EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 3
232-237
Year: 2017

CATALASE AND MALONDIALDEHYDE LEVELS: POSSIBLE MARKERS FOR TYPE 2 DIABETES MELLITUS

Dr. Chandrika Rao*[1], Dr. Sanjana Rao[2], Dr. Kishan Prasad H.L.[3] and Dr. Vidya B.[4]

[1]MBBS, DCP, PhD (Path), Assistant Professor, Department of Pathology, K. S. Hegde Medical Academy, Deralakatte, Mangalore.
[2]Intern, K S Hegde Medical Academy, Deralakatte, Mangalore.
[3]MBBS, MD (Path), Associate Professor, Department of Pathology, K. S. Hegde Medical Academy Deralakatte, Mangalore.
[4]Intern, K S Hegde Medical Academy, Deralakatte, Mangalore.

***Corresponding Author: Dr. Chandrika Rao**
MBBS, DCP, PhD (Path), Assistant Professor, Department of Pathology, K. S. Hegde Medical Academy, Deralakatte, Mangalore.

Article Received on 01/01/2017 **Article Revised on 21/01/2017** **Article Accepted on 12/02/2017**

ABSTRACT
Background: Diabetes is a chronic and common metabolic disorder with a rapidly increasing prevalence due to population growth, aging, urbanisation and obesity due to physical inactivity characterized by hyperglycemia. Catalase (CAT) is an antioxidative enzyme which plays an important role against oxidative stress-generated complications such as diabetes and cardiovascular diseases. To estimate catalase and malondialdehyde (MDA) levels in type 2 diabetes mellitus (DM) patients. To analyze the relation between catalase, MDA and fasting blood sugar (FBS) in type 2 DM. **Methods:** The study included hundred, age and sex matched subjects. Blood samples of all subjects were analyzed for fasting blood sugar, catalase and malondialdehyde. **Results:** Catalase levels were significantly decreased and malondialdehyde levels were significantly increased in type 2 diabetes mellitus as compared to controls. There is positive correlation between MDA and FBS in type 2 diabetes mellitus. **Conclusion:** Diabetes patients are susceptible to oxidative stress with free radical mediated lipid peroxidation. The results show significant decrease in antioxidant enzyme and increase in MDA in type 2 diabetes mellitus as compared to normal healthy subjects. This suggests that the antioxidant enzyme production is affected in type 2 diabetes mellitus patients leading to higher risk of cell organ damage.

KEY WORDS: Type 2 Diabetes mellitus, catalase, malondialdehyde, oxidative stress, lipid peroxidation.

INTRODUCTION
Diabetes is a chronic and common metabolic disorder with a rapidly increasing prevalence due to population growth, aging, urbanisation, and the increase of obesity due to physical inactivity.[1] It is characterized by hyperglycemia associated with glycosuria, polydypsia and polyphagia. Type 2 diabetes mellitus (DM) is caused by a collection of pathophysiological mechanisms, among which insulin resistance and beta-cell failure play important roles.[2] Diabetes leads to a variety of complications including peripheral vascular diseases, nephropathy, neuropathy, retinopathy, morbidity, and/or mortality.[1]

Oxidative stress plays a pivotal role in cellular injury from hyperglycemia. A certain amount of oxidative stress/reactive oxygen species (ROS) is necessary for the normal metabolic processes since ROS play various regulatory roles in cells. ROS are produced by neutrophils and macrophages during the process of respiratory burst in order to eliminate antigens .They also serve as stimulating signals of several genes which encode transcription factors, differentiation, and development as well as stimulating cell-cell adhesion, cell signalling, involvement in vasoregulation, fibroblast proliferation, and increased expression of antioxidant enzymes. However over- and/or uncontrolled production of ROS are deleterious.[2] Oxidative stress is produced either by increased production of reactive oxygen species or by decreased efficiency of antioxidant defences. Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals. Glucose oxidation is believed to be the main source of free radicals. In its enediol form, glucose is oxidized in a transition-metal dependent reaction to an enediol radical anion that is converted into reactive ketoaldehydes and to superoxide anion radicals. The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals. Superoxide

anion radicals can also react with nitric oxide to form reactive peroxynitrite radicals.[3]

Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals. Another important source of free radicals in diabetes is the interaction of glucose with proteins leading to the formation of an Amadori product and then advanced glycation endproducts (AGEs). These AGEs, via their receptors, inactivate enzymes and alter their structures and functions, promote free radical formation, and quench and block antiproliferative effects of nitric oxide. By increasing intracellular oxidative stress, AGEs activate the transcription factor NF- κB, thus promoting up-regulation of various NF- κB controlled target genes. NF-κB enhances production of nitric oxide, which is believed to be a mediator of islet beta cell damage.[3]

While on the one hand hyperglycemia engenders free radicals, on the other hand it also impairs the endogenous antioxidant defense system in many ways during diabetes. Antioxidant defense mechanisms involve both enzymatic and nonenzymatic strategies. Common antioxidants include the vitamins A, C and E, glutathione and the enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase.[3]

Intracellular antioxidant defence mechanism is mainly provided by antioxidant enzymes by catalyzing the decomposition of reactive oxygen species. The major antioxidant enzymes include superoxide dismutase, catalase and gluthaione peroxidase. Oxidative stress acts as mediator of insulin resistance and its progression to glucose intolerance and installation of diabetes mellitus, subsequently favouring the appearance of atherosclerotic complications and contributes to rise in many micro- and macrovascular complications.[1]

Catalase is an antioxidative enzyme present nearly in all living organisms. It plays an important role against oxidative stress-generated complications such as diabetes and cardiovascular diseases. Catalase acts as main regulator of hydrogen peroxide metabolism. Hydrogen peroxide is a highly reactive small molecule formed as natural by-product of energy metabolism. Excessive concentration of hydrogen peroxide may cause significant damages to proteins, DNA, RNA, and lipids. Catalase enzymatically processes hydrogen peroxide into oxygen and water and thus neutralizes it. Increased risk of diabetes has been documented in patients with catalase deficiency. The deficiency of this enzyme leads, in the β-cell, to an increase in oxidative stress and ultimately to a failure of this cell type. β-cells are rich in mitochondria, and thus this organelle might be a source of ROS.[1]

Lipid peroxidation is the oxidative degradation of lipids, free radicals take electrons from lipids of the cell membrane leading to cell injury.[3] Lipids are reported as one of the primary targets of ROS.[1] This is a free radical chain reaction and involves 3 steps. Initiation takes place by reactive oxygen species to produce fatty acid radical and water. The fatty acid radical produced is not very stable so it reacts with molecular oxygen to produce peroxyl-fatty acid radical. This radical too isn't stable so it reacts with another free fatty acid to give a different fatty acid radical and lipid peroxide. Termination takes place when two radicals react to produce a non radical species. Antioxidants scavenge free radicals and prevent lipid peroxidation and protect cell membrane.[4]

Hydroperoxides have toxic effects on cells both directly and through degradation to highly toxic hydroxyl radicals. They may also react with transition metals like iron or copper to form stable aldehydes, such as malondialdehyde (MDA), that damage cell membranes. Peroxidation of lipids produces highly reactive aldehydes, including MDA, acrolein, 4-hydroxynonenal (HNE), 4-oxononenal (ONE) and isolevuglandins. It has been reported that peroxyl radicals can remove hydrogen from lipids, producing hydroperoxides that further propagate the free-radical pathway. MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress.[1] The aim of this study was to assess the levels of catalase and malondialdehyde and its association with fasting blood sugar in patients with type 2 diabetes mellitus.

MATERIAL AND METHODS

The study protocol was approved from the institutional ethical committee K. S. Hegde Medical Academy, Mangalore, India. Fifty subjects with type 2 Diabetes mellitus and 50 non diabetic subjects of age group 20 – 60 years, of either sex were included in the study after an informed and written consent duly signed by each participant. Subjects suffering from hypertension and renal disorders were excluded.

Taking aseptic precautions blood samples; approximately 5 ml is collected without anticoagulant in appropriate sterile vials by venous arm puncture for MDA and catalase analysis. For FBS analysis, the patient is kept fasting overnight and 2ml of blood is collected in fluoride containing vacutainer. The serum is separated by centrifugation at 2500rpm for 15 minutes and stored at 4˚C. Fasting blood sugar was analyzed by GOD-PAP method. For catalase estimation 3ml of hydrogen peroxide buffer and 3ml of distilled water is taken in different cuvettes. 10µl of the sample is added to both the cuvettes. The change in absorbance is measured at 240nm wavelength. For MDA estimation 100µl of the serum sample is made upto 500µl with distilled water. 1ml of the TCA-TBA-HCl reagent is added and kept in boiling water bath for 15minutes. Cooled and centrifuged. The supernatant is taken and the optical density of the pink colour developed is measured at 535nm in the spectrophotometer.

