Glutathione and Glutathione Peroxidase in Type 1 Diabetic Patients

Atip Likidlilid MSc*, Natchai Patchanans MSc*,
Saiphon Poldee MSc*, Thavatchai Peerapatdit MD **

** Department of Biochemistry, Faculty of Medicine, Siriraj Hospital, Mahidol University*
*** Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University*

Objective: *Diabetic (DM) patients are claimed to be under oxidative stress because of hyperglycemia. The influence of free radical production by this hyperglycemic induction may involve cardiovascular complications in diabetes. The present study aimed to compare the glutathione (GSH) level and glutathione peroxidase (GPx) activity in type 1 DM and a normal healthy group.*

Material and Method: *GSH level and GPx activity were determined in red cells of 20 subjects of type 1 DM containing fasting plasma glucose (FPG) $\geq$ 140 mg/dL. Twenty healthy normal subjects with normal plasma glucose level (FPG $\leq$ 110 mg/dL) and matched for gender and age served as the control group. These oxidative stress parameters of type 1 DM were compared to a control group by unpaired student's t-test. The association of these parameters with FPG was performed by Pearson product moment correlation.*

Results: *The level of red cell GSH was significantly lower in type 1 DM (p = 0.011) but red cell GPx activity was significantly increased (p = 0.003) when compared to age-matched normal control. The decrement of red cell GSH may be due to the higher rate of consumption of GSH, increasing GPx activity or a reduction of pentose phosphate pathway, stimulated by insulin, resulting in lowered GSH recycle. The correlation between FPG and GSH in type 1 diabetic patients compared with healthy normal subjects was also observed and it was found that there was a negative correlation, but not found between FPG and GPx activity.*

Conclusion: *The present finding suggested that type 1 DM patients were susceptible to oxidative stress and higher blood glucose level had an association with free-radical-mediated lipid peroxidation. Therefore, any means that can reduce oxidative stress may be beneficial for slow progression of cardiovascular complication in type 1 diabetic patients.*

Keywords: *Type 1 diabetes, Oxidative stress, Glutathione, Glutathione peroxidase, Cardiovascular complication*

J Med Assoc Thai 2007; 90 (9): 1759-67
Full text. e-Journal: *http://www.medassocthai.org/journal*

The development of type 1 diabetes required genetic susceptibility but not genetic in the usual Mendelian sense[1]. Rather, several genetic alterations, most but probably not all of which are located on chromosome six within the major histocompatibility complex, result in an increased likelihood of β-cell damage[2-7]. The mechanism of β-cell damage and destruction is thought to be the result of an organ-specific autoimmune process in which the immune system reacts abnormally against the body's own insulin secreting β-cells in the pancreatic islets. Substantial evidence suggests that antigens released from the β-cell are seen as foreign proteins by the macrophage or antigen presenting cell (APC) that presents the altered antigen to a highly specialized HLA-linked receptor in a helper T cell. This process then initiates an active cellular and humoral response involving antibody production and lymphokine release[8,9]. The final biochemical mediator of this toxic process is probably nitric oxide (NO)[10]. NO will react with O_2 to produce peroxynitrite ($ONOO^-$) which can initiate the destruction of β-cells through the lipid peroxidation process[11].

The epidemiologic studies of type 1 diabetes have shown that a high incidence was found in the Scandinavian countries, intermediate levels in much of

Correspondence to : Peerapatdit T, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. E-mail: sitpp@mahidol.ac.th

the West, and very low levels in the Far Eastern countries, including Japan, China, Korea, and Thailand[12-15]. This distribution has an extraordinary similarity to that seen in the worldwide distribution of atherosclerosis and of morbidity and mortality from coronary heart disease (CHD)[16]. The atherosclerotic risk and type1 DM incidence may correlate with the oxidative stress[17].

Several studies have suggested that chronic hyperglycemia and oxidative stress may associate with diabetes and its complications[18]. The oxidative stress results from increased free radical formation and/or decreased antioxidants in the body. The free radicals or sometimes specifically called reactive oxygen species (ROS) can be influenced by exogenous agents, radiation, trauma, drug activation, oxygen excess, or by endogenous mechanisms such as oxidative metabolism, transition metals catalyzed reactions, microbial killing by phagocytic cell, and inflammation. Moreover, there are more mechanisms that induce oxidative stress in diabetic patients than in normal individuals; glucose autoxidation, non-enzymatic glycation of protein, and polyol pathway[19]. These pathways enhance the generation of ROS that lead to tissue damage and cause several complex syndromes in diabetic patients such as cataracts, renal dysfunction, nerve damage, and atherosclerosis. Especially, the atherosclerosis leads to the CHD, which is the major cause of death among diabetics[20,21].

