Reduced Free Radical Scavengers and Chronic Renal Failure

LEENA ONG-AJYOOTH, M.D.*, SOMPONG ONG-AJYOOTH, B.Sc. (Pharm), M.S.**,
KANYA TIENSONG, M.S.**, SANGA NILWARANGKUR, MD.*

Abstract

Endogenous oxygen radical scavengers such as glutathione peroxidase (GSH-Px), catalase (CAT), glutathione and vitamin E are powerful regulatory systems against free radical toxicity. These oxidative injuries are increased in patients with chronic renal failure leading to various abnormalities including anemia. In this study, activities of GSH-Px, CAT, glutathione and vitamin E were measured in the erythrocytes of 54 chronic renal failure patients compared with 32 healthy controls. GSH-Px activities were lower significantly from controls (20.5 ± 6.79 *vs* 28.3 ± 9.0 u/gHb, $p < 0.001$). Erythrocytes CAT (6.52 ± 2.3 *vs* 7.54 ± 1.9 u/gHb, $p < 0.05$), glutathione (63.59 ± 20.2 *vs* 75.1 ± 6.3 mg/dl, $p < 0.05$) vit. E (2.23 ± 0.53 *vs* 3.38 ± 0.44 g/ml RBC, $p < 0.001$) were also lower in the patients group. Plasma malondialdehyde (MDA) known as lipid peroxidation product was higher significantly than controls ($p < 0.001$). Abnormal erythrocyte osmotic fragility test, expressed by glycerol lysis time (GLT_{50}) was found in the patients group ($p < 0.001$) and correlated significantly with RBC vitamin E.

Results demontrated defects in erythrocytes enzymatic antioxidant defense mechanism in chronic renal failure patients. To improve antioxidant systems seems to be promising in preventing hemolysis and anemia in these patients.

Anemia is an important problem in patients with chronic renal disease. Although it has a multifactorial origin, its common causes are decreased erythropoietin production[1] and shortened red cell survival[2] present with hemolysis[3]. Lipid peroxidation of erythrocyte membranes by toxic oxygen-free radicals (OFR) has been suggested to play a major role in RBC hemolysis[4,5]. The products of lipid peroxidation molondialdehyde (MDA) are detected in blood and have been used as a measure of oxidative stress.

* Renal Unit, Department of Medicine,
** Biochemistry, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

It is known that vitamin E blocks the peroxidation of the polyunsaturated fatty acid constituents of cell membrance include red blood cells[6,7]. Increased sensitivity of red blood cells to oxidative damage is therefore an index of vitamin E deficiency[8]. In addition, administration of vitamin E can significantly improve the anemia of sickle cell disease[9] or thalassemia[10]. Very little information is available on vitamin E, oxidative stress, and anemia in chronic renal failure patients. In the following investigations, we measure activities of antioxidant enzyme systems: glutathione peroxidase GSH-Px, glutathione, catalase, vitamin E in RBC and plasma MDA of chronic renal failure patients of different stages and to evaluate the effects of these parameters on the RBC osmotic fragility.

MATERIAL AND METHOD

Patients

Fifty-four patients with renal disease participated in this study. The clinical data is shown in Table 1. They were divided into 5 groups according to their serum creatinine levels.

Goup I comprised patients with serum creatinine less than 2 mg/dl; Group II with serum creatinine from 2.1 to 4 mg/dl; Group III with serum creatinine from 4.1 to 8 mg/dl; Group IV with serum creatinine from 8.1 to 12 mg/dl; and Group V with serum creatinine more than 12 mg/dl. There were 33 cases of glomerulonephritis, 11 cases of nephrosclerosis, 7 cases of chronic tubulointerstitial nephritis and one case of polycystic kidney disease. There were 2 cases with unclassified etiology. Exclusion criteria were patients with diabetes mellitus, chronic respiratory insufficiency, alcoholics, smokers and intercurrent infections. Those who had received a blood transfusion within 3 months before entering this study were also excluded. Iron and vitamin supplements were stopped for 2 weeks before blood and urine determinations. Thirty-two age and sex matched volunteers from the hospital staff and personnel constituted the normal, healthy controls (group 0).