Statistical Analysis: This is performed using descriptive statistics mean, standard deviation and correlation in SPSS version 20.0. Independent sample't' test is used. Level of significance is 5%.

RESULTS

In the present study, 100 subjects were included. Out of which 50 were Type 2 DM patients confirmed by biochemical investigations as per WHO criteria and 50 were non-diabetic apparently healthy control subjects. Serum CAT, FBS and MDA levels was measured in these subjects. (Table 1, figure 1).

The mean serum level of catalase in the study group was 22.91±5.71µmol/L; in control group it was 26.85±6.81 µmol/L. Catalase was significantly lower among diabetics. (Table 2, figure 2).

The mean serum level of MDA in study group was 1.66±0.57 µmol/L, in control group it was 0.95±0.33 µmol/L. MDA was significantly higher among diabetics. (Table 3, figure 3).

There is a positive correlation between FBS and MDA (r = -0.205, p= 0.040) (Table 4) (figure 4) There is a no significant association between fasting blood sugar and catalase levels in type 2 DM subjects.

Table 1: Fasting blood sugar levels of controls and patients with Type 2 diabetes mellitus

FBS level (mg/dl)	Group	Number of subjects (n)	Mean	Standard deviation	"t" value	Significance (p value)
	Control	50	101.26	11.48	-5.226	<0.001 S
	Case	50	135.30	44.61		

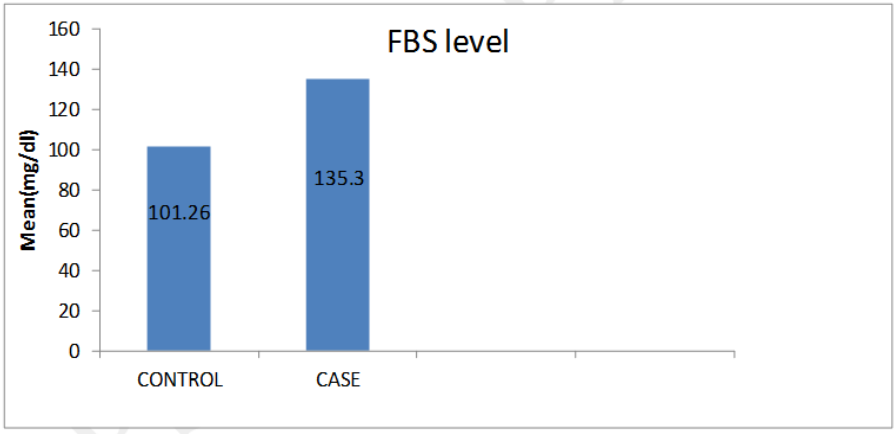

Figure 1: Graphical representation of fasting blood sugar levels in normal and diabetes patients

Table 2: Antioxidant enzyme (CAT) status in controls and patients with Type 2 diabetes mellitus

CAT(µmol/L)	Group	Number of subjects (n)	Mean	Standard deviation	"t" value	Significance (p value)
control	1	50	26.85	6.81	3.131	0.002 S
Study	2	50	22.91	5.71		

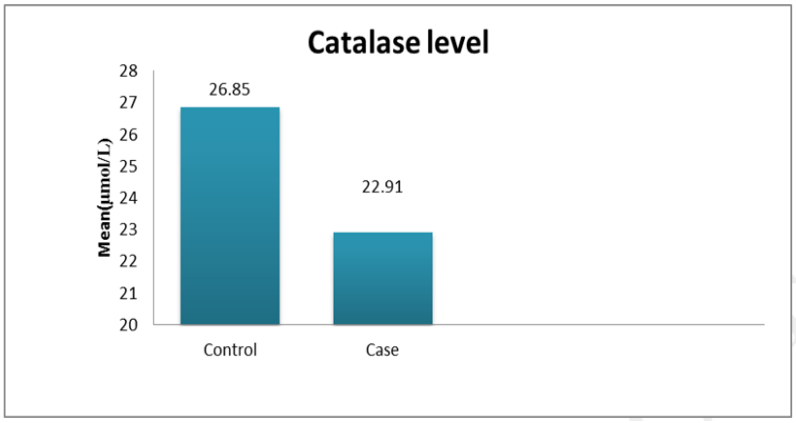

Figure 2: Graphical representation of catalase levels in normal and diabetes patients