However, the action of ROS can be prevented by the antioxidant defense system. In the human body, there are antioxidant enzymes such as superoxide dismutase (SOD), the enzyme which dismutates superoxide ($O_2^{\cdot-}$) to hydrogen peroxide (H_2O_2), and the other two H_2O_2-scavenging enzymes - glutathione peroxidase (GPx) and catalase (CAT) - that convert H_2O_2 to water. In addition, there are endogenous non-enzymatic antioxidants like glutathione (GSH) and exogenous antioxidant vitamins from the diet such as fruits and vegetables. These non-enzymatic antioxidants act as terminators of free radicals' chain reactions caused by lipid peroxidation[11].

The GSH has the advantage of antioxidant property in that it is a cofactor of GPx enzyme and is involved in the detoxification of ROS, whereas, exogenous antioxidant vitamins acts only as detoxification of ROS. GSH precursor has been successfully used in diabetes to attenuate renal damage and peripheral neuropathy in experimental models[22,23] but its effects on cardiac damage remain unknown. Therefore, the aim of the present study was to investigate the level of glutathione and GPx activity in type 1 DM compared to the normal subjects to be the knowledge base for understanding the progression of pathogenic mechanisms of cardiovascular complication in type 1 diabetes.

Material and Method

Subjects

Twenty normal healthy subjects were the control group with fasting plasma glucose (FPG) $\leq$ 110 mg/dL. The ages ranged from 18 to 48 years. Twenty Type 1 diabetic subjects whose FPG $\geq$ 140 mg/dL. The ages ranged from 14 to 55 years. Venous blood samples for the successive biochemical determinations were withdrawn at fasting in the morning. Blood biochemistry determinations were performed using the enzymatic reagent kit as the common methods of the authors' laboratory. All the subjects gave their consent to the present study approved by the ethical clearance committee on human rights, Faculty of Medicine Siriraj Hospital, Mahidol University. Normal eligible subjects were recruited from subjects who attended for a routine medical check at the Department of Preventive and Social Medicine, Faculty of Medicine Siriraj Hospital. They were defined as healthy by physical examination (weight, height, and blood pressure measurements, chest X ray, respiratory, and eye examination) and laboratory tests [complete blood count, blood urea nitrogen, creatinine, uric acid, fasting blood glucose, liver function tests, total cholesterol, triglyceride, and high density lipoprotein cholesterol (HDL-C)]. Subjects with hypertension, diabetes mellitus, cardiovascular diseases, renal or hepatic diseases, inflammation, injury, or trauma in the previous month were excluded from the present study. Type 1 diabetic subjects were selected from the medical outpatient department and diabetic clinics in Siriraj Hospital. All patients with any renal dysfunction, (i.e. raised blood urea and serum creatinine levels), with coexistent illness (i.e. infections), liver diseases, respiratory tract diseases, congestive heart failure, acute myocardial infarction, proliferative retinopathy, with diabetic macroangiopathic complications (i.e. coronary artery disease, peripheral vascular disease and stroke: diagnosed by clinical history and examination) were excluded from the present study. All patients had been on insulin treatment for more than 6 months. The control group and patients who had been supplemented with antioxidants were also excluded.

Erythrocyte reduced glutathione assay

The reduced glutathione (GSH) in red cells was performed as described by Beutler[24].

Table 1. Database of age, gender, FPG, hematological data and lipid profiles in normal healthy subjects and type 1 diabetic patients (Mean ± SEM)

	Normal subjects (n = 20)	Type 1 diabetes (n = 20)	p-value
Age	30.45 ± 2.60	34.25 ± 3.40	0.381
Gender (M/F)	13/7	9/11	
FPG (mg/dL)	96.71 ± 3.52	210.58 ± 21.35	<0.001
Hb (g/dL)	16.07 ± 0.45	13.80 ± 0.60	0.005
Hct (%)	45.79 ± 0.86	45.69 ± 1.30	0.952
Total cholesterol (mg/dL)	189.81 ± 9.61	214.48 ± 13.28	0.140
LDL cholesterol (mg/dL)	96.80 ± 8.51	131.81 ± 11.65	0.020
VLDL cholesterol (mg/dL)	21.15 ± 2.74	29.37 ± 4.63	0.136
Triglyceride (mg/dL)	105.73 ± 13.65	146.89 ± 23.14	0.140
HDL cholesterol (mg/dL)	71.86 ± 4.09	53.30 ± 3.57	0.002

In brief, 3% TCA was added to the 0.1 ml of packed red cells and mixed, 0.25 ml followed by 0.9 ml of cold distilled water. The suspension was centrifuged at 1870 x g for 5 min and supernatant was assayed glutathione by adding 1 ml of 0.3 M phosphate buffer, pH 7.0. Then, 0.125 ml of 40 mg% DTNB solution was added and the mixture was left to stand at 4ºC for 5 min. The optical density was read at 412 nm against an unknown blank. The GSH level was obtained from a standard curve that was prepared by using 1.25, 25, 50, 100, and 200 mg/dL of standard reduced GSH from Sigma in distilled water.

