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Oxidative Stress and 3243 A/G Mitochondrial Dna Mutation In Maternally Inherited Type 2 Diabetes Mellitus

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Abstract: ***Aims: The imbalance between the activities of antioxidant enzymes and free radicals gives rise to oxidative stress. Increased oxidative stress can induce other serious complications in diabetic patients. Mutations in mitochondrial DNA can cause oxidative stress and type 2 diabetes mellitus. Hence, the present study was undertaken to evaluate the levels of antioxidant enzymes, lipid peroxidation and mitochondrial DNA 3243 A/G mutation in families with a history of maternally inherited type 2 diabetes mellitus. Method: This study included two diabetic families and one normal healthy family. The level of catalase, Mn/Fe SOD, Cu/Zn SOD, nitric oxide and malonaldehyde formation were studied via various biochemical standard methods. DNA isolation and mutation analysis were done as per standard protocols and methods. Result: As compared to control samples, a significant decrease in the activities of catalase (family 1 p<0.01, family 2 p<0.01), Mn/Fe SOD (family 1 p<0.01, family 2 p< 0.05), Cu/Zn SOD (family 1 p<0.001, family 2 p<0.01) was observed. Significantly increased concentrations of malonaldehyde (family 1 p< 0.05, family 2 p<0.05) and nitric oxide (family 1 p<0.05, family 2 p<0.05) was also observed. Both diabetic families represent altered antioxidant enzymes status. This study corroborates an absence of the mutation. Conclusion: High oxidative stress was shown in both the families with diabetes, but we did not find a mutation in mitochondrial DNA.***

Keywords: Oxidative stress, Antioxidant Enzymes, Free Radicals, Mitochondrial DNA.

1. Introduction

Diabetes mellitus is now prevalent everywhere around the world. In the year 2000 the prevalence of the diabetes in the world was 171 million, which is expected to rise up to 366 million in the year 2030 [1,2]. Mitochondria are dynamic organelles and found almost in every cell and play a central role in ATP generation via oxidative phosphorylation. [3]. Oxidation of glucose generates reducing equivalents like FADH2 and NADH, which transfer their electron to the electron transport chain. But the electron transport chain found to be leaked sometimes which gives rise to an electron leakage during its passage from complex I to complexes IV. These leaked electrons can form free radicals like $ONOO^-$, $OH^\bullet$ ion, $O^\bullet$ superoxide anion, H_2O_2 etc; referred as ROS (reactive oxygen species), which are chemically highly reactive and can react with various cellular organelles, proteins, DNA (deoxyribonucleic acid), cell membranes etc. and damage them. This intracellular damage initiates the cascade of cellular non functioning [4,5,6].

Aerobic animals have generated a defence mechanism against free radicals through various antioxidant enzymes, including Catalase, Superoxide dismutase (SOD), Glutathione peroxidase etc. [7]. Among these, Cu/Zn SOD is a cytoplasmic enzyme, whereas Mn/Fe SOD found in mitochondria [8]. Mitochondria are the main site for respiration and inherited maternally by their offspring's. Mitochondrial DNA with nuclear DNA codes for the polypeptides of the electron transport chain (ETC). Mutated mitochondrial DNA generates an aberrant ETC and reported as a causative agent for type 2 diabetes mellitus. Inheritance of such defective mitochondria could increase the oxidative stress in families [9, 10, 11, 12,13].

Lipid peroxidation is a chain process; generated due to leakage of electrons. It determines the free radicals attack and the cell damage by them [14]. Nitric oxide is a potent free radical which is formed from the conversion of L-Arginine to L Citrulline via nitric oxide synthase enzyme. Excess concentration of NO plays a key role in cardiovascular and other cell damages [15]. In diabetes mellitus oxidative stress is generated by an imbalance between free radicals and its scavenging systems. An aberrant activity of an antioxidant enzyme accelerates oxidative stress in diabetes patients, which can cause serious complications [16,17,18].

