RESEARCH ARTICLE

Evaluation of Glutathione dependent antioxidant enzymes and A 8344 G mitochondrial DNA mutation in maternally inherited type 2 diabetes mellitus.

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ABSTRACT

Diabetes mellitus is characterised by hyperglycaemia often accompanied with polydipsia and polyurea. In recent past decade's mitochondria has attended much prominence in the field of type 2 diabetes mellitus. Mutations in mitochondrial DNA lower the ATP production and increases the ROS generation responsible for insulin resistance and type 2 diabetes mellitus. At present many point mutations have been reported for type 2 diabetes mellitus. The present study was undertaken to evaluate the status of glutathione dependent antioxidant enzymes and mitochondrial DNA mutation A/G at position 8344, which may responsible for maternally inherited type 2 diabetes mellitus.

Keywords: Mitochondrial DNA, Reactive Oxygen Species (ROS), mutation, type 2 diabetes mellitus.

INTRODUCTION

Diabetes Mellitus (DM) is prevalent around the world and an alarming epidemic in India. The prevalence of DM in world in the year 2000 was 171 million which is expected to rise up to 366 million by the year 2030 (Wild S et al. 2004). Mitochondria are the dynamic organelles prevalent almost in every living cell and the main source of ATP via oxidative phosphorylation. Mitochondria contain its own genome measures about 16,569 base pairs and code the thirteen polypeptides of the electron transport chain (ETC) (Lamson D W 2002, Dongre U J 2015). Moreover, mtDNA lack in histone proteins and DNA repair enzymes making it more susceptible to mutations. Mutated mtDNA can generate more free radicals (ROS), which may damage other cellular organs. Mutations in mtDNA at position A 8344 G codes defective polypeptides of the ETC, which may leaks an electrons and decreases the production of ATP. Decreased ATP concentration opens the ATP sensitive potassium channel, which causes depolarization of the cell, prevent intracellular calcium entry and therefore resist the insulin release from insulin stored vesicles (Lamson D W 2002).

MATERIALS AND METHODS

Sample Collection and Family history:
This study included eighteen samples from three families; family 1 and family 2 are diabetic whereas family 3 is non diabetic (healthy control) between the age group of 18 to 70 years. Signed consents were taken from all patients and healthy control individuals from "Swami Vivekanand Medical Mission Hospital" Nagpur, Maharashtra, India.

Inclusion Criteria: Families with a history of maternally inherited diabetes.

Exclusion Criteria: Any kind of paternal history of type 2 diabetes mellitus, type 1 diabetes mellitus, Juvenile diabetes mellitus.

Sample Preparation: 2 ml blood sample from each participant was collected in EDTA vacutainer tubes; from each tube 0.3 ml of blood sample was used to isolate DNA and the remaining blood sample was centrifuged at 3000×g for 15 minutes for the collection of plasma. The plasma samples were recentrifuged at 3000×g for the same time and were collected in new vials to avoid the carryover of blood cells. All samples were stored at -20˚C until further analysis.

Enzymatic Analysis All standard methods were used to determine the concentration of various antioxidant enzymes. Activity of Glutathione Reductase was assayed by the method of Racker et. al. (1955), Glutathione Peroxidise was estimated by the method of Rotruck et. al. (1973), Glutathione -S- Transferase was assayed as per Habig et. al. (1974) and protein was estimated by Lowery method (1951).

Statistical Analysis: Statistical analyses were done using MS-Excel and Med Calc Software. All results were expressed in Mean ± SD. The two tailed probability student "t" test was used to differentiate between the two diabetic families assuming unequal variance. P< 0.05 was taken as a standard for significance difference.

Isolation of Mitochondrial DNA and Identification of mutation: DNA was isolated by using whole blood DNA isolation Kit (Ge Nei catalogue No: 612102300011730). Mutation was identified by mismatch PCR. Total 223 nucleotide sequence was amplified using mismatch PCR as per the method of Lee H. C. (1997). Presence of the mutation will generate two bands of 197 and 26 bps, while absence of mutation will generate 223 bp DNA bands.