Table 3: Lipid peroxidation (MDA) status in controls and patients with Type 2 diabetes mellitus

MDA (µM/L)	Group	Number of subjects (n)	Mean	Standard deviation	"t" value	Significance (p value)
control	1	50	0.95	0.33	-7.56	<0.001 S
study	2	50	1.66	0.57		

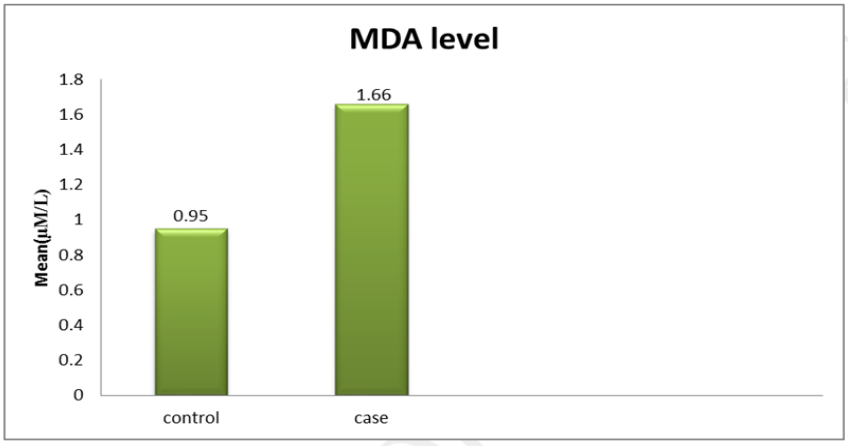

Figure 3: Graphical representation of malondialdehyde levels in normal and diabetes patients

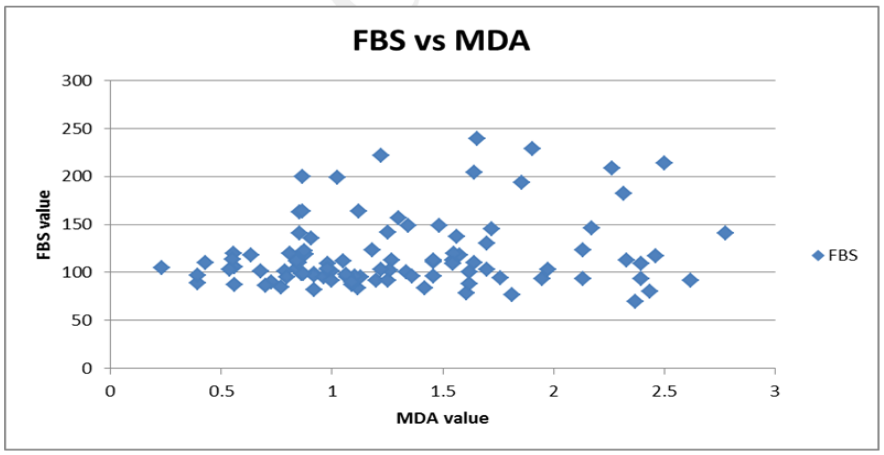

Figure 4: Scatter diagram showing correlation between fasting blood sugar level and malondialdehyde in type 2 diabetes mellitus patients

Table 4: Correlation between fasting blood sugar level, malondialdehyde and catalase in type 2 diabetes mellitus patients

	Number of subjects (n)	Pearson correlation(r value)	Significance (p value)
FBS vs CAT	50	-0.179	0.074 NS
FBS vs MDA	50	0.205	0.040 S

DISCUSSION

Many studies have shown increased oxidative stress is present in diabetic subjects.[5, 9-11] Present study provides further evidence that there is presence of oxidative stress with alteration in antioxidant enzyme (CAT) activity and increase in lipid peroxidation (MDA levels) in Type 2 diabetic patients.