A mitochondrial DNA mutation at position 3243 A/G transcribes the aberrant tRNA Leucine, which may code, the defective polypeptide of an electron transport chain [19]. Mitochondrial DNA mutation A/G at position 3243 in mitochondrial DNA is not only associated with type 2 diabetes mellitus but also it can cause Mitochondrial Encephalopathy Lactic Acidosis and Stroke like Syndrome (MELAS) [20]. Many studies manifest the involvement of mitochondrial DNA 3243A/G mutation in maternally inherited type 2 diabetes mellitus. Because of high frequency rate amongst the worldwide population, we selected this mutation for study [21,22,23].

2. Material and Method

Sample Collections 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. Samples were collected after taking a signed consent form from the patients. All the samples and family history of patients were taken from "Diabetes Hospital" located at, 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 samples were collected in EDTA vacutainer tubes; from each tube 0.3 ml of blood sample was used to isolate DNA immediately 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 the samples were stored at -20˚C until further analysis.

Enzymatic Analysis

All standard methods were used to determine the concentration of various antioxidant enzymes. Catalase was estimated according to the method of Aebi et. al. (1983) [24]. In plasma samples, the addition of 2mM cyanide inhibited Cu/Zn SOD and MnSOD were unaffected by it, whereas the addition of 50 micro L ice cold Choroform/Ethanol inactivated Mn/Fe SOD [25,26]. Both Cu/Zn SOD and Mn/Fe SOD were assayed as per Marklund and Marklund (1974) [27]. TBARS (Thio Barbituric Acid Reactive Substances) was measured according to J. Stocks et. al. (1971) [28]. The concentration of Nitric Oxide was measured as per Green's et al (1982) [29]. And the concentration of protein was estimated according to Lawery O.H. et. al. (1951) [30].

Isolation of Mitochondrial DNA

DNA was isolated by using whole blood DNA isolation Kit (Ge Nei catalogue No: 612102300011730)

Identification of Mutation:

Total 581 nucleotides containing DNA amplification were carried out using ready to use Master Mix Bioline (Catalogue No.BIO-33057) by PCR (Polymerase Chain Reaction) method. Mutation analysis was done according to the method given by Ouweland J.M.W. [31]. The 20 nucleotides containing each forward and reverse primers were taken from nucleotide sequence 3029 to 3048 as FORWARD 5' AAGGTTCGTTTGTTCAACGA 3' and from 3591 to 3610 as REVERSE 5' GGCCTAGGTTGAGGTTGACC 3' as per revised Cambridge Reference sequence (rCRS) of the human mitochondrial DNA (MITOMAP) and performed pBLAST online tool, using NCBI (National Centre for Biotechnology Information) gene sequences. Primers were synthesized through IDT scientific technologies. PCR reactions were carried out in the 12 µL reaction mixture consisting of 1 µL of DNA template (100ng), 2µL Forward primer (100ng), 2µL reverse primer (100ng) and 7µL of the Bioline master mix. Protocol condition consisted, incubation at 94˚C for 3 minutes, followed by 94˚C for 30 seconds, 55˚C for 30 seconds, 72˚C for 45 seconds and a final incubation at 72˚C for 5 minutes in Biorad Thermal Cycler (USA). Amplified samples were then preceded for RFLP (Restriction Fragment Length Polymorphism); digested by Restriction Enzyme ApaI GeNie at 37˚C for approximately 24 hours. Resultant amplicons were subsequently electrophoresed using 2% agarose gel and visualized by ethidium bromide, with DNA ladder (100 to 1000 bp) GeNei (Catalogue No. GeNei 612652671001730) in the Gel-Doc system (Bio-Rad USA). The digested amplicons will give 367 bp and 214 bp containing DNA fragments if A/G mutation is present at position 3243 in mitochondrial DNA. A single 581 bp DNA band represents an absence of the mutation.

Statistical Analysis:

Med Calc statistical software was used for statistical analysis of oxidative stress. All results were expressed in Mean ± SD. The two tailed probability student's T test was used to differentiate between the two diabetic families assuming unequal variance. $P < 0.05$ was a standard for significance difference.

3. Result

Table 1 shows the general data about the diabetic family 1 and 2 and normal control samples selected for the study. Oxidative stress markers are represented in table 2.

