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Alterations of Erythrocyte and Plasma Lipid Peroxides as well as Antioxidant Mechanism in Patients with Type II Diabetes Mellitus (NIDDM)

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Abstract: It has been maintained that free oxygen radicals (FOR) that occur in cell have important roles in the ethiopathogenesis of diabet, and that cell damage progressing as a result of their accumulation is responsible for the development of diabetic complications. In recent years, along with free oxygen radicals, antioxidant mechanisms and the substances influencing as free radical cleaner have been investigated in connection with diabet. With this respect, in a total of 30 patients with type II Diabetes Mellitus (NIDDM), 15 with diabetic retinopathy and 15 without diabetic retinopathy, and in 20 healthy subjects, we measured glutathione (GSH), catalase and antiperoxidant superoxide dismutase (SOD) levels as well as plasma and eritrosit levels of Malondialdehid (MDA), which is the last product of oxydation of polyunsaturated fatty acids.

Erythrocyte and plasma MDA levels of patients with diabetic retinopathy were higher relative to those withouut diabetic retinopathy ($P<0.001$, $P<0.005$). In both diabetic groups, erythrocyte and plasma levels were observed to have increased considerably when compared with those in the control group ($P<0.001$). It was determined that, while erthrocyte SOD, catalase and glutathione levels in he groups with diabetic retinopathy decreased significantly relative to those in the control group, a slight decrease occurred in the SOD levels of the group without diabetic retinopathy with respect to controls, and that this, however, was statistically insignificant ($P<0.05$). Still, the decrease in the catalase and glutathione levels was found to be significant ($P<0.02$).

These results indicate that accompaniment of inhibited antioxidant defence systems with increased lipid peroxide levels leading to damage in endotelial cell membran is important not only in the progress of diabete but also in the development of diabetic complications.

Key Words: Lipid peroxidation, Superoxide dismutase, catalase, glutathione.

Introduction

It is maintained that erythrocyte lipid peroxide levels are increased in diabetes and that high plasma lipid peroxides (LPO) in diabetes may result from oxidative destruction of erythrocyte membrane lipids (1). Erythrocytes are unique biological entities that contain molecular oxygen, ferrous ions and poliunsaturated fatty acids at high concentrations.

In erythrocytes, auto-oxidation of oxyhemoglobine into methemoglobine, interaction of hemoglobine with redox drogs and ksenobiotics, Fenton and Haber-Weiss reactions catalyzed with metal (Fe) are factors that may lead to free radical production (2). Bound-dissociation energies of alillic hydrogenes found in the structure of phospholipids in erythrocyte membranes are quite low. Therefore, polyunsaturated fatty acids are more sensitive to oxidative damage, but erythrocytes are resistant to oxidative damage. Because, in their structure, there are enzymes and molecules such as catalase, SOD, glutathione peroxidase (GSH-Px) and glutathione, which are found in antioxidation mechanism (2,3). Of these, catalase catalyses the destruction of H_2O_2 and other hydrogen donors. This enzyme found at the highest concentration in human liver and kidney is at lower levels in serum. Other antioxidant enzyme SOD is an enzyme that eliminates superoxide radicals effectively, being identical with erythrocuprein (4). GSH redox cycle, one of antioxidant systems, constitutes the main system to reduce hyperoxides that are formed in the cell. Key enzyme of cycle is glutathione peroxidase, substrat of which is reduced glutathione (GSH) (5).

LPO products are damaging endothelial and intima cells, increasing thrombocyte aggregation and inhibiting prostacyclyn synthesis. By increasing the comsuption of antioxidant substances such as SOD,

