital at Bengaluru. Thirty one diabetics of both genders who had a strong family history of diabetes and the age of onset

being between 20 to 30 years were selected. Juvenile diabetes and non-insulin dependent diabetes mellitus type-II (NIDDM) patients were excluded from the study.[3] Age and gender matched controls were selected and it was ensured that the patients were not ketotic at presentation.

Spectrophotometric analysis of serum samples of patients with MODY and controls was done to assess the levels of oxidative stress markers. The serum levels of protein carbonyls were estimated by oxyblots and malondialdehyde (MDA) by thiobarbituric acid reaction (TBAR) assay. Oxyblots were carried out on serum samples by reprivatizing with dinitrophenyl hydrazine (DNPH) in the presence of 12% SDS for 20 min at room temperature. The reaction was stopped by neutralization with 2M Tris in 30% glycerol and 10µl of the sample was spotted in triplicate on nitrocellulose membrane and probed with anti-DNP antibody. Nonderivatized samples did not show anti-DNP immunoreactivity confirming the specificity of the antibody.[3]

Oxidants assay

Lipid peroxidation was measured by estimation of MDA by TBAR method. Serum samples were added to a mixture containing 0.75 ml of acetic acid (pH 3.5, 20% v/v), 0.1 ml SDS (8%, w/v) and 0.75 ml of thiobarbituric acid (0.8%, w/v) and heated in a boiling water bath for 45 min. The adducts formed were extracted into 1.5 ml of 1-butanol and centrifuged at 2,500 rpm (10 min) and their absorbance was measured at 532 nm. The amount of MDA formed was calculated using the molar extinction coefficient (24m mol/cm).[3]

Antioxidants assay

The catalase assay was made by reaction mixture containing 15µg protein (serum sample) mixed with 900 l phosphate buffer (0.1 M, pH 7.0) and 50 l of H2O2(8.8 mM) and the decrease in absorbance at 240 nm was followed for 5 min. The enzyme activity was expressed as mol H2O2 consumed/min/mg protein (e = 43.6 mM-1 cm-1).[3]

Superoxide Dismutase (SOD) activity was assayed using its inhibitory action on quercetin oxidation based on the method described earlier with minor modifications. The final reaction mixture contained 30 mM Tris HCl (pH 9.1), 0.5 mM EDTA, 50 mM TEMED, 0.05 mM quercetin and 10 l of serum sample containing 10 g of protein. The reaction was monitored at 406 nm for 10 min. One unit of SOD activity was defined as the amount of enzyme (per mg protein) that inhibits quercetin oxidation reaction by 50% of maximal value.[3]

Statistical analysis

The significance of differences in the assays of serum levels of oxidants and antioxidants between patients with MODY and age and sex matched were assessed by Mann-Whitney test. A p value of < 0.05 was considered as statistically significant.

Results

The assay values of the oxidative stress markers are presented as mean ± SD in table 1.

Table 1. Oxidative stress marker levels in MODY (n=31)

Stress Marker	Mean Levels	SD
SOD	**U/L**	
Control	59.7	13.4
MODY	47.3	16.8
Catalase	**U/L**	**SD**
Control	9.6	5.2
MODY	44.4	11.8
MDA	**u Mole/L**	**SD**
Control	3.16	1.62
MODY	0.84	0.29
Protein Carbonyls	**Arbitrary values**	**SD**
Control	21.5	4.3
MODY	33.2	8.5

It is observed that SOD levels are decreased significantly in MODY patients compared to control subjects ($p<0.05$) (Fig 1).

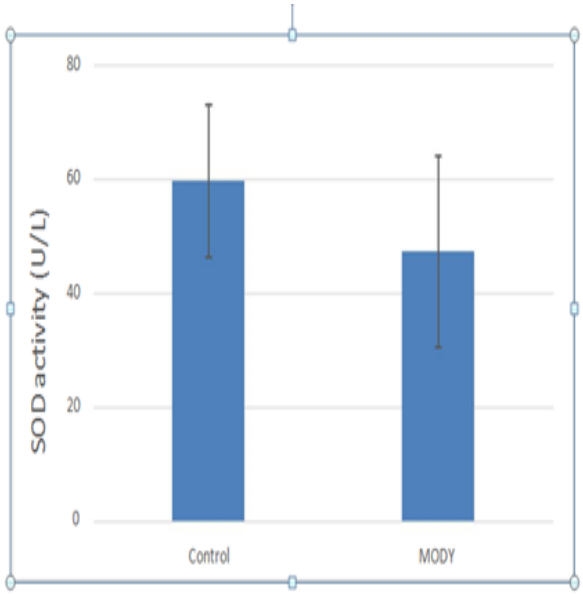

Fig 1. Serum SOD levels in MODY patients and control subjects

It is observed that Catalase levels are increased significantly to compensate increased levels of ROS, in MODY patients compared to control subjects ($p<0.05$) (Fig 2).