This study demonstrates that, as compared to normal control subjects, the activity of catalase is significantly decreased not only in family 1 ($p<0.01$) but also in family 2 ($p<0.01$) [Table 2, Fig (A)]. Likewise the significant decrease in Mn/Fe SOD activity has been reported in family 1 ($p<0.01$) and family 2 ($p<0.05$) [Table 2, Fig (B)]. In addition to this the activity of Cu/Zn SOD was significantly lower in family1 ($p<0.001$) as well as in family 2 ($p< 0.01$) [Table 2, Fig (C)]. The levels of MDA were significantly increased in both familiy1 ($p<0.05$) and family 2 ($p<0.05$) [Table 2, Fig (D)]. The concentration of nitric oxide was found significantly increased in family1 ($p<0.05$) and family 2 ($p<0.05$) [Table 2, Fig (E)].

Table 1: General Data of Selected Families.

PARAMETERS	TOTAL Obs.	CONTROL MEAN±SD (No. Obs. 6)	FAMILY1 MEAN±SD (No. Obs. 6)	FAMILY2 MEAN±SD (No. Obs. 6)
AGE (YEARS)	18	43.16±11.99	43.83±15.99	46.66±16.07
ONSET	12		39.83±9.94	39.83±9.17
WEIGHT (Kg)	18	60.33±6.59	75.83±9.23	71.50±15.43
FPGLU (Mg/dL)	18	81.00±12.93	189.00±18.31	195.33±28.07

Obs. = Observations, No.Obs. = Number of observations, FPGLU = Fasting Plasma Glucose.

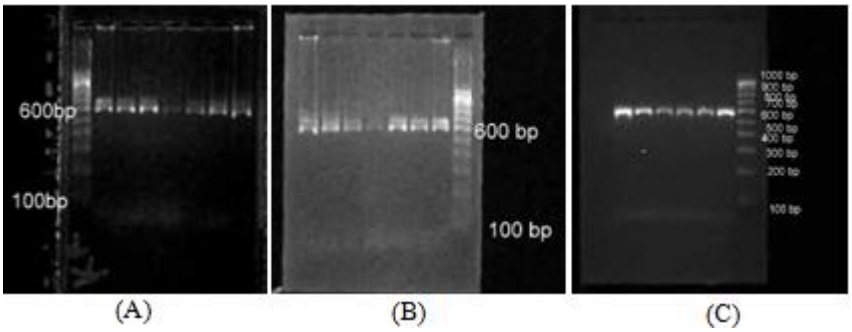
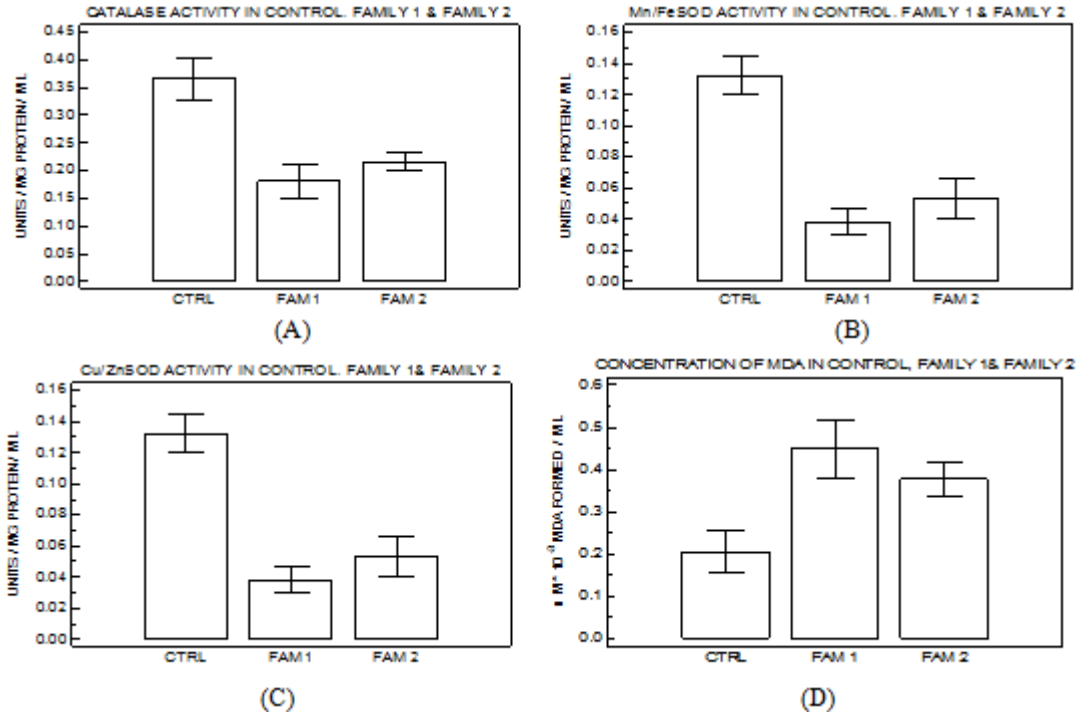