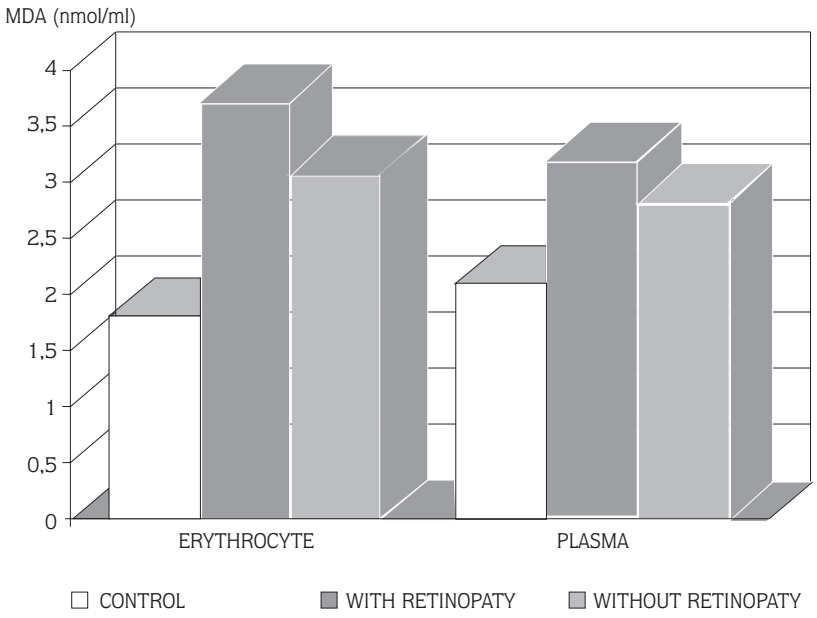

Figure 1. Showing of the alterations in MDA levels in diabetics with retinopathy and without retinopaty relative to those of the control group.

catalase, glutathione peroxidase, glutathione and vitamin E, superoxide radicals (SOR) and lipid peroxidation products make the defense systems within the cell insufficient and can also inhibit the activity of SOD (6).

Starting out from these preliminary data, are investigated th levels of plasma and erythrocyte MDA, alterations in the levels of erythrocyte SOD, catalase and glutathione, and thereby the effects of these alterations upon the complications developing in diabetes.

Materials and Methods

In thus study, 30 patients hospitalized in Diyarbakyr SSK Hospital with diagnosis of type II Diabetes mellitus, 15 patients of whom developed retinopathy and the other 15 patients did not developed retinopathy, and 20 healty subjects (10 male, 10 female) whose ages were varying between 43-53, and who did not use any drugs, alcohol and cigarettes, without any history of disease were included in the study. Of diabetics that were composed of 18 female and 12 male, retinopathy group had age varying between 44-58 and diabetic duration was 5-15 years, while the group without retinopathy had age varying between 42-55, and diabetic duration was 3-12 years. Varitations in diabetes duration and ages are presented in Table 1. Most of the patients were administered oral antidiabetic or insulin undermedical treatment. Heparinized blood samples were taken from each patient fasting overnight (before insulin administration in those using insulin).

The erythrocytes package was prepared by washing the erythrocytes fractioned blood plasma taken by heparin with 0.15 mol/L NaCI solution at a rate of 1:5 three times, and by centrifuge them at 3000 rpm for ten minutes each time. The measurements of SOD, catalase, GSH and erythrocyte MDA were conducted in erythrocyte; those of plasma MDA level were carried out in plasma.

Erythrocyte LPO was measured by Stocks and Dormandy's thiobarbituric acid (TBA) method (7); plasma LPO by TBA method modified from Takeuchi (8). Erythrocyte GSH was measured by 2-nitrobenzoic acid (DTNB) method from Beutler (9); Erythrocyte SOD was measured by modified Winterbourn and Hawkins' method based upon reduction of nitroblue tetrasolium (NBT) (10).

Catalase levels were determined by Aebi's modified colorimetric method (11). It is based upon alteration of H_2O_2 optic density, depending upon enzymatic decomposition of H_2O_2 (by the effect of catalase in the sample). Data were changed to k/g Hb after "k" value was determined, taking suitable absorbans for each analysis according to calculated regression. Drabkin's method was used to determine erythrocyte hemoglobin.

In the statistical evaluation of the results, the difference between averages of the two experiments series was determined by "student's t" test.