Method

Fresh heparinized blood in trace elements free tubes was centrifuged at 2,500 rpm for 10 minutes at 4°C. Plasma subdivided and frozen until analysis. Buffy coat was discarded. The red cells obtained were washed 3 times with cold (4°C) 5 nM phosphate buffer saline. The hemolysate for the determination of glutathione peroxidase and catalase activities was prepared from the clean packed red cells. The glutathione peroxidase, glutathione and catalase activity was measured by the method of Beutler[11-13]. Malondialdehyde (MDA) was determined by TBA method[14]. Vitamin E was measured by high performance liquid chromatography[15]. Serum and urine creatinine were measured by a modified alkaline picrate method[16], and urine protein by Biuret method[17]. Osmotic fragility test was measured by glycerol lysis time (GLT_{50})[18].

Statistical analysis

All results are expressed as the mean ± S.D. The data were analysed using unpaired two-tailed student's *t*-test and linear regression analysis. Statistical significance level was defined as $P < 0.05$.

RESULTS

Anemia was seen in patients of group 2 when creatinine began to rise and worsen gradually when renal function deteriorated as shown in Table 1 and Fig. 1. Antioxidant enzyme activities measured in this study (Table 2) showed clear cut decrease in glutathione peroxidase GSH-Px between the patients and controls (20.48 ± 6.79 *vs* 28.26 ± 9 u/gHb, $p < 0.001$), catalase (6.52 ± 2.3 *vs* 7.54 ± 1.9 u/gHb, $p < 0.05$ and glutathione (63.59 ± 20.2 *vs* 75 ± 6 mg/dl, $p < 0.05$). The plasma malondialdehyde was significantly high in every group of patients compared with controls ($p < 0.001$). Fig. 2 demonstrates vitamin E in red blood cells of chronic renal failure patients and controls (2.23 ± 0.52 *vs* 3.38 ± 0.45 g/ml RBC, $p < 0.0001$). These datas confirmed the existence of impaired antioxidant systems in chronic renal disease patients. Red blood cell osmotic fragility determined by measuring as glycerol lysis time (GLT_{50}) was also abnormally rapid significant in the patient groups. ($p < 0.001$) as shown in Fig. 3.

In addition, the red blood cells vitamin E correlated significantly with GLT_{50} ($r = 0.71$, $p = 0.003$) as presented in Fig. 4.

DISCUSSION

Anemia in chronic renal failure is partially attributed to decreased red blood cell formation due to lack of erythropoietin[1] and partially due to increased red blood cells destruction.

Table 1. Clinical data of the study subjects (mean ± S.D.)

	Controls	Patients					
		group 1	group 2	group 3	group 4	group 5	Total
Number	32	12	8	13	13	8	54
Age, years	30.69 ± 8.63	38.00 ± 10.86	46.12 ± 16.5	49.93 ± 14.06	48.93 ± 12.89	45.14 ± 17.75	46 ± 14
Sex, M : F	16 : 16	5 : 7	5 : 3	3 : 11	1 : 11	4 : 3	35 : 51
Etiology							
Chronic GN.	-	8	4	7	8	6	33
Nephrosclerosis	-	1	2	3	5	-	11
Chronic tubulointerstitial nephritis	-	3	-	2	-	2	7
Polycystic kidney	-	-	-	1	-	-	1
Unclassified	-	-	2	-	-	-	2
Hct (%)	42.06 ± 4.79	40.97 ± 7.5	32.41 ± 6.54***	27.00 ± 5.12***	21.99 ± 6.49***	20.17 ± 4.07***	28.3 ± 9.6***
Serum creatinine (mg/dl)	1.02 ± 0.15	1.28 ± 0.28***	3.12 ± 0.58***	5.30 ± 1.13***	9.62 ± 1.36***	19.84 ± 6.55***	7.3 ± 6.6***
Serum albumin (g/dl)	4.88 ± 0.25	3.88 ± 1.34***	4.07 ± 0.93***	4.3 ± 0.68***	4.34 ± 0.241***	4.16 ± 0.53***	4.1 ± 0.8***
Ccr (ml/min)	89.03 ± 18.47	60.75 ± 23.14***	20.37 ± 6.68***	10.86 ± 4.02***	5.09 ± 1.40***	2.83 ± 2.08***	21.9 ± 25.4***

$P < 0.05$*, $P < 0.01$**, $P < 0.001$**

Table 2. Plasma MDA, enzymatic antioxidant systems and glycerol lysis time in red blood cells of the patients.