Erythrocyte glutathione peroxidase assay

The GPx activity in red cells was performed according to the instruction of glutathione peroxidase reagent kit (Ransel), No. RS504 from Randox Laborarories, UK.

Statistical analysis

Descriptive statistics were expressed as mean ± SEM (standard error of the mean). The SPSS for windows program was used for statistical analysis. Since the data obtained were normally distributed, the independent student's t-test (unpaired t-test) was performed to compare type 1 diabetic patients with their healthy normal groups. The Pearson product moment correlation coefficient was used to assess the relationship among these oxidative stress values and FPG of type 1 diabetic patients and healthy normal subjects. Differences were considered significant when $p < 0.05$.

Results

Database characteristics of type 1 DM

Table 1 shows the database of age, gender, FPG, and lipid profile between the normal healthy group and type 1 DM patients. This table shows that FPG in type 1 DM patients was significantly higher than that in the normal group. The study of plasma lipid profile showed total cholesterol, VLDL-cholesterol and triglycerides in type 1 DM patients were not significantly different from the normal healthy group. This may be due to the treatment of cholesterol-lowering drug to maintain the blood lipid level in diabetic subjects. However, the significant increase of LDL-cholesterol and significant decrease of HDL-cholesterol were also found in type 1 DM subjects. The increasing of LDL-cholesterol and decreasing of HDL-cholesterol may the major risk factors to exacerbate the cardiovascular complication in this group of diabetes. In addition, hemoglobin (Hb) concentration was also significantly lower in type 1 DM compared to normal control subjects, whereas, hematocrit (Hct) was not different.

Table 2. Red blood cell GSH and GPx activity in normal subjects and type 1 DM (mean ± SEM)

Subjects	GSH (mg/gHb)	GPx (u/gHb)
Normal (n = 20)	1.27 ± 0.07	25.18 ± 1.55
Type 1 DM (n = 20)	1.03 ± 0.06	35.83 ± 2.97
p-value	0.011	0.003

Red blood cell GSH level and GPx activity

The mean level of red cell GSH that functions as an antioxidant and a cofactor of enzyme GPx and GPx activity are summarized in Table 2. The level of GSH was significantly lower in type 1 diabetes comparing with normal subjects ($p = 0.011$) (Fig. 1). Conversely,

the red cell GPx activity was significantly higher than the normal group (p = 0.003) (Fig. 2).

The demographic data for Pearson correlation analysis among FPG, GSH and GPx activity in type 1 diabetic patients and normal subjects were also performed. There were negative correlations between FPG and GSH in type 1 diabetic subjects and in total subjects (r = - 0.589, p = 0.006; r = -0.460, p = 0.001, respectively) whereas normal subjects showed no correlation (r = 0.344, p = 0.138) and have a trend to increase the GSH level (Fig. 3). No correlation was observed with FPG and GPx in normal subjects, type 1

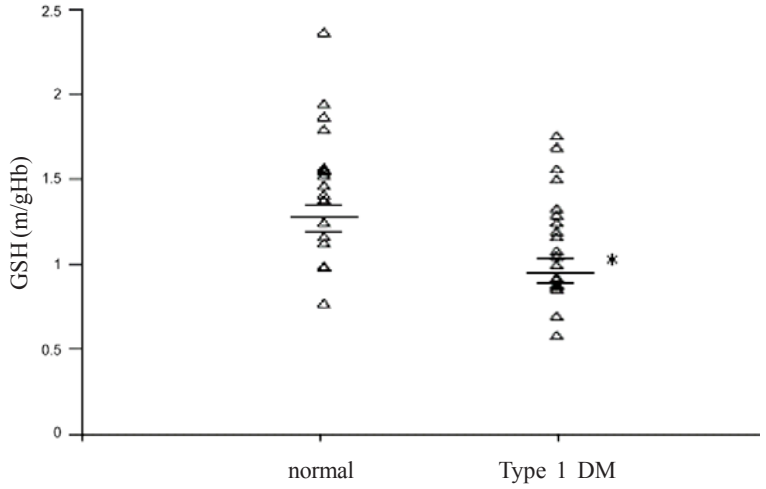

Fig. 1 Erythrocyte reduced glutathione in normal healthy subjects (n = 20) and type 1 diabetes (n = 20)
Results are given in mg/gHb and expressed as the mean ± SD
The horizontal bar represents the mean value and * shows the level of significance