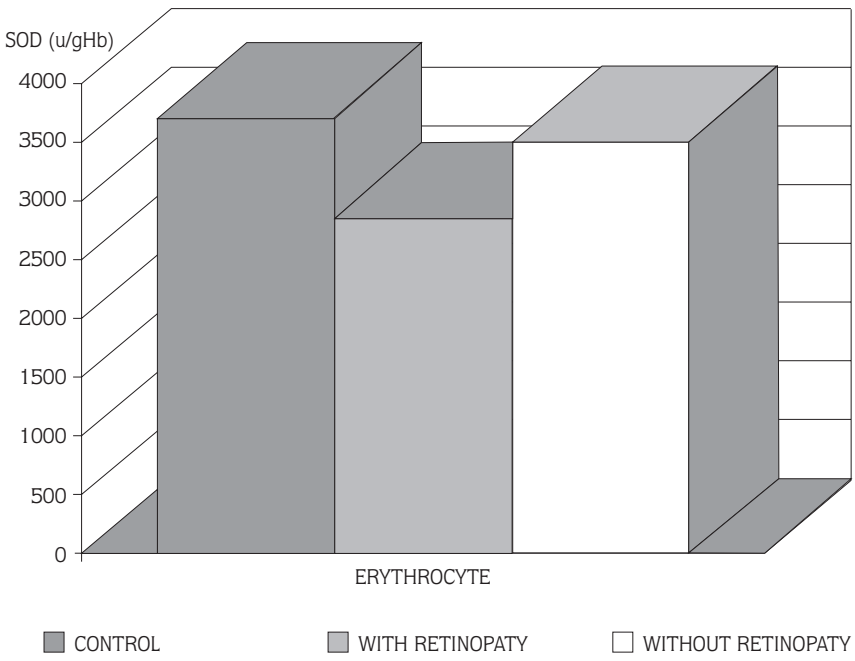

Figure 2. Showing of the alterations in erythrocyte SOD activities in diabetics with retinopaty and without retinopaty relative to those of the control group

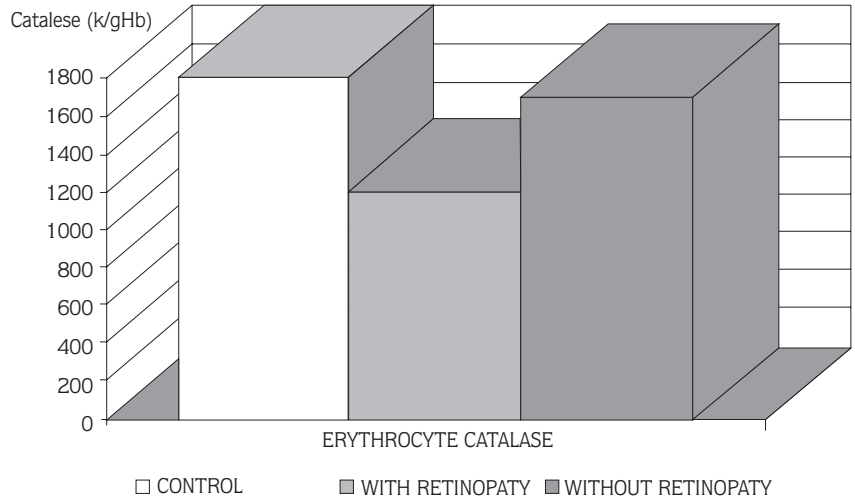

Figure 3. Showing of the alterations in erythrocyte catalase activities in dtiabetics with retinopaty and without retinopaty relative to those of the conrol group

Results

The results obtined from control and both diabetic groups are shown statistically in Table 1-4 According to these;

(a) The difference between the age averages of control and diabetics, and those of diabetics with and without retinopathy as well as the difference between diabetic duration were not found statistically significant ($P>0.05$) (Table 1).

(b) SOD, catalase, glutathione levels of diabetics are significantly lower ($P<0.001$), while glucose, erythrocytes and plasme MDA levels were significantly higher, when compared with those of control group ($P<0.001$) (Table 2).

(c) Glucose, erythrocyte and plasma MDA levels of the group with retinopathy were significantly higher ($P<0.001$, $P<0.005$), whereas SOD, catalase and glutathione levels were significantly lower relative to those of the group without retinopathy ($P<0.001$) (Table 3).