	Controls	Patients					
		total	group 1	group 2	group 3	group 4	group 5
Erythrocyte							
GPX (U/gHb)	28.26 ± 9.01	20.48 ± 6.79**	15.85 ± 8.33*	21.61 ± 5.63*	19.14 ± 7.63*	25.53 ± 2.57*	20.29 ± 2.86
Catalase (U/gHb)	7.54 ± 1.91	6.52 ± 2.31*	6.38 ± 2.50	7.00 ± 1.94	5.23 ± 2.61*	6.51 ± 1.47	8.36 ± 2.02
Glutathione (mg/dl)	75.08 ± 6.32	63.59 ± 20.26*	60.73 ± 21.81	59.96 ± 9.37	70.56 ± 20.05	66.60 ± 24.31	55.31 ± 9.29*
Vit. E (g/ml PRC)	3.38 ± 0.45	2.23 ± 0.52**	2.17 ± 4.41**	2.32 ± 0.79**	2.36 ± 0.36**	2.25 ± 0.53**	1.97 ± 0.56**
GLT_{50} (sec.)	283 ± 40	71 ± 24**	72 ± 17**	71 ± 30**	59 ± 20**	65 ± 22**	86 ± 23**
Plasma MDA (μmol/l)	7.39 ± 3.39**	92.94 ± 61.66**	123.5 ± 54.47**	146.43 ± 84.44**	83.85 ± 61.38**	64.23 ± 35.7**	69.38 ± 45.55**

Values are mean ± S.D., * $P < 0.05$, ** $P < 0.0001$, *versus* controls.

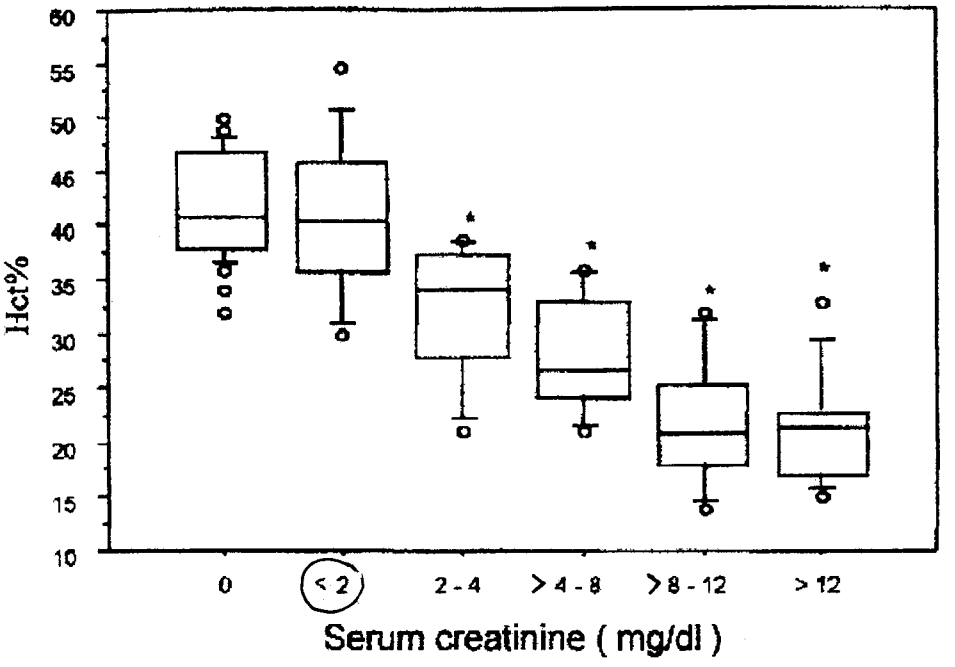

Fig. 1. Relationship between hematocrit in control and chronic renal failure patients. (*P < 0.0001)