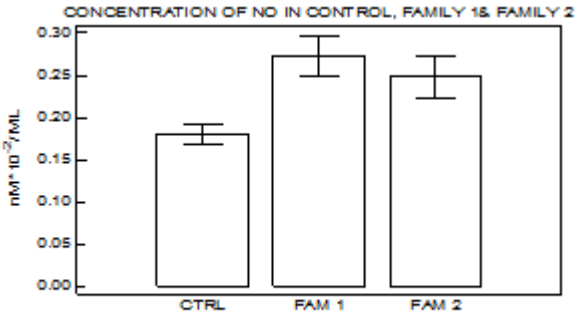
Figure 1: A 3243 A/G mutation analysis in Family 1(A), family 2 and in control healthy subjects (C). The intact 581 bp containing DNA bands were observed. In panel A, first well contains DNA ladder and form well 2 to 8 contains patient's amplified DNA samples except well number 5. In panel B, last well contains DNA ladder and form well 1 to 7 contains patient's amplified DNA samples except well number 4. In panel C, last well contains a DNA ladder and from well 2 to 7 contains patient's amplified DNA samples.

Figure 1 represents the 3243A/G mutation analysis in both diabetic family 1, 2 and in control samples. Electrophoresis showed 581 bp containing DNA bands when they were compared to control DNA ladder. This showed that there was no 3243A/G mutation in mitochondrial DNA of maternally inherited type 2 diabetes mellitus.

4. Discussion

Mutated mitochondria can generate more free radicals. The free radicals are highly reactive in nature due to its unsatisfied valencies [32] and hence it can act as a potent source in the pathogenesis of diabetes mellitus [33,34]. The various antioxidant enzymes and other antioxidants like vitamin E, vitamin C, carotene, etc. play a vital role in free radicals scavenging mechanism [35,36]. Oxidative stress is an imbalance between the free radicals and antioxidant enzymes. Many studies revealed that oxidative stress in diabetes mellitus gives rise to an increase concentration of free radicals and diminishes the activity of antioxidant enzymes [37-39].





(E)

Figure 2: The activity of various antioxidant enzymes, including catalase represented by a graph (A), the Mn/FeSOD and Cu/Zn SOD represented by (B) and (C) respectively; similarly the concentration TBARS shown by (D) and the concentration of Nitric Oxide shown by (E) in normal control subjects and in patients with diabetes of family 1 and family 2.

We evaluated the levels of antioxidant enzymes in maternally inherited type 2 diabetes mellitus and in a healthy control subjects. The results of this study, clearly corroborates the oxidative stress in both family 1 and family 2. Catalase is the important antioxidant enzyme found nearly in all cells [40] which converts hydrogen peroxide into water molecule and oxygen [41]. Hydrogen peroxide if not removed from the biological samples it may convert into hydroxyl radicals, which may impart more oxidative stress. Hydroxyl radicals are the potent free radical, which is reported as the deadliest free radical which has ever been found. Hence catalase plays an important role in free radical scavenging [42]. Statistical analysis affirms that not only in family 1 but also in family 2 there is a significant decrease in the activity of catalase enzyme in both the diabetic families. This is in accordance with various other studies [43,44]

Like the catalase enzyme, Manganese/ Iron Superoxide Dismutase and Copper/ Zinc containing Superoxide Dismutase also plays a central role in the defence mechanism against the free radicals damage [45]. The main function of the Superoxide Dismutase enzyme is to eradicate superoxide anion which can cause damage to cells, cellular membranes, DNA and other biological organelles. Superoxide Dismutase converts superoxide anion radical into the hydrogen peroxide molecule [46,47]. Many studies showed that there is decreased activity of Mn/Fe SOD and Cu/Zn SOD in type 2 diabetes mellitus than normal control samples [48]. This study also shows a significant decrease in the activity of Mn/Fe SOD and Cu/Zn SOD in patients with type 2 diabetes.