(d) Erythrocyte MDA and plasma MDA levels of the group without retinopathy were significantly high-

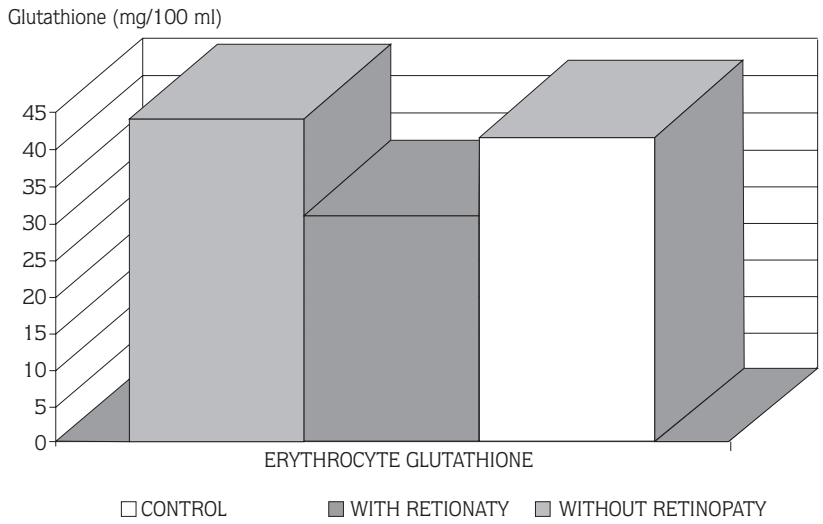

Figure 4. Showing of the alterations in erythrocyte glutathione activities in diabetics with retinopaty and without retinopaty relative to those of the control group

er (P<0.001) (Figure 1), catalase and glutathione levels were lower (P<0.02) when compared to control group (Figure 3, 4). Although SOD levels were lower relative to those of control group, the difference was not found statistically significant P>0.05 (Table 4) (Figure 2).

(e) Glucose, erythrocyte and MDA levels of the group with retinopathy were significantly higher (P<0.001) (Figure 1); SOD, catalase and GSH levels were significantly lower, when compared to control group (P<0.001) (Table 4) (Figure 2, 3, 4).

Discussion

Alterations in increased lipid peroxidation free radicals and antioxidant defense systems have been investigated as related with diabetes in recent years. It was demonstrated that free fatty acids increasing in the lack of insulin contributed to increased plasma LPO levels. Insulin reduces hepatocyte LPO production. In addition, since insulin activates glutathione peroxidase that breaks LPO, hepatocytes can not remove LPO in the lack of insulin (12).

By most researchers, it was demonstrated that lipid peroxides (MDA) were higher in diabetics with retinopathy relative to diabetics without retinopathy (1,13-16). We also identified higher plasma and erythrocyte LPO (MDA) levels in the group with retinopathy, compared with those in the group without retinopathy. There are a few biochemical mechanism that explain the reason for this rise. The increasing of blood free fatty acid levels depending on lypolys increase results in increase in MDA production. In the excessive production of free radicals, it leads to microvascular lesions. Thees lesions are associated with disfunction of biologic antioxidant systems (17, 18, 19). It has been seen that this damage is more severe in ketotic period. This was attirbuted to the decreasing of cytoplasmic NADPH due to blockage of pentose phosphate shant and disfunction of glutathione of syntetase (18).

Increased LPO levels of diabetic individuals may take origin from peroxidative damage of membrane lipids. Jenning et al. (13) have showen that increased free radical activity leads to an increased trombotic tendency and a reduction in prostocyclyn stimulating factor, depending on increasing trombocyte reactivity in diabetics (especially with retinopathy), and they have also demonstrated that intracellular SOD activity is reduced in patients with retinopathy (13). Most researchers actually have shown that prostanoids and lipid peroxides accepted as on index of intravascular free radicals are effective in the beginning and development of microangiopathy and that valuable results can be achieved in the prevention of diabetic microangiopathy progress through lipid control along with glisemic control (13,15,16,20,21).