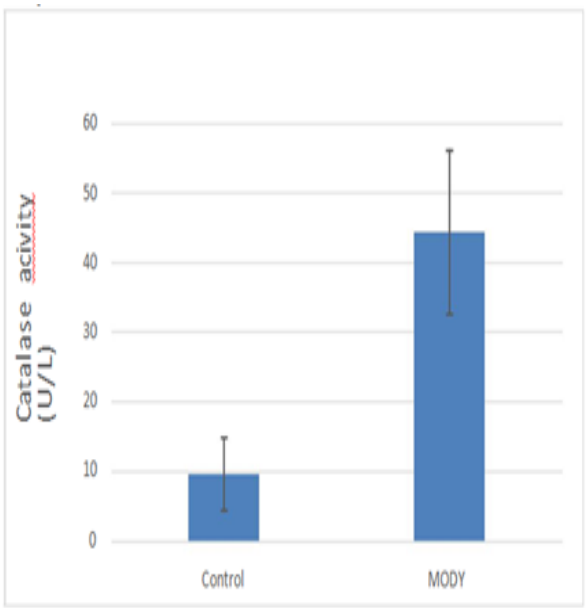

Fig 2. Serum Catalase levels in MODY patients and control subjects

It is observed that MDA levels are decreased significantly in MODY patients compared to control subjects ($p<0.05$) (Fig 3).

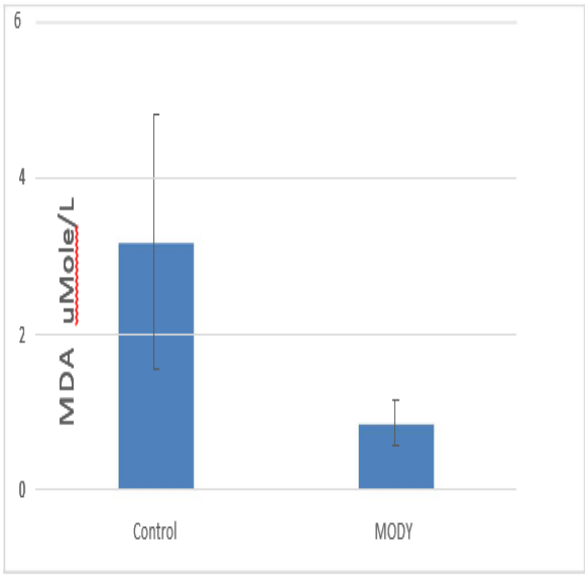

Fig 3. Serum MDA levels in MODY patients and control subjects

It is observed that protein carbonyl levels are increased significantly in MODY patients compared to control subjects. ($p<0.05$) (Fig 4).

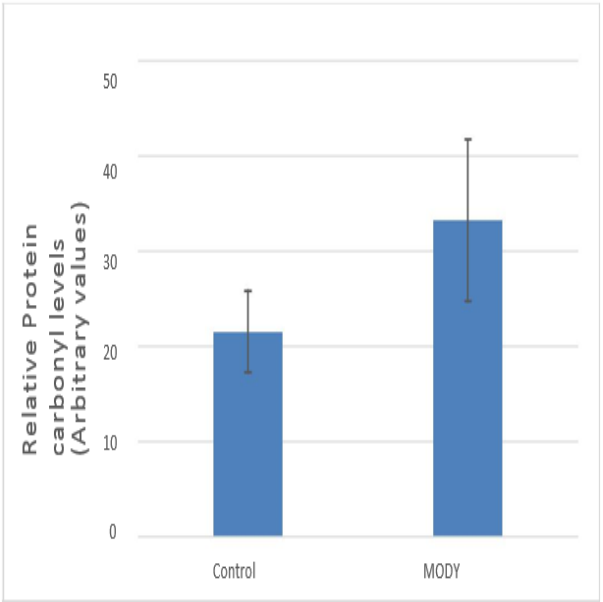

Fig 4. Serum protein carbonyls levels in MODY patients and control subjects

Discussion

Spectrophotometric analysis of serum samples of MODY patients found that the oxidative marker levels are altered. From the data observed in the patients with MODY it was found that serum contained elevated levels of protein carbonyls and decreased levels of MDA (fig 1 & 2, Table 1) compared to age matched controls as seen in few similar other studies.[7] Our study data also suggests that serum catalase levels are increased to nullify excess reative oxygen species formed by oxidative stress and SOD levels are significantly decreased compared to the age matched controls. This may be due to increased oxidative stress causing excessive production of reactive oxygen species [ROS] which may reduce antioxidant levels. These accumulated free radicals may damage DNA and cause dysregulation of gene expression and may be a risk factor for MODY.

Conclusion

Our study strongly suggests the use of assessment of serum oxidative stress markers such as Catalase, SOD, MDA and protein carbonyls in the detection of MODY in young diabetics with a significant family history of the disease.

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