Malonaldehyde formation is an indication of increased lipid peroxidation in diabetes mellitus [49]. It is used to determine the oxidative stress through determining the balance between free radicals and antioxidant enzymes [50]. We analysed the significant increase in the concentration of malonaldehyde in both diabetic families. The increased concentration of malonaldehyde indicates an oxidative stress in patients with diabetes. Various other studies showed an increase in oxidative stress in diabetes mellitus [43,51,52].

Statistics showed somewhat similar results for the concentration of nitric oxide to those of malonaldehyde. Nitric oxide may react with superoxide anion and can form peroxinitrite molecules, which can harm cellular mechanism. Hence, it is very essential to maintain the concentrations of free radicals like nitric oxide and superoxide radicals [53]. Although, the life span of nitric oxide is very less, increased concentration can induce an oxidative stress and may play a pivotal role in diabetes mellitus [54]. The present study, exhibited significant increase in the concentration of nitric oxide in both the families of diabetic patients. Certain other studies have also demonstrated an increase concentration of nitric oxide in patients with diabetes than control [55,56].

Table 2 : A Statistical analysis of various antioxidant enzymes, lipid peroxidation and nitric oxide.

ENZYMES	*GROUPS*	*MEAN±SD*	*P-VALUE*
CATALASE	CONTROL	0.3659±0.0931	
(Units/Mg protein/ML)	FAMILY1	0.1805±0.0752	<0.01
	FAMILY2	0.2160±0.0400	<0.01
Mn/Fe SOD	CONTROL	0.0376±0.0074	
(Units/Mg protein/ML)	FAMILY1	0.0244±0.0064	<0.01
	FAMILY2	0.0215±0.0115	<0.05
Cu/Zn SOD	CONTROL	0.1325±0.0308	
(Units/Mg protein/ML)	FAMILY1	0.0379±0.0203	<0.001
	FAMILY2	0.0532±0.0305	<0.01
MDA	CONTROL	0.2048±0.1247	
($nM \times 10^{-3}$ / ML)	FAMILY1	0.4487±0.1656	<0.05
	FAMILY2	0.3767±0.1022	<0.05
NO	CONTROL	0.1800±0.0303	
($nM \times 10^{-2}$ / ML)	FAMILY1	0.2483±0.0611	<0.05
	FAMILY2	0.2733±0.0592	<0.05

Mitochondrial DNA lacks histone proteins and DNA repair mechanisms, which increases the chances of its damage by free radicals [57]. Colossal work on mitochondrial DNA exhibited its role in maternally inherited type 2 diabetes mellitus [21,22,23]. For last few decades, researchers have been working on mitochondrial DNA to identify the various mitochondrial DNA mutations, which can play a pivotal role in the pathogenesis of type 2 diabetes mellitus. Over more than 40 different mitochondrial DNA mutations associated with type 2 Diabetes Mellitus have been identified yet [58]. In this study, the absence of 3243 A/G mutation has been observed in the selected families (Fig: 2), this was according to the study of Naveed AK et.al. [59].

5. Conclusion

This study cogitated on oxidative stress and 3243 A/G mitochondrial DNA mutation in two families with a history of maternally inherited type 2 diabetes mellitus. Decrease activities of various antioxidant enzymes like catalase, Mn/Fe SOD, Cu/Zn SOD with increase concentration of nitric oxide and lipid peroxidation providing a straight forward evidence of oxidative stress in both diabetic families. Increased oxidative stress can cause other complications like DNA and cellular damage in the patients. Therefore, monitoring oxidative stress in patients with maternally inherited type 2 diabetes mellitus could be of utmost importance to prevent these complications. The undertaken study did not observe a 3243A/G mutation in patients with a history of maternally inherited type 2 diabetes mellitus, other mutations meight be present in selected families.