In a number of studies, it was established that abnormalities in blood parameters of patient with diabetic complication and non-regulated well (Retionpathy, neuropathy, nephropaty, peripheral vascular disorders, coronary or cerebral diseases and the like) were much more when compared with those patients well regulated and without complication. (20, 22) Since lipid peroxides play a major role in he formation of vascular tissue damage, it is suggested that LPO,

increasing in diabetes can be effective in the pathogenesis of diabetic angiopathy. (20,22) It has been indicated that free fatty acids increasing in the lack of insulin contribute to high plasma LPO levels. (22)

One of the intracellular protective mechanisms against free radicals that form peroxidation in membrane lipids is glutathione and redox system. Reduced GSH is a non-specific reduction agent and plays on important role in oxidation mechanisms. In cell metabolism, it performs important funcitons in the prevention of sulphyhydrile groups of various proteins and lipoproteins in cell membrane. GSH participates in antioxidative defense system as free radical in activator (19). It is accepted that, as thrombocyte GSH level is decreased in diabetes, increased tromboxan A_2 (TXA_2) synthesis prepares ground for vascular complications by increasing thrombocyte activity. In the existence of GSH, which is a cofactor of GSH-Px (Glutathione peroxidase) enzyme it inhibites siklooxygenesis by removing LPO from the medium. However, LPO accumulated in the cell stimulates TXA_2 synthesis, in the lack of GSH, or when GSH-Px activity is decreased (23). Some researchers recognized that SOD and catalase levels in well-regülated diabetics did not exhibit difference with respect to control group (21,24).

It has been suggested that excessive free radical production causes microvasculer lesions, and that they are related with disfunction of biological antioxidant systems of these lesions (18). In the lack of SOD, hydroxil radical singlet oxygen produced through fenton reaction and dismutation of superoxide by its own accord initiate lipid peroxidation in especially unsaturated fatty acids. These two radicals are the most toxic and effective radicals that lead to lipid peroxidation. Therefore, it is accepted that these two radicals are essentially responsible for changes in the membranes of tumour cells (25,26).

Hayakawa et al (20), have reported that increased lipid peroxide causes damage in endothelial cell membranes, that inhibition of antioxidants marks this damage and that this status is important in the pathogenesis of diabetic complications (20).

As a results, we are of the opinion that;

1. Increase in lipid peroxides accepted as an index for intravascular free radicals is effective in the initiation and evolution of microangiopathy in diabetics,

2. An inefficiency occurs in antioxidant defense systems, depending upon lipid peroxidation increase or inhibition of antioxidant defense systems.

Most investigators have observed that erythrocyte glutathione levels are decreased in diabetic individuals (1,5,18,23). We have also observed that GSH activity has decreased in both diabetic groups. However the decrease in GSH levels was clearer in the group with retinopathy.

It has demonstrated by most researchers that SOD and catalase activities were reduced in non-regülated diabetics, but unchanged in well-regulated diabetics (17,18,20,21).

In our study, while SOD and catalase levels were significantly decreased in he group with retinopathy, the decrease determined in SOD levels of the group without retinopathy was found to be insignificant. However, the decrease identified in catalase levels of the group without retinopathy was found significant.

References

1. Uzel N, Sivas A, Uysal M, Öz H. Erythocyte lipid peroxidation and glutathione peroxidase activities in patients with diabetes mellitus. Horm Metabol Res 9:89-90, 1987.
2. Freeman BA, Crapo JD. Biology of disease. Free radicals and tissue injury. Lab Invest 47: 412-426, 1982.
3. Gutteridge JMC, Halliwell B. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem Sci 15: 129-135, 1990.
4. Sözmen EY, Onat T, Tanyalçın T, Erlaçin S. Age correlated modifications of antioxidant enzyme activities in human erytrocytes. Journal of Biochemistry XVIII, 3, 83-89, 1993.
5. Costagliola C. Oxidative state of glutathione in red blood cells and plasma of diabetic patients Clin Physiol Biochem 8: 204, 1990.
6. Loeper I, Goy J, Rozenstain L, Bedu O, Moisson. Lipid peroxidation and protctive enzymes during myocardial infarction. Clin Chim Acta 196: 119, 1991.
7. Stocks J and Dormandy TL. The autoxidation of human red cell lipids induced by hydrogen peroxide. Brit J Hematol 20: 95-111, 1971.
8. Takeuchi N, Tanaka F, Katayama Y, Matsumiya K and Yamamura Y. Effects of a-tocop-herol on thiobarbituric acid reactive substanes in serum and hepatic subcelluler organelles and lipid metabolism. Exp Geront 11: 179-185, 1976.
9. Beutler E, Duran O and Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 61 (5): 882-888, 1963.

