

**CORRELATION BETWEEN HEXOKINASE AND MDA IN TYPE 2 DIABETES
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ABSTRACT

Hexokinase catalyses the first step of glycolysis and located in the inner mitochondrial membrane. MDA is used as a marker for oxidative stress which is imbalance between free radicals and their scavenging enzymatic and non enzymatic antioxidants. For present study, we selected patients having history of maternally inherited type 2 diabetes mellitus, which are more prone to oxidative stress induced cellular damages due to mitochondrial dysfunctions. Increased oxidative stress may cause hexokinase inactivation, which may lead to severity of diabetes due to inappropriate glucose metabolism. Therefore, this study has been undertaken to evaluate activity of hexokinase enzyme in oxidative stress.

KEYWORDS: Hexokinase, MDA, type 2 diabetes mellitus, glycolysis.**INTRODUCTION**

Diabetes mellitus is an alarming disease in India and widespread around the globe. In India, more than 40 million people have been affected with diabetes mellitus, however approximately about 171 million people were affected with diabetes mellitus upto the year 2000, which is expected to rise 366 million worldwide by the year 2030 unless urgent preventive steps are taken.^[1,2] In the last few decades research on diabetes mellitus was strictly focused on insulin related drugs and therapies, but in the last two decades mitochondria has attended much prominence in the field of diabetes mellitus via various metabolic pathways and its genomic mutations.^[3]

Mitochondria are dynamic organelles predominantly occur almost in every living cell and considered to the main powerhouse of the cell; since it involve in ATP production by the process of oxidative phosphorylation. It has been proved that, mitochondria can inherit in families via female germline, since paternal mitochondria marked for ubiquitous destruction in early embryonic stages of the development. ATPs generated by mitochondria meets the cellular energetic demands, but this process may also leads to harmful agents like free radicals, responsible for maximum cellular damages including proteins, DNA, cell membranes etc.^[4-8]

Mitochondria have their own genome and codes for the 13 polypeptides of the Electron Transport Chain (ETC) in association with the nuclear genome. During the process of ETC, when electrons flow thorough it some electrons may get leaked and forms superoxide radicals

and alters the status of enzymatic and non enzymatic antioxidants leads to increase cellular oxidative stress. However, since mitochondria lacks in histone proteins and other DNA repair enzymes, mitochondrial DNA is more vulnerable to oxidative stress induced mutations, which forms abnormal polypeptides of the ETC, causing more electrons leakage and high oxidative stress.^[8] This phenomenon is called as Mitochondrial "Vicous Cycle" reported as one of the culprit for type 2 diabetes mellitus and other physiological conditions and diseases.^[9]

Malondialdehyde (MDA) the end product of lipid peroxidation has been reported as a marker for oxidative stress.^[10,11] Hexokinase catalyzes the first step of glycolysis via glucose phosphorylation. It is associated with the outer surface of the external membrane through specific binding to a porin. This association allows hexokinase, direct utilization of ATP generated during respiration.^[12] However, as this enzyme localise in the vicinity of the mitochondria, we have undertaken this study to investigate the effect of oxidative stress on an activity of hexokinase.

MATERIALS AND METHODS**1. Family History and sample collection**

As, mitochondria inherited via female germline only, we have selected 25 patients from 4 families having history of maternally inherited type 2 diabetes mellitus (Ages ranging from 18-75 years). Blood samples were collected after taking signed consents from studied individuals and completed under ethical supervision of "Swami Vivekanand Medical Mission Hospital" Nagpur.

2. Inclusion Criteria

Families with a history of maternally inherited diabetes.

Exclusion Criteria

Any kind of paternal history of type 2 diabetes mellitus, Juvenile diabetes mellitus, type 1 diabetes mellitus etc.

3. Sample Preparation

1 ml blood sample was collected in EDTA vacutainer tubes from all studied individuals and centrifuged at 3000×g for 15 minutes to collect plasma. The plasma samples were recentrifuged at 3000×g for same time to avoid the carryover of blood cells and were collected in new vials and stored at -20°C.

4. Evaluation of Hexokinase activity and determination of MDA

Activity of hexokinase was estimated using the method given by Singh A.^[13] A decrease in absorbance at 560

nm was recorded/ mg protein/ ml/ minute. The formation of MDA (n mole/ mg protein/ ml /hour), determines the level of lipid peroxidation. MDA was assayed in the form of Thio Barbituric Acid Reactive Substances (TBARS) as per the method of Stocks J et al.^[14]

5. Statistical Analysis

Statistical analyses were done using Med Calc Statistical software. Correlation was studied between selected parameters by Spearman's coefficient Rank correlation. All results were reported in Mean±SEM and level of significance was considered to be ≤ 0.05.

RESULTS AND DISCUSSION

Table 1 shows the mean activity of hexokinase (0.005603±0.000568) and concentration of MDA (0.01429±0.0008332) in all studied 25 patients.

Table: 1 Table showing activity of hexokinase and concentration of MDA.

SR	Parameters	Mean ± SEM n=25
1	Hexokinase	0.005603±0.000568
2	MDA	0.01429±0.0008332

Table: 2 Correlation analysis of Hexokinase with MDA

SR	Parameters	Spearman's coefficient of Rank Correlation	p- Value
1	Hexokinase	-0.659	0.0013
2	MDA		

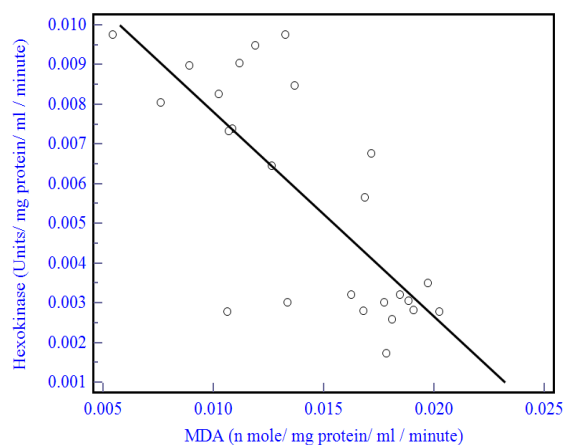


Fig 1: Figure showing inverse correlation analysis between Hexokinase activity and MDA in studied individuals.

Table 2 showed the significant correlation between an activity of hexokinase enzyme and MDA (-0.659; P=0.0013). We found significant inverse correlation between an activity of hexokinase and MDA, suggesting that when oxidative stress increases an activity of hexokinase decreases. This is possibly due to the fact that, hexokinase enzyme is located in the region of mitochondria, and studied patients are having a history of maternal inheritance type 2 diabetes mellitus expected high oxidative stress due to abnormal mitochondria. Since, we have selected patients suffering from

maternally inherited type 2 diabetes mellitus, significantly strong decreased mean activity of hexokinase enzyme would increase the abnormal glucose metabolism in studied patients and may lead to increase higher risk of micro and macrovascular complications.

CONCLUSION

Significant inverse correlation between MDA and hexokinase suggested that oxidative stress may decrease activity of hexokinase.

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