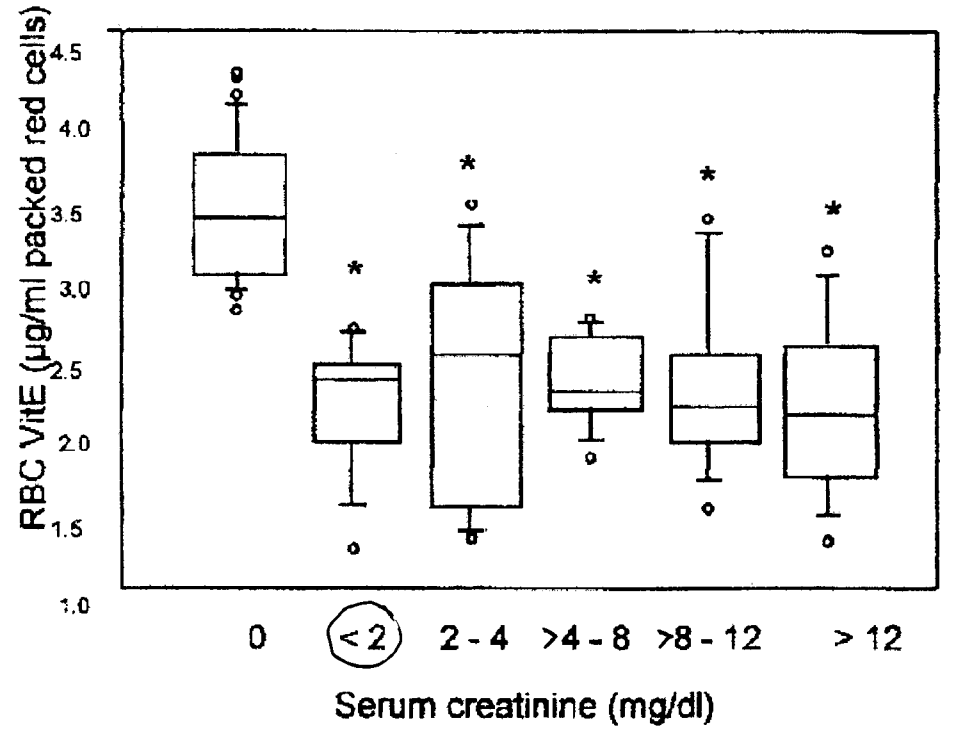

Fig. 2. RBC vit. E in controls and CRF patients. (*P < 0.0001)

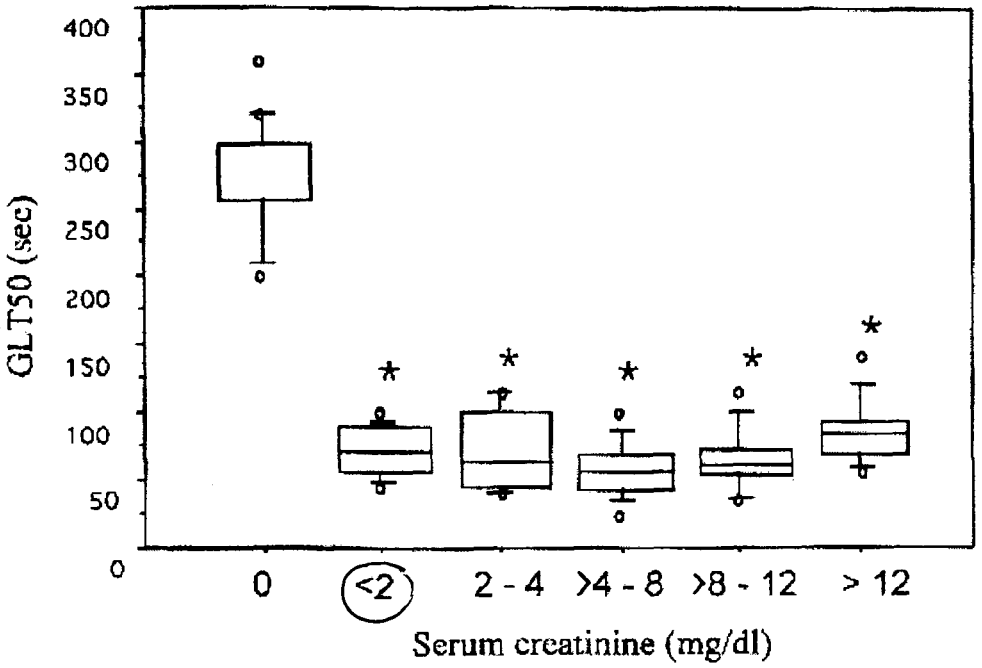

Fig. 3. Osmotic fragility in RBC of controls and CRF patients. (*P < 0.0001)