10. Winterbourn CC, Hawkins RE, Brian M. Carrell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 85 (2): 337, 1975.

11. Aebi H. Catalase in vitro. Methods in enzymology 105:121, 1984.

12. Kosugi K, Harano Y, Kashiwagi A, Suzuki M. Effect of insülin synthesis and release of lipid peroxide by cultured hepatocytes isolated from normal and diabetic rats. Experentia 40: 394-6, 1984.

13. Jennings PE, McLaren M, Scott NA, Saniabadi AR, Belch JJ. The relationship of oxidative stress to thrombotic tnedency in type 1 diabetic patients with rtinopathy. Diabet Med 8(9): 860-5, 1991.

14. Gallau G, Ruelland A, Legras B, Maugendre D, Allanninc H, Cloarec L. Plasma malondial-dehyde in type 1 and type 2 diabetic patients. Clin Chim Acta 214 (2): 227-34, 1993.

15. Augustin AJ, Breipohy W, Boker T, Llutz J, Spitznas M. Increased lipid peroxide levels and myeloperoxidase activity in the vitreous of patients suffering from proliferative diabetic retinopathy. Graefes Arch Clin Exp Ophthalmol 231 (11): 647-50, 1993.

16. Katoh K. Possible relevance of lipid peroxidation and thromboxane production to the initation and/or evolution of microangiopathy in non-hyperlipidemic type 2 diabetes mellitus Diabetes. Res Clin Pract 18 (2): 89-98, 1992.

17. Cousins RJ: Absorbtion, transport and hepatic metabloism of copper and zinc: Special reference to MTN and CP, Am Physiol Soc, 238-309, 1985.

18. Faure P, Corticelli P, Richard MJ, Arnaud J, Coudray C, Halimi S, Favier A and Roussel AM. Lipid peroxidation and trace element status indiabetic ketotic patients: Influence of Insulin therapy. Clin Chem 39/5, 789-793, 1993.

19. Kynlaw WB: Abnormal zinc metabolism in diabetes mellitus. Am J Med, 75: 273-277, 1983.

20. Hayakawa M, Kuzuya F. Free radicals and diabetes mellitus. Nippon Ronen Igakkai Zasshi 27 (2): 149-54, 1990.

21. Balashova TS, Golega EN, Rud'ko IA, Balabolkin MI, Kubatieve AA. Lipid peroxidation and the antioxidant protectino of the erythrocytes in diabetes mellitus patients. Tre Arkh 65 (10): 23-7, 1993.

22. Kosugi K, Harano Y, Kashiwagi A, Suzuki M: Effect of insulin synthesis and release of lipid peroxide by cultured hepatocyets isolated from normal and diabetic rats. Experentia, 40: 394-6, 1984.

23. Dingiloğlu N, Özmen D, Bayındır O, Kutay F, Yılmaz C. Erythrocyte and plosma lipid peroxides, erythrocyte GSH and glucose-6-P dehydrogenase levels in diabetics. Journal of Biochemistry XVIII: (3), 13-18, 1993.

24. Volkovova K, Chorvathova V, Jurcovicova M, Koszeghyova L, Bobek P. Antioxidative state of the myocardium and kidneys in acute diabetic rats. Physio Res 42 (4): 251-5, 1993.

25. Kılınç K. Oxygen radicals and superoxide dismutase in cancer. Biochemistry XI (3), 59-76, 1986.

26. Collier A, Wilson R, Bradley H, Thomson JA, Small M. Free radical activity in type 2 diabetes. Diabet Med 7(1): 27-30, 1990.