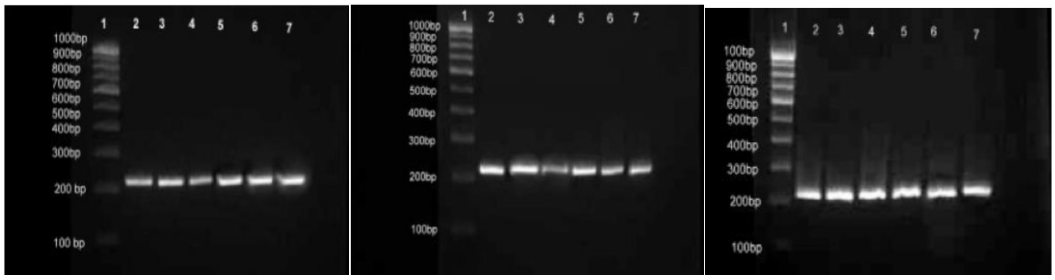
RESULT AND DISCUSSION

Results showed the absence of the 8433 A/G mutation not only in selected DM families but also in control healthy individuals. This was according the study of Wang S (2013).

Table 1: Statistical analysis of sought parameters.

PARAMETERS	GROUPS	MEAN±SD
Glutathione Reducatse (Units/mg protein/ ml / minute)	Control	0.020±0.019
	Family 1	0.034±0.019*
	Family 2	0.033±0.025*
Glutathione S Transferase (Units/mg protein/ ml / minute)	Control	35.63±3.05
	Family 1	28.36±4.90**
	Family 2	33.16±7.08*
Glutathione Peroxidase (Units/mg protein/ ml / minute)	Control	172.32±36.45
	Family 1	102.65±80.00**
	Family2	99.12±45.84***
Thio Barbituric Acid (nM of MDA/ mg protein/ ml / minute)	Control	0.0012×10^{-5}±7.26
	Family 1	0.0019×10^{-4}±5.49**
	Family 2	0.0020×10^{-4}±3.95***
Nitric Oxide (nM/ml)	Control	4.54±1.44
	Family 1	24.10±6.12****
	Family 2	15.77±2.68****
Hydrogen Peroxide (µM/ ml)	Control	8.26±0.066
	Family 1	5.80±0.16**
	Family 2	5.16±0.31**

*>0.05; **<0.01; p***<0.001; p****<0.0001



(A) **(B)** **(C)**

Fig 1: (A) shows mutation analysis of healthy control individuals, **(B)** shows mutation analysis of family 1 and **(C)** shows mutation analysis of family 2. The molecular weights of all amplicons were found to be 233 bp, shows an absence of the mutation.

Glutathione Reductase catalyzes the reduction of glutathione disulphide (GSSG) to the sulfhydryl form glutathione (GSH), which is a critical molecule in resisting oxidative stress and maintaining the reducing environment of the cell. It was found that the activity of glutathione reductase did not varied significantly in both family 1 and family 2 ($p>0.05$).

Glutathione –S- Transferase is an enzyme that catalyses the conjugation of glutathione to electrophilic centres on a wide variety of substrates in order to make the compounds more water soluble. This activity detoxifies endogenous compounds such as peroxidised lipids and enables the breakdown of xenobiotics. Through statistical analysis it was found that the activity of Glutathione –S- Transferase differed significantly in family 1 ($p<0.01$) as compared to healthy control individuals while in family 2 the activity did not differed significantly ($p>0.05$).

Like Glutathione –S- Transferase, Glutathione Peroxidase also plays a central role in defence mechanism against the free radical damage. The main function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. The activity of glutathione peroxidase differed significantly in both family 1 ($p<0.01$) and family 2 ($p<0.001$).

Lipid peroxidation refers to the oxidative degradation of lipids which is measured through the formation of TBARS (Thiobarbituric Acid Reactive Substances). For both the families formation of TBARS differed significantly. The p value for family1 was ($p<0.01$) and for family 2 it was (<0.001). Nitric oxide is another free radical which can generate an oxidative stress if the concentration gets increased. The concentration of nitric oxide varied significantly in both the families ($p<0.0001$). We found significantly decreased concentration of hydrogen peroxide in both families ($p<0.01$).

CONCLUSION

Altered levels of antioxidant enzymes and free radicals corroborated oxidatrive stress. However, this study did not show 8344 A/G mutation in selected families diagnosed and type 2 diabetes and having history of maternally inherited type 2 diabetes mellitus.

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