The evaluation of antioxidant enzyme CAT in Type 2 Diabetes mellitus patients and normal subjects indicated impaired antioxidant status in diabetic patients which is a definite sign of oxidative stress. Statistically significant decrease in CAT activities when compared to controls (p = 0.002).Verma S et al[2], Kumawat M[5] reported increase in CAT activity in type 2 diabetes mellitus. Goth et al[12] suggested that decreased catalyse activity may be due to elevated hydrogen peroxide, could contribute to oxidative destruction of pancreatic β cells, to decrease insulin secretion, insulin effectiveness and to the onset of diabetes. There are conflicting reports of decrease,[13-16] increase[2, 5, 7, 17-20] and no change in catalase activity under diabetic condition.[21-22] The onset of diabetes for catalase deficient patients appeared more than 10 years earlier than for normocatalasemic subjects.[23]

The MDA levels were significantly higher in type 2 diabetes mellitus patients compared to normal subjects (p=0.000), similar to findings of earlier studies.[2, 5-7, 15, 24-25] A study conducted in Mumbai, observed that freshly diagnosed diabetics under no medications had higher MDA levels across sexes compared with the finding in the control group.[26] In another study, MDA values increased with an increase in the levels of plasma glucose and duration in patients with diabetes without complications and in diabetics with ischemic heart disease.[13] A Spanish study on children and adolescent showed that the level of estimated MDA was higher with the earlier onset and prolonged duration of diabetes, and these levels continued to rise during the course of the disease.[27] The hyperglycaemia associated with hyperlipidemia could be causative factor for the increased production of free radical and lipid peroxidation (MDA level).[5] The extreme production of reactive oxygen species (ROS) has been suggested as a common result leading to intensified oxidative damage at the level of lipid peroxidation and peak in diabetic nephropathy in association with diabetes. [28,29]

A significant correlation is observed between MDA and CAT levels in diabetic and non diabetic subjects. No similar study was found to support the same. No significant correlation is observed between CAT and FBS levels in type 2 diabetes mellitus patients, this is in contrast to the study report by Likid lilid et al.[7]

Significant association is found between MDA and FBS level in type 2 diabetes patients. A study conducted in Thailand noted significant positive correlation between the MDA level and FBS in poorly controlled type 2 diabetes mellitus and type 2 diabetes mellitus complicated with coronary heart disease.[7]

These findings confirmed the evidence that diabetes patients are susceptible to oxidative stress with free radical mediated lipid peroxidation. The results also showed significant decrease in antioxidant enzyme (CAT) in type 2 diabetes mellitus as compared to normal healthy subjects. This suggests that the antioxidant enzyme production is affected in type 2 diabetes mellitus patients leading to higher risk of cell organ damage. Therefore, detection of oxidative stress levels can be used as a marker for monitoring type 2 diabetes mellitus in our population. The decreased efficiency of cellular antioxidant mechanisms in type 2 diabetes mellitus patients with enhanced lipid peroxidation may constitute the pathogenic link between hyperglycemia and development of endothelial dysfunction and higher risk of cell organ damage.