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References

[1] Wild S, Gojka R, Green A, Sciref R, King H. Global prevalence of diabetes estimates for the year 2000 and projection for 2030. Diabetes Care 2004;27:1047-1053.
[2] World Health Organization (WHO). Facts and figures. http://www.who.int/diabetes/facts/world_figures/en/
[3] Jean-Claude HEnquin. Triggering and amplifying pathways of regulation of insulin secretion by glucose. Diabetes 2000;49: 1751-1760.
[4] Cadenas E, Davies K J. Mitochondria free radical generation, oxidative stress and aging. Free Radical Biology and Medicine 2000;29:222-230.
[5] Ide T, Tsutsui H, Kinugawa S, Utsumi H, Kang D, Hattori N, et al. Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. Circulation Research 1999;85:357-363.
[6] Machlin L J and Bendich A. Free radical tissue damage: protective role and antioxidant nutrients. The FASEB journal 1987;6:441-445.
[7] Halliwell B. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant physiology 2006;141:312-322.
[8] Keller J.N., Kindy M S, Holtsberg F W, Clair DKS, Yeb HC, Grmeyer A, et.al. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: Suppresion of peroxynitrite production, lipid peroxidationa nd mitochondrial dysfunction. The Journal of Neuroscience 1998;18:687-697.
[9] Miki T, Nagashima K and Seino S. The structure and function of ATP sensitive potassium channel in insulin secreting pancreatic beta cells. Journal of Molecular Endocrinology 1999;22:113-123.
[10] Craig T J, Ashcroft F M and Proks P. How ATP inhibits the open kATP channel. J. Gen Physiol 2008;132:131-144.
[11] Ohkubo K, Yamano A, Nagashima M, Mori Y, Anzai K, Akehi Y, et al. Mitochondrial gene mutation in the tRNA Leu(UUR) Region and diabetes: Prevalence and Clinical Phenotypes in Japan. Clinical Chemistry 2001;47:1641-1648.
[12] Lynn S, Wardell T, Johanson MA, Chinnery PF, Dally ME, Walkar M, et al. Mitochondrial Diabetes: investigation and identification of a novel mutation. Diabetes 1998;47:1800-1802.
[13] Dongre U J, Meshram VG. Is mitochondrial DNA responsible for maternally inherited type 2 diabetes mellitus: A hypothetical review. Int. J. Pharm Sci. Rev. Res 2014;28:179-187.
[14] Min B and Ahn D.V. Mechanism of lipid peroxidation in meat and meat products-A review. Food Sci Biotechnol 2005;14:152-163.
[15] HAbib S and Ali A. Biochemistry of nitric oxide. Ind J Clin Biochem 2011;26:3-17.
[16] Flekac M, Skrha J, Higartova J, Lacinova Z and Jarolimkova M. Gene polymorphism of superoxide dismutase and catalase in dibetes mellitus. BMC Medical genetics 2008;30: 1-9.
[17] Dongre UJ & Meshram VG. A statistical analysis of antioxidants and biochemical parameters in maternally inherited type 2 diabetes mellitus. Asiatic Journal of Biotechnology Resources 2014;4:94-96.
[18] Dongre UJ & Meshram VG. Evaluation of glutathione dependant antioxidant enzymes in maternally inherited type 2 diabetes mellitus. J. Pharm. Sci & Res. 2015;7:137-140.
[19] Moraes, C.T., F. Ciacci, E. Bonilla, C. Jansn, M. Hirano, N. Rao, R.E. Lovelace, L.P. Rowland, E.A. Schon, and DiMauro. Two novel pathogenic mitochondrial DNA mutation affected organelle number and protein synthesis. Is the tRNALeu(UUR) gene an etiologocal hot spot?. Journal of Clinical Investigation 1993;92: 2906-2915.
[20] Goto, Y., I. Nonaka, and S. Horai. A mutation in the tRNA Leu(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature 1990;348: 651-653.
[21] Takashi K, Kodawari H, Mori Y, Tobe K, Sakuta R, Suzuki Y, et. al. A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. N Engl J Med 1994;330: 962-968.
[22] Ohkubo K, Yamano A, Nagashima M, Mori Y, Anzai K, Akehi Y, et al. Mitochondrial gene mutation in the tRNA Leu(UUR) Region and diabetes: Prevalence and Clinical Phenotypes in Japan, Clinical Chemistry, 2001;47:1641-1648.
[23] Duraisamy P, Elango S, Vishwananadha VP, Balamurugan R. Prevalence of mitochondrial t RNA gene mutation and their association with specific clinical phenotypes in patients with type 2 diabetes mellitus of Coimbatore. Genetic testing and Molecular Biomarkers 2010;14: 49-55.
[24] Aebi H, wyss SR, Scherz B, Gross J. Properties of erythrocyte catalase from homozygotes and heterozygotes for Swiss type acatalasemia. Biochemical Genetics 1976;14:791-807.