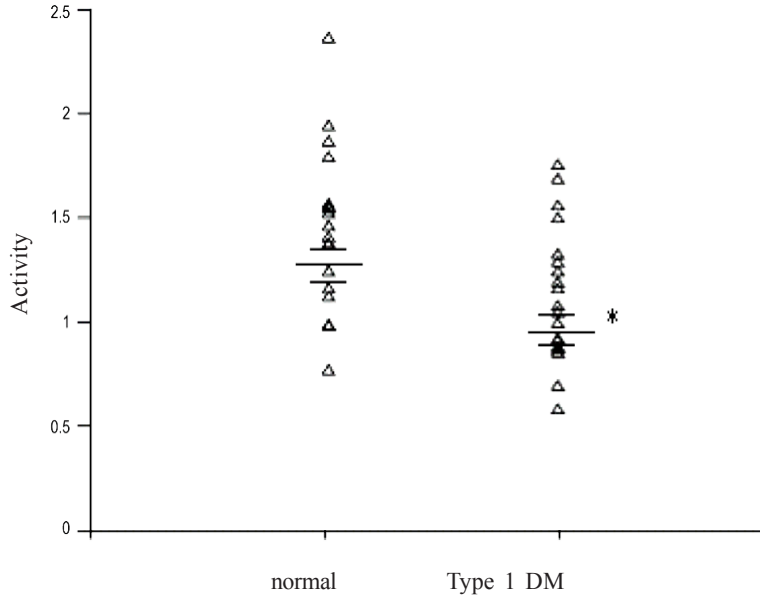

Fig. 2 Erythrocyte glutathione peroxidase activity in normal healthy subjects (n = 20) and type 1 diabetes (n = 20)
Results are given in U/gHb and expressed as the mean ± SD
The horizontal bar represents the mean value and * shows the level of significance

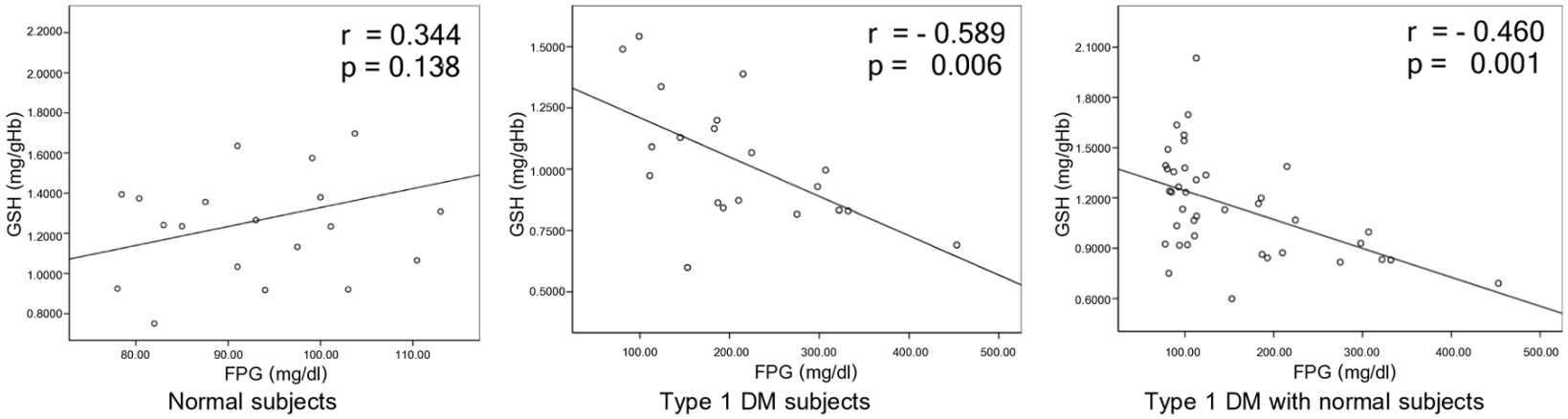

Fig. 3 Correlation analysis between fasting plasma glucose and reduced glutathione in normal subjects, type 1 diabetic patients and type 1 diabetic patients with normal subjects