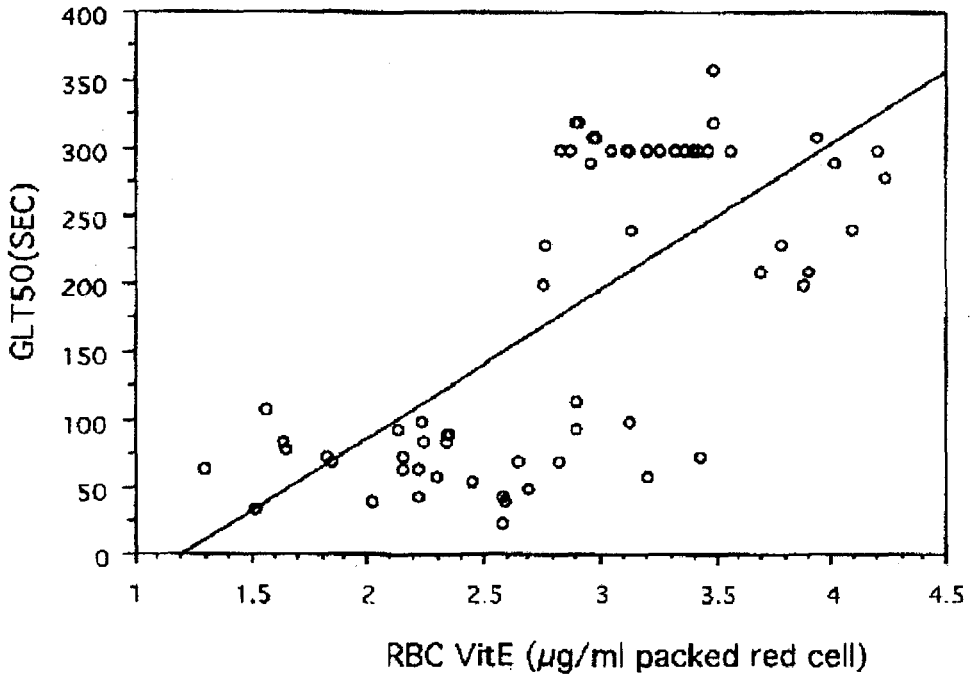

Fig. 4. Relationship between red blood cell vitamin E and GLT_{50}. (R = 0.706, P = 0.0003)

Increased red blood cell hemolysis due to uremic toxin inhibition of glucose 6-phosphate dehydrogenase activity, which leads to reduced NADPH and GSH levels, was previously reported[19,20]. The reduced levels of antioxidant NADPH, GSH and vitamin E are all indicative of a hyperoxygenative state in these patients which could be caused from excessive aluminium[21], one of uremic toxins leading to generate oxygen free radicals and accelerate peroxidation[22]. The structure and functional integrity of red blood cell is conditioned by the production of energy and maintenance of the reductive state of the cell. The maintenance of the reductive state is compromised by a continuous release, within the red blood cell, of superoxide radicals such as superoxide anion (O^-_2), hydroxyl radical $(OH)^-$ and hydrogen peroxide (H_2O_2). The superoxide radicals are known to be produced, within the red blood cell, by the spontaneons oxidation of oxyhemoglobin[23], of isolated hemoglobin chains and by hemolytic drugs such as phenylhydrazine[24]. In abnormal release of activated oxygen species in red blood cell is believed to be responsible for extensive cellular damage such as the hemoglobin precipitation as Heinz bodies and peroxidation of erythrocyte membrane. Nevertheless, red blood cells possess several lines of defence against oxidative stress. Reduced glutathione (GSH), together with its related enzymes, represents one of the major scavenger systems of activated oxygen species in red blood cells. Besides, endogenous oxygen radical scavengers such as glutathione peroxidase (GSH-Px), catalase, glutathione and vitamin E are powerful regulatory systems against free radical toxicity. These oxidative injuries are increased in patients with chronic renal failure leading to various abnormalities including anemia.