[25] Crouch RK, Gandy SE, Kimsey G, Galibraith RA, Galibraith GMP, Buse MG. The inhibition of islets superoxide dismutase by daibetogenic drugs. Diabetes 1981,30;235-241.
[26] Ken CF, Lee CC, Duan KJ, Lin CT. Unusual stability of manganese superoxide dismutase from a new species, tatumella Ptyseas CT: its gene structure, expression and enzymatic properties. Protein Expression and Purification 2004;40:42-50.
[27] Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem 1974;47:469-474.
[28] J. Stocks, T.L.Dormandy. Auto oxidation of Human red cell lipids induced by hydrogen peroxide, British Journal of Heamatology 1971, 20;95-111.
[29] Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [15N] nitrate in biological fluids. Anal Biochem 1982;126:131-138.
[30] Lowery O.H., N.J. Rosebrugh, A.L. Farr and R.J. Randall. Protein measurement with folin-phenol reagent. J. Biol Chem 1951: 265.
[31] Van Den Ouweland JMW, Lunkes HHPJ, Ruitenbeek W, Sandkuiji LA, De Vijilder MF, Struyvenberg PAA, et.al. Mutation in mitochondrial tRNA Leu (UUR) gene in a large pedigree with maternally transmitted type 2 diabetes mellitus and deafness. Nature Genetics 1992;1: 368-371.
[32] Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress and aging. Free Radical Biology and Medicine 2000;3: 222-230.
[33] Griesmacher A, Kindhouser M, Andert S E, Schreiner W, Toma C, Knoebl P, et. al. Enhanced serum levels of thiobarbituric acid reactive substance in diabetes mellitus. American Journal of Medicine 1995;98:469-475.
[34] Giron MD, Salto R, Gonzalez Y, Giron J A, Nieto N, Periago J, et. al. Modulation of hepatic and intestinal glutathione s transferase and other antioxidant enzymeby dietary lipids in streptozotocin diabetic rats. Chemosphere 1999;38: 3003-3013.
[35] Ramanathan M, Jiaswal Ak and Bhattacharya SK. Superoxide dismutase, catalase and glutathione peroxidise activities in the brain of strptozotocin induced diabetic rats. Ind. J. Exp. Biol 1999;37:182-183.
[36] Merzouk S, Hichami A, Madani S, Merzouk H, Berrouiguet AY, Prost J, Moutairou K, Chabane-Sari and Khan NA. Antioxidant status and levels of different vitamins determined by high performance liquid chromatography in diabetic subjects with multiple complications. Gen. Physiol. Biophys 2003;22:15-27.
[37] Kangralkar V.A., Patil SD, Bandivadekar RM. Oxidative stress and diabetes: A review. International Journal of Pharmaceutical applications 2010;1: 38-45.
[38] Godin DV, Wohaeib SA, Garnett ME, Goumeniouk AD. Antioxidant enzyme alterations in experimental and clinical diabetes. Mol Cell Biochem 1988; 84:223-233.
[39] Noda Y, Mori A, Packer L. Gliclazide scavengers hydroxyl, superoxide and nitric oxide radicals: an ESR study. Mol Pathol. Pharmacol 1999; 96:115-124.
[40] P. Chelikani, I Fita and P.C. Loewen. Diversity of structures and properties among catalases. Cellular and Molecular Life Sciences 2004;61:192-208.
[41] Takemoto K, Tanaka M, Iwata H et.al. Low catalase activity in blood is associated with the diabetes caused by alloxan. Clinica Chimica Acta 2009;407:43-46.
[42] Tiedge M, Lortz S, Modey R, Lenzen S. Complimentary action of antioxidant enzymes in the protection of bioengineered insulin producing RIN m5F cells against the toxicity of reactive oxygen species. Diabetes 1998; 47:1578-1585.