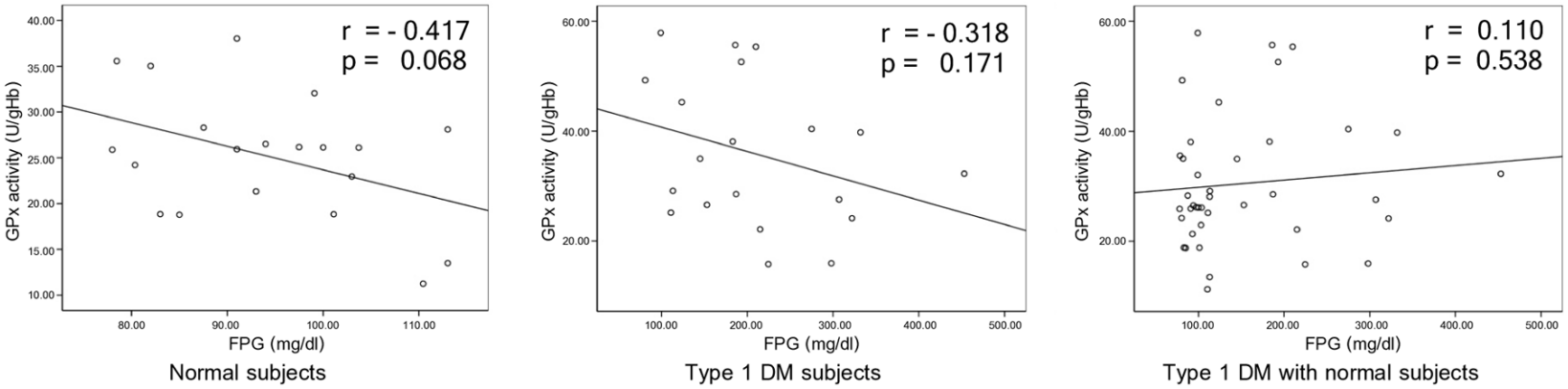

Fig. 4 Correlation analysis between fasting plasma glucose and glutathione peroxidase activity in normal subjects, type 1 diabetic patients and type 1 diabetic patients with normal subjects

diabetic patients and total subjects (r = -0.417, p = 0.068; r = -0.318, p = 0.171; r=0.110, p = 0.538, respectively) (Fig. 4).

Discussion

Chronic hyperglycemia and oxidative stress in diabetic patients may be associated with long-term damage, dysfunction, and failure of various organs. These chronic complications include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputation, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms. In addition to these microvascular diseases (microangiopathies), the macrovascular disease (macroangiopathy) such as atherosclerosis was also found. The atherosclerotic vascular disease is the cause of mortality and significant morbidity in diabetes. This chronic hyperglycemia can initiate the $O_2^{\cdot-}$ production by glucose autoxidation, non-enzymatic glycation of proteins, and polyol pathway as described earlier. The $O_2^{\cdot-}$ can be converted to H_2O_2 and $OH^{\bullet}$, respectively. The hyperactivity of these ROS is directed against lipids and proteins as well as nucleic acid[25] and resulting in structural modification and fragmentation that cause cell damage in diabetes[24].

Antioxidant defense system, however, in the blood can protect tissues and organs against the ROS. The antioxidant systems are composed of enzymatic and non-enzymatic antioxidants. There are three main antioxidant enzymes such as SOD, CAT, and GPx. Study in the erythrocyte GPx activity in type 1 DM showed that GPx activity was markedly increased corresponding to the other reports with type 2 diabetes[27-29]. The increment of GPx activity may be explained by which the GPx is an inducible enzyme and was stimulated by ROS[30]. The increased production of ROS may promote the GPx activity for adaptive process of combating excessive peroxidative damage. In another mechanism, GPx is a selenium-dependent enzyme, diabetic tissue may retain selenium in the cells and thereby increases GPx activity[29]. The result from the patients of β-thalassemia/hemoglobin E as reported by Likidlilid et al[31] demonstrated the higher level of selenium in red cells and lower level in plasma. The thalassemic patients are also known to be under the oxidative damage similar to the diabetic patients. However, Gebre-Medhin et al[32] observed an increased level of plasma selenium in diabetic children. Therefore, a selenium turnover study is required to confirm whether a compensatory mechanism sets in for saving tissue selenium levels in diabetes. Another factor that increases GPx activity is dietary factors of the PUFA (polyunsaturated fatty acid) intake[33] because PUFA within the cells are prone to peroxidative damage by ROS. The increasing of hydroperoxide, the product of lipo- peroxidative damage by ROS, is also known as GPx activation[34].

GSH is a ubiquitous tripeptide that presents in red cells and participates in GPx reaction. When H_2O_2 is detoxified by GPx, the GSH is simultaneously converted to the oxidized form (GSSG). In the present study, the authors found that GSH levels in type 1 DM patients were significantly lower than that in their same age-matched control subjects. These results are in good agreement with other studies[35-37]. As already mentioned, GSH serves as an essential cofactor for the enzyme GPx and formed oxidized glutathione (GSSG) during the enzyme processes. Thus, increasing in GPx activities imply higher consumption of GSH. Other mechanisms that may explain the GSH reduction in diabetes are that the GSH is regenerated by the enzyme glutathione reductase, using reducing equivalents from NADPH. The NADPH is generated in red blood cells through the pentose phosphate pathway, which is stimulated by insulin[38], and in DM NADPH production may be sluggish, probably resulting in lowered glutathione reductase activity and reduced GSH recycle. The enzyme glutathione reductase was found to be decreased in type 2 diabetic patients as reported by Dincer et al[39]. Moreover in diabetes mellitus, the increased sorbitol synthesis via the polyol pathway occurred. This elevated sorbitol production caused the NADPH depletion that was required by aldose reductase enzyme in this pathway. This deficiency will also limit the GSH recycle[39].