The results obtained in this study have clearly demonstrated that the levels of antioxidant enzyme activities in red blood cells (glutathione, catalase, glutathione peroxidase and vitamin E) are reduced significantly than normal controls. The present study confirmed the altered status of enzymatic antioxidant systems associated with increased lipid peroxidation. The reduced glutathione peroxidase, catalase, glutathione and increased lipid peroxidation all agree with previous results[25-28]. Since the observation by Rose and Gyorgy[29] that vitamin E can act as antioxidant which can reduce hemolysis of red cells caused by oxidant stress, additional evidence of a wide variety of roles in membrane - related activities and pathologic processes secondary to a deficiency has been reported [30]. Vitamin E status has been traditionally determined by measuring plasma vitamin E levels and there have been few investigations of α-tocopherol in red cells owing to technical difficulties of the assay. There is also considerable evidence that the level in the red cell needs to be directly measured rather than plasma when indicating hemolysis[31].

In this study we found low levels of RBC vitamin E in all groups of the patients. Our findings suggest that anemia in chronic renal failure can partly be attributed to vitamin E deficiency in RBC membranes which accompany increased rates of lipid and protein oxidation, destruction of membrane function, and inactivation of membrane enzymes. Evidence shown in this study is decreased RBC vitamin E, low levels of enzymatic antioxidant systems, rapid glycerol lysis time (GLT_{50}) leading to rapid hemolysis and anemia. The levels of vitamin E also correlalate with GLT_{50} which indicate that oxidative damage will be minimized while vitamin E is still present. The reasons why chronic renal failure patients have vitamin E deficiency are postulated in several explanations. Some reports demonstrate diets low in vitamin E[25,32]. A local deficiency of vitamin E can arise rapidly in membranes under conditions of intense oxidative stress occur in renal injury. Since free radical-mediated damage has been implicated in cellular and extracellular changes that occur over time in the development of chronic renal diseases, the replenishment of vitamin E in depleted membranes therefore requires days to weeks. Besides, the administration of a diet that is modestly enriched in vitamin E can protect against the development of progressive renal damage in chronic puromycin aminonucleoside nephropatly in experimental animals[32]. In human, the effect of vitamin E therapy on plasma and erythrocyte lipid peroxidation in chronic hemodialysis patients was reported to be promising[34]. However, the beneficial effect of vitamin E in improving anemia in chronic renal failure patients needs to be confirmed in large prospective studies.

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ระบบป้องกันอนุมูลออกซิเจนอิสระกับความเปราะของผิวเม็ดเลือดแดงในผู้ป่วยไตวายเรื้อรัง

ลีนา องอาจยุทธ, พ.บ., สมพงษ์ องอาจยุทธ, วท.ม.**,*
*กัลญา เทียนส่ง, วท.ม.**, สง่า นิลวรางกูร, พ.บ.**

จากการศึกษาระบบป้องกันอนุมูลออกซิเจนอิสระ ด้วยกลูตาไธโอน เปอร์ออกซิเดส (GSH-Px), คาตาเลส และวิตามิน อี ในเม็ดเลือดแดง ผู้ป่วยไตวายเรื้อรังจำนวน 54 ราย เปรียบเทียบกับคนปกติ 32 ราย พบว่าการทำงานของ GSH-Px, คาตาเลส, กลูตาไธโอน ต่ำในผู้ป่วยอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$) ระดับมาโลนไดอัลดีไฮด์ (MDA) ในพลาสม่า ซึ่งเป็นผลลัพธ์ของ lipid peroxidation สูงอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$) ความเปราะของเม็ดเลือดแดงวัดโดย glycerol lysis time (GLT_{50}) พบว่าผิดปกติชัดเจน ($p < 0.001$) และสัมพันธ์กับระดับวิตามิน อี ของเม็ดเลือดแดงด้วย ผลการศึกษาแสดงถึงกลไกของการเกิดซีดในผู้ป่วยไตวายเรื้อรัง ว่าส่วนหนึ่งมาจากความผิดปกติของระบบป้องกันอนุมูลออกซิเจนอิสระ การช่วยระบบป้องกันนี้ให้ดีขึ้น จะช่วยไม่ให้ผู้ป่วยไตวายเรื้อรังซีดได้อีกทางหนึ่ง

* ภาควิชาอายุรศาสตร์,
** ภาควิชาชีวเคมี, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10700