[43] Sundaram RK, Bhaskar A, Vijayalingam S, Vishwanathan M, Mohan R, Shanmugasundaram. Antioxidant status and lipid peroxidation in type 2 diabetes mellitus with and without complications. Clinical Science 1996;90:255-260.
[44] Marica JB, Veljko B, Jadranka B, Zeljko M, Ivan J, Zeljko R. Impact of glycemic control on antioxidant enzyme activity in patients with type 2 diabetes mellitus. Diabetologia Croatica 2004;33:131-135.
[45] Zelko IN, mariani JJ, Folz RJ. Superoxide dismutase multienzyme family: a comparison of the Cu/Zn SOD (SOD1), MnSOD (SOD2) ans EC SOD (SOD3) gene structures evolution and expression. Free Radical Biology and Medicine 2002; 33:337-349.
[46] Faraci FM, Didion SP. Vascular protection: Superoxide dismutase isoform I the vessel wall. Atherosclerosis Thrombosis and Vascular Biology 2004;24:1367-1373.
[47] Wang X, Tao L and Hai CX. Redox-regulating role of insulin: the essence of insulin effect. Molecular and Cellular Endocrinilogy 2012;349:111-127.
[48] Hamed S, Brenner B, Aharon A, Daoud D, Roguin A. Nitric oxide and SOD modulate endothelial proginator cell function in type 2 diabetes mellitus. Cardiovascular Diabetology 2009;8:56.
[49] Gallou G, Ruelland A, Legars B, MAugender D. Allannic H and Cloarec L. Plasma malonaldehyde in type 1 nad type 2 diabetic patients. Clin.Chim. Acta 1993;214:227-234.
[50] Altomare E, Vendemiale G, Chicco D, Procacci V and Cirelli F. Increased lipid peroxidation in type 2 poorly controlled diabetic patients. Diabetes metab 1992;18:264-271.
[51] Pasaoglu H, Banu S,Bukan N. Lipid peroxidation and resistance to oxidation n patients with type 2 diabetes mellitus. Tohoku J. Exp. Med 2004;203:211-218.
[52] Gupta S, Chari S. Proxidant and antioxidant status in patients of type 2 diabetes mellitus IHD. Indian Journal of Clinicla Biochemistry 2006;21:118-122.
[53] Guzik TJ, West NEJ, Pillai R, Taggart DP, Channon KM. Nitric Oxide modulates superoxide release and peroxinitrite formation in human blood vessels. Hypertention 2002;39:1088-1094.
[54] Giugliano D, Ceriello A. Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996;96:257-267.
[55] Bhatia S, Shukla R, MAdhu V, Kaur J, Prabhu KM. Antioxidant status, lipid peroxidation and nitric oxide end products in patients of type 2 diabetes mellitus with neuropathy. Clinical biochemistry 2003;36:557-562.
[56] Maejima K, Nakano S, Himeno M, Ichi S T, Makishi H, Ito T, Nakagawa A, et. al. Incraesed basal level of plasma nitric oxide in type 2 diabetic

subjects:Relationship to microvascular complications. Journal of Diabetes and its complications 2001;15:135-143.

[57] Chistiakov DA, Sobenin IA, Bobryshev YV, Orekhov AN. Mitochondrial dysfunction and mitochondrial DNA mutation in atherosclerotic complications in diabetes. World J. Cardiol 2012;5:148:156.

[58] Lamson, D.W., and S.M. Plaza. Mitochondrial factors in the pathogenesis of diabetes: a hypothesis for treatment. Alternative Medicine Review 2002;7: 94-111.

[59] Naveed AK, Wahid M, Naveed A. Mitochondrial t RNA Leu (UUR) gene mutation and maternally inherited diabetes mellitus in Pakistani population. International Journal of Diabetes Mellitus 2009;1: 11-15.