To study the association between FPG and parameters of oxidative stress in type 1 DM and normal subjects, The Pearson product moment correlation was performed. From the value of correlation coefficient (r) between FPG and GSH, the authors found that there was a negative fair correlation (r = -0.46, p = 0.001) reflecting some association of the GSH with increased oxidative stress. However, the association between FPG and GPx activity showed no correlation, this implied that the GPx enzyme might be glycated by hyperglycemic state[40] resulting in fragmentation of this enzyme[19,26]. This indicated that the glycated form of GPx may be less active. As already described, Wolff et al[41] reported that glycated protein produced ROS. Mullarkey et al[42] also suggested that increased glycation of proteins may produce ROS and accelerate atherogenesis because of oxidative modifications of

vascular membrane lipids. The production of ROS by the glycated proteins, on the one hand, and the inactivation of GPx activity by glycation, on the other hand, may enhance the accumulation of ROS leading to the serious complications in diabetes[43,44].

Conclusion

In conclusion, the present study supported the hypothesis that hyperglycemia activated cellular and tissue damage by oxidative stress. However, there were compensatory mechanisms for defense against the ROS. Normalization of oxidative stress was not achieved in the diabetic patients. Thus, any means that can reduce the oxidative damage may be beneficial for treatment of diabetic patients in the future.

Acknowledgements

The authors wish to thank all of the patients and normal subjects who participated in this experiment. The authors also wish to thank Ms. Anchaleekorn Somkasettrin for her assistance in statistical analysis and preparing the manuscript. Some parts of this study were presented at The ASEAN Federation of Endocrine Society (AFES), Manila Philippines on 7-10 December 2005.

References

1. Rossini AA, Greiner DL, Friedman HP, Mordes JP. Immunopathogenesis of diabetes mellitus. Diabetes Rev 1993; 1: 43-75.
2. Segurado OG, Arnaiz-Villena A, Wank R, Schendel DJ. The multifactorial nature of MHC-linked susceptibility to insulin-dependent diabetes. Autoimmunity 1993; 15: 85-9.
3. Kumar D, Gemayel NS, Deapen D, Kapadia D, Yamashita PH, Lee M, et al. North-American twins with IDDM. Genetic, etiological, and clinical significance of disease concordance according to age, zygosity, and the interval after diagnosis in first twin. Diabetes 1993; 42: 1351-63.
4. Serjeantson SW, Court J, Mackay IR, Matheson B, Rowley MJ, Tuomi T, et al. HLA-DQ genotypes are associated with autoimmunity to glutamic acid decarboxylase in insulin-dependent diabetes mellitus patients. Hum Immunol 1993; 38: 97-104.
5. Penny MA, Mijovic CH, Cavan DA, Jacobs KH, Jenkins D, Fletcher J, et al. An investigation of the association between HLA-DQ heterodimers and type 1 (insulin-dependent) diabetes mellitus in five racial groups. Hum Immunol 1993; 38: 179-83.
6. Cruickshanks KJ, Jobim LF, Lawler-Heavner J, Neville TG, Gay EC, Chase HP, et al. Ethnic differences in human leukocyte antigen markers of susceptibility to IDDM. Diabetes Care 1994; 17: 132-7.
7. van Endert PM, Liblau RS, Patel SD, Fugger L, Lopez T, Pociot F, et al. Major histocompatibility complex-encoded antigen processing gene polymorphism in IDDM. Diabetes 1994; 43: 110-7.
8. Drell DW, Notkins AL. Multiple immunological abnormalities in patients with type 1 (insulin-dependent) diabetes mellitus. Diabetologia 1987; 30: 132-43.
9. Laron Z, Karp M. Genetic and environmental risk factors for type 1 diabetes (IDDM),including a discussion on the autoimmune basis. London: Freund Publishing House; 1992.
10. Kolb H, Kolb-Bachofen V. Nitric oxide: a pathogenetic factor in autoimmunity. Immunol Today 1992; 13: 157-60.
11. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 3rd ed. Oxford: Oxford University Press, 1999.
12. WHO Multinational Project for Childhood Diabetes. WHO Diamond Project Group. Diabetes Care 1990; 13: 1062-8.
13. Tuomilehto J, Dabee J, Karvonen M, Dowse GK, Gareeboo H, Virtala E, et al. Incidence of IDDM in Mauritian children and adolescents from 1986 to 1990. Diabetes Care 1993; 16: 1588-91.
14. Kocova M, Trucco M, Konstantinova M, Dorman JS. A cold spot of IDDM incidence in Europe. Macedonia. Diabetes Care 1993; 16: 1236-40.
15. Karvonen M, Tuomilehto J, Libman I, LaPorte R. A review of the recent epidemiological data on the worldwide incidence of type 1 (insulin-dependent) diabetes mellitus. World Health Organization DIAMOND Project Group. Diabetologia 1993; 36: 883-92.
16. Reusens B, Dahri S, Snocek A, Bennis-Taleb N, Remacle C, Hoet JJ. Long-term consequences of diabetes and its complication may have a fetal origin: experimental and epidemiological evidence. In: Cowett RM, editor. Diabetes, Nestle Nutrition Workshop Series, Vol. 35. New York: Raven Press; 1995: 187-98.
17. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996; 19: 257-67.
18. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40: 405-12.