REFERENCES

1. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of Oxidative Stress during Diabetes Mellitus. J Biomarkers, 2013; 1-8.
2. Verma S, Sagar N , Pushpank V, Shukla K N, Abbas M, Banerjee M. Antioxidant enzyme levels as marker for type 2 diabetes mellitus. International Journal Bioassays, 2013; 02: 685-690.
3. Maritim AC, Sanders JB, Watkins III. Diabetes, Oxidative Stress and Antioxidants: A Review. J Biochem Molecular Toxicology, 2003; 17: 24-38.
4. Bhatia S, Shukla R, Vonkata MS, Kaur GJ, Madhava PK. Antioxidant Status, lipid peroxidation and nitric oxide and products in patients of type 2 diabetes mellitus with nephropathy. Clin Biochem 2003; 35: 557-62.
5. Kumawat M, Sharma T, Singh I, Singh N, Ghalaut VS, Vardey S, Shankar V. Antioxidant Enzymes and Lipid Peroxidation in Type 2 Diabetes Mellitus Patients with and without Nephropathy. North American J Med Sciences 2013; 5: 213-19.
6. Bhutia Y, Ghosh A, Sherpa M, Pal R, Pradip M. Serum malondialdehyde level: Surrogate Stress Marker In The Sikkimese Diabetics. J Natural Science, Biology and Medicine 2011; 2: 107-12.
7. Likidlilid A, Patchanans N, Peera Patdit T, Sriratanasathavorn C. J Med Assoc Thai 2010; 93: 682-93.
8. Dwivedi J, Sarkar P. International Journal of Pharma and Biosciences 2010; 1: 340-50.
9. West IC. Radical and oxidative stress in diabetes. Diabetes Med 2000; 17: 171-80.
10. Jakus V. The role of free radicals, oxidative stress and antioxidant systems in diabetic vascular diasease. Bratisl Lek Listy 2000; 101: 541-51.
11. Ceriello A. Acute hyperglycemia and oxidative stress generation. Diabet Med 1997; 14: 45-9.
12. Góth L, Lenkey A, Bigler WL. Blood Catalase Deficiency and Diabetes in Hungary. Diabetic care, 2001; 21: 1839 – 50.
13. Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. Antioxidant status and lipid peoxidation in type 2 diabetes mellitus with and without complications. Clin Sci 1996; 90: 255-60.
14. Parthiban A, Vijayalingam S, Shanmugasundaram KR, Mohan R. Oxidative stress and the development of diabetic complications-antioxidants and lipid peroxidation in erythrocytes and cell membrane.Cell Biol Int 1995; 19: 987-93.
15. Ramakrishna V, Jailkhani R. Oxidative stress in noninsulin-dependent diabetes mellitus (NIDDM) patients. Acta Diabetol 2008; 45: 41-6.
16. Kuppusamy UR, Indran M, Ahmad T, Wong SW, Tan SY, Mahmood AA. Comparison of oxidative damage in Malaysian end-stage renal disease patients with or without non-insulin-dependent diabetes mellitus. Clin Chim Acta 2005; 351: 197-201.
17. Selvam R, Anuradha CV.Lipid peroxidation and antiperoxidative enzyme changes in erythrocytes in diabetes mellitus. Indian J Biochem Biophys 1998; 25: 268-272.
18. VanderJagt DJ, Harrison JM, Ratliff DM, Hunsaker LA, Vander Jagt DL. Oxidative stress indices in

IDDM subjects with and without long-term diabetic complications. Clin Biochem 2001; 34: 265-70.
19. Matkovics B, Kotorman M, Varga IS, Hai DQ, Salgo L, Novak Z. Pro-, antioxidant and rheologic studies in the blood of type 2 diabetic patients. Acta Physiol Hung 1997; 85: 107-12.
20. Kesavulu MM, Rao BK, Giri R, Vijaya J, Subramanyam G, Apparao C. Lipid peroxidation and antioxidant enzyme status in Type 2 diabetics with coronary heart disease. Diabetes Res Clin Pract 2001; 53: 33-9.
21. Peuchant E, Delmas-Beauvieux MC, Couchouron A, Dubourg L, Thomas MJ, Perromat A et al. Short-term insulin therapy and normoglycemia. Effects on erythrocyte lipid peroxidation in NIDDM patients. Diabetes Care 1997; 20: 202-7.
22. Song F, Jia W, Yao Y, Hu Y, Lei L, Lin J et al. Oxidative stress, antioxidant status and DNA damage in patietnts with impaired glucose regulation and newly diagnosed Type 2 diabetes. Clin Sci 2007; 112: 599-06.
23. László Góth.Catalase Deficiency and Type 2 Diabetes. Diabetes Care 2008; 31: e93.
24. Whiting PH, Kalansooriya A, Holbrook I, Haddad F, Jennings PE. The relationship between chronic glycaemic control and oxidative stress in type 2 diabetes mellitus. Br J Biomed Sci 2008; 65: 71-4.
25. Hanachi P, Moghadam RH, Latiff ah AL. Investigation of Lipid Profiles and Lipid Peroxidation in patients with Type 2 Diabetes. Eur J Sci Res 2009; 28: 6-13.
26. Reshamwala SM, Patil ND. Biochemical changes in erythrocytemembrane in uncontrolled type 2 diabetes mellitus. Indian J Biochem Biophys 2005; 42: 250-3.
27. Domínguez C, Ruiz E, Gussinye M. Oxidative Stress at Onset and in Early Stages of Type 1 Diabetes in Children and Adolescents. Diabetes Care 1998; 21: 1736-42.
28. Kedziora-Karnatowska KZ, Lucia M, Blaszcyk Y, Pawlak W. Effect of aminoguanidin on erythrocyte lipid peoxidation and activities of antioxidant enzymes in experimental diabetes. Clin Chem Lab Med 1996; 36: 771-5.
29. Ha H, Lee H. Reactive oxygen species amplify glucose signaling in renal cells cultured under high glucose and in diabetic kidney. Nephrology 2005; 10: 7-10.