19. Takahashi M. Taniguchi N. Glycation and free radicals-fragmentation of Cu, Zn-SOD due to reactive oxygen species. J Act Oxyg Free Rad 1993; 4: 134-41.
20. DeFronzo RA. Current therapy of diabetes mellitus. St. Louis, MO: Mosby; 1998.
21. Mitchell BD. Macrovascular disease in diabetes. In: Leslie RD, Robbins DC, editors. Diabetes: clinical science in practice. Cambridge: Cambridge University Press; 1995: 222-36.
22. Odetti P, Pesce C, Traverso N, Menini S, Maineri EP, Cosso L, et al. Comparative trial of N-acetylcysteine, taurine, and oxerutin on skin and kidney damage in long-term experimental diabetes. Diabetes 2003; 52: 499-505.
23. Sagara M, Satoh J, Wada R, Yagihashi S, Takahashi K, Fukuzawa M, et al. Inhibition of development of peripheral neuropathy in streptozotocin-induced diabetic rats with N-acetylcysteine. Diabetologia 1996; 39: 263-9.
24. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882-8.
25. Wolff SP, Garner A, Dean RT. Free radicals, lipid and protein degradation. Trends Biochem Sci 1986; 11: 27-31.
26. Ookawara T, Kawamura N, Kitagawa Y, Taniguchi N. Site-specific and random fragmentation of Cu, Zn-superoxide dismutase by glycation reaction. Implication of reactive oxygen species. J Biol Chem 1992; 267: 18505-10.
27. 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.
28. Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci (Lond) 1996; 90: 255-60.
29. Matkovics B, Varga SI, Szabo L, Witas H. The effect of diabetes on the activities of the peroxide metabolism enzymes. Horm Metab Res 1982; 14: 77-9.
30. Mena P, Maynar M, Gutierrez JM, Maynar J, Timon J, Campillo JE. Erythrocyte free radical scavenger enzymes in bicycle professional racers. Adaptation to training. Int J Sports Med 1991; 12: 563-6.
31. Roemsungnoen J, Likidlilid A. Antioxidant status and tissue damage in beta-thalassemia/hemoglobin E [M.Sc. thesis]. Bangkok: Faculty of Graduate Studies, Mahidol University; 1995.
32. Gebre-Medhin M, Ewald U, Plantin LO, Tuvemo T. Elevated serum selenium in diabetic children. Acta Paediatr Scand 1984; 73: 109-14.
33. Mutanen ML, Mykkanen HM. Effect of dietary fat on plasma glutathione peroxidase levels and intestinal absorption of 75Se-labeled sodium selenite in chicks. J Nutr 1984; 114: 829-34.
34. Bellisola G, Galassini S, Moschini G, Poli G, Perona G, Guidi G. Selenium and glutathione peroxidase variations induced by polyunsaturated fatty acids oral supplementation in humans. Clin Chim Acta 1992; 205: 75-85.
35. Giugliano D, Ceriello A, Paolisso G. Diabetes mellitus, hypertension, and cardiovascular disease: which role for oxidative stress? Metabolism 1995; 44: 363-8.
36. Ceriello A, Bortolotti N, Pirisi M, Crescentini A, Tonutti L, Motz E, et al. Total plasma antioxidant capacity predicts thrombosis-prone status in NIDDM patients. Diabetes Care 1997; 20: 1589-93.
37. Seghrouchni I, Drai J, Bannier E, Riviere J, Calmard P, Garcia I, et al. Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; insulin treatment efficiency. Clin Chim Acta 2002; 321: 89-96.
38. Weber G, Convery HJ. Insulin: inducer of glucose 6-phosphate dehydrogenase. Life Sci 1966; 5: 1139-46.
39. Dincer Y, Alademir Z, Ilkova H, Akcay T. Susceptibility of glutatione and glutathione-related antioxidant activity to hydrogen peroxide in patients with type 2 diabetes: effect of glycemic control. Clin Biochem 2002; 35: 297-301.
40. Kawamura N, Ookawara T, Suzuki K, Konishi K, Mino M, Taniguchi N. Increased glycated Cu,Zn-superoxide dismutase levels in erythrocytes of patients with insulin-dependent diabetis mellitus. J Clin Endocrinol Metab 1992; 74: 1352-4.
41. Wolff SP, Bascal ZA, Hunt JV. Autoxidative glycosylations: free radicals and glycation theory. In: Baynes JW, Monnier VM, editors. The millard reaction in aging, diabetes and nutrition. New York: AR Liss; 1989: 259-75.
42. Mullarkey CJ, Edelstein D, Brownlee M. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun 1990; 173: 932-9.
43. Tankova T, Koev D, Dakovska L, Kirilov G. The

effect of repaglinide on insulin secretion and oxidative stress in type 2 diabetic patients. Diabetes Res Clin Pract 2003; 59: 43-9.

44. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006; 440: 944-8.

ระดับกลูตาไธโอนและกลูตาไธโอนเปอร์ออกซิเดสในผู้ป่วยเบาหวานชนิดที่ 1

อธิป ลิขิตลิลิต, ณัฏฐ์ชัย ภัทร์ชนันท์, สายฝน ผลดี, ธวัชชัย พีรพัฒน์ดิษฐ์

วัตถุประสงค์: *ภาวะน้ำตาลในเลือดสูงในผู้ป่วยเบาหวานชนิดที่ 1 เป็นสาเหตุสำคัญที่ทำให้เกิดภาวะออกซิเดทีฟสเตรสอิทธิพลของการสร้างอนุมูลอิสระที่มีสาเหตุจากการมีน้ำตาลในเลือดสูงนี้มักจะเกี่ยวข้องกับการเกิดภาวะโรคแทรกซ้อนในผู้ป่วยเบาหวานโดยเฉพาะโรคหลอดเลือดหัวใจตีบ ดังนั้นการศึกษานี้จึงมีจุดมุ่งหมายเพื่อเปรียบเทียบระดับกลูตาไธโอนและการทำงานของเอนไซม์กลูตาไธโอนเปอร์ออกซิเดสในผู้ป่วยเบาหวานชนิดที่ 1 และคนปกติ*

วัสดุและวิธีการ: *ทำการวิเคราะห์ระดับกลูตาไธโอนและการทำงานของเอนไซม์กลูตาไธโอนเปอร์ออกซิเดสในเม็ดเลือดแดงของผู้ป่วยเบาหวานชนิดที่ 1 จำนวน 20 คนที่มีระดับน้ำตาลในพลาสมาในระยะอดอาหารมากกว่า 140 มก./ดล. และคนปกติจำนวน 20 คนที่มีจำนวนของเพศและอายุใกล้เคียงกัน และมีระดับน้ำตาลในพลาสมาในระยะอดอาหารน้อยกว่า 110 มก./ดล. เป็นกลุ่มควบคุม ข้อมูลที่ได้จะถูกวิเคราะห์โดย unpaired student's t-test และสหสัมพันธ์ของค่าเหล่านี้ระหว่างผู้ป่วยเบาหวานชนิดที่ 1 กับคนปกติจะถูกวิเคราะห์โดย pearson product moment correlation*

ผลการศึกษา: *จากการศึกษาพบว่าระดับกลูตาไธโอนในเม็ดเลือดแดงของผู้ป่วยเบาหวานชนิดที่ 1 มีระดับต่ำกว่าคนปกติอย่างมีนัยสำคัญ ($p = 0.011$) แต่การทำงานของระดับเอนไซม์กลูตาไธโอนเปอร์ออกซิเดสสูงกว่าคนปกติอย่างมีนัยสำคัญ ($p = 0.003$) ระดับที่ลดลงของกลูตาไธโอนในเม็ดเลือดแดงของผู้ป่วยเบาหวานชนิดที่ 1 นี้อาจเนื่องมาจากมีการใช้กลูตาไธโอนมากสำหรับการทำงานของเอนไซม์กลูตาไธโอนเปอร์ออกซิเดสที่เพิ่มขึ้น หรือ อาจจะมีสาเหตุจากการทำงานของเอนไซม์ในวิถีเพนโตสฟอสเฟตที่ลดลงอันเนื่องมาจากการขาดอินซูลินในการกระตุ้นวิถีนี้ทำให้มีการสังเคราะห์กลูตาไธโอนลดลง และจากการศึกษาสหสัมพันธ์ระหว่างระดับน้ำตาลในพลาสมาในระยะอดอาหารกับระดับของกลูตาไธโอน พบว่าสหสัมพันธ์นี้จะเป็นเชิงลบและมีนัยสำคัญทางสถิติ ขณะที่ไม่พบสหสัมพันธ์ระหว่างระดับน้ำตาลในพลาสมาในระยะอดอาหารกับเอนไซม์กลูตาไธโอนเปอร์ออกซิเดส*

สรุป: *การศึกษานี้ชี้แนะว่าผู้ป่วยเบาหวานชนิดที่ 1 อยู่ภายใต้ภาวะออกซิเดทีฟสเตรส และระดับน้ำตาลกลูโคสในเลือดที่เพิ่มสูงขึ้นก็เป็นสาเหตุให้อนุมูลอิสระไปทำลายเซลล์โดยกระบวนการ lipid peroxidation ดังนั้นวิธีการใด ๆ ที่สามารถลดภาวะออกซิเดทีฟสเตรสในผู้ป่วยเบาหวานชนิดที่ 1 ก็อาจจะเป็นประโยชน์ต่อการลดภาวะแทรกซ้อนของการเกิดโรคหลอดเลือดหัวใจตีบในผู้ป่วยได้*