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ชื่อวิทยานิพนธ์ ภาวะโภชนาการของซีลีเนียมในผู้ป่วยธาลัสซีเมีย

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บทคัดย่อ

ซีลีเนียม เป็นส่วนประกอบของเอนไซม์ glutathione peroxidase (GSH-Px) ซึ่งเป็นเอนไซม์ที่ช่วยป้องกันเซลล์ไม่ให้ถูกทำลายโดยกระบวนการออกซิเดชั่นต่าง ๆ ที่เกิดขึ้นในร่างกาย การศึกษานี้เป็นการประเมินภาวะโภชนาการของซีลีเนียม, ความสัมพันธ์ระหว่างซีลีเนียมกับวิตามินอี และผลของการให้วิตามินอีต่อระดับเอนไซม์ GSH-Px ในผู้ป่วยธาลัสซีเมีย ผู้ป่วยธาลัสซีเมียที่ทำการศึกษาได้แก่ Hb H disease 10 ราย, B-thalassemia/Hb E 30 ราย และ B-thalassemia major 2 ราย เปรียบเทียบกับเด็กปกติ 14 ราย ผู้ป่วยทั้งหมดไม่ได้รับเลือดอย่างน้อย 3 เดือนก่อนการศึกษา ผลการศึกษาพบว่าระดับซีลีเนียมในเม็ดเลือดแดงของผู้ป่วยธาลัสซีเมียทั้ง 3 กลุ่ม ไม่ต่างจากกลุ่มเด็กปกติ แสดงว่าผู้ป่วยธาลัสซีเมียเหล่านี้มีภาวะโภชนาการของซีลีเนียมอยู่ในระดับปกติ เมื่อดูการทำงานของเอนไซม์ GSH-Px ในเม็ดเลือดแดงของผู้ป่วยธาลัสซีเมียทั้ง 3 กลุ่ม พบว่าสูงกว่าปกติ อธิบายได้ว่าเม็ดเลือดแดงของผู้ป่วยเหล่านี้อาจมีการปรับตัว โดยเพิ่มการทำงานของเอนไซม์ GSH-Px เพื่อป้องกันตัวเองจากการถูกทำลายโดยปฏิกิริยาออกซิเดชั่นต่าง ๆ นอกจากนี้ยังพบว่าผู้ป่วยธาลัสซีเมียทั้ง 3 กลุ่ม มีภาวะโภชนาการของวิตามินอีต่ำกว่ากลุ่มเด็กปกติ ทั้งนี้อาจเนื่องจากผู้ป่วยเหล่านี้มีการนำวิตามินอีไปใช้ในกระบวนการป้องกัน lipid peroxidation ของเม็ดเลือดแดงมากกว่าปกติ

สำหรับความสัมพันธ์ระหว่างวิตามินอีกับซีลีเนียม ศึกษาโดยดูความสัมพันธ์ของวิตามินอีในพลาสม่ากับการทำงานของเอนไซม์ GSH-Px ในเม็ดเลือดแดง พบว่าในกลุ่มเด็กปกติและกลุ่ม Hb H disease ความสัมพันธ์ระหว่างตัวแปรทั้งสองเป็นแบบผกผัน แสดงว่าในภาวะที่มี

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วิตามินอีต่ำ ร่างกายจะมีการปรับตัวโดยการเพิ่มการทำงานของเอนไซม์ GSH-Px มากขึ้น แต่ในกลุ่ม B-thalassemia/Hb E ซึ่งส่วนใหญ่มีวิตามินอีต่ำมาก พบว่าไม่มีความสัมพันธ์ระหว่างตัวแปรทั้งสอง อาจเนื่องจากเอนไซม์ GSH-Px ของผู้ป่วยกลุ่มนี้ปรับตัวถึงระดับสูงสุดแล้ว ถึงแม้ระดับวิตามินอีในเลือดจะต่ำมากก็ไม่สามารถทำให้เอนไซม์ทำงานเพิ่มขึ้นได้อีก

เมื่อเสริมวิตามินอีให้กับผู้ป่วยธาลัสซีเมีย 5 ราย ที่มีระดับวิตามินอีต่ำมาก และมีเปอร์เซนต์การแตกของเม็ดเลือดแดงสูง พบว่าหลังจากเสริมวิตามินอี 200 มก./วัน ประมาณ 6-8 สัปดาห์ ระดับวิตามินอีในพลาสม่าของผู้ป่วยสูงขึ้นถึงระดับปกติ หรือมากกว่า และพบว่าการทำงานของเอนไซม์ GSH-Px ในเม็ดเลือดแดงลดลง นอกจากนี้เมื่อทดสอบการแตกของเม็ดเลือดแดง โดย H_2O_2 hemolysis test ไม่พบว่ามีการแตกของเม็ดเลือดแดงในผู้ป่วยทั้ง 5 ราย ผลการทดลองนี้สนับสนุนสมมติฐานที่กล่าวว่า วิตามินอีและซีลีเนียมมีความสัมพันธ์กันในการป้องกันเซลล์จากการถูกทำลายโดยปฏิกิริยาออกซิเดชั่นต่าง ๆ ในร่างกาย

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ABSTRACT

Selenium, an essential component of glutathione peroxidase (GSH-Px) and vitamin E guard against peroxidative damage of erythrocytes. The objectives of this study are to investigate selenium status, its relationship with vitamin E and the effect of vitamin E supplementation on erythrocyte GSH-Px activity in thalassemias. Forty-two thalassemic patients and 14 non thalassemic children participated in the study. The thalassemic patients consisted of 10 with Hb H disease, 30 with β-thalassemia/Hb E and 2 with β-thalassemia major. They were not given blood transfusion within 3 months prior to this study. The mean GSH-Px activity in all thalassemic patients was significantly ($P<0.05$) higher than that of the control group, but no difference was

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found in the mean erythrocyte selenium concentration. These findings suggested normal status of selenium in thalassemic patients. The elevation of GSH-Px activity shown in thalassemic erythrocytes may result from the adaptation of the erythrocytes' defense mechanism to prevent themselves from the oxidative damage. In addition, the patients exhibited significantly ($P<0.05$) lower plasma vitamin E concentration than normal subjects. The increased vitamin E uptake into the pathological erythrocytes and other tissues in order to counteract the oxidative damage may be the plausible explanation.

Inverse correlation between plasma vitamin E level and erythrocyte GSH-Px activity was observed among the normal subjects and Hb H disease patients. This indicates the adaptive physiological response of GSH-Px in the condition of low vitamin E status. No correlation of these two parameters was found in β-thalassemia/Hb E patients which might be due to markedly low vitamin E status as well as the possibility that GSH-Px might reach its maximum adaptive activity. Supplementation with vitamin E 200 mg/day for 6-8 weeks was conducted in five thalassemic subjects with low vitamin E status. Significant increase of plasma vitamin E to normal level and decreased erythrocyte GSH-Px activity were found. The findings agree with the postulated mechanism of vitamin E-selenium relationship in the prevention of peroxidative damage.

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TABLE OF CONTENTS

	Page
ABSTRACT	i
LIST OF TABLES	x
LIST OF FIGURES	xii
CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	3
2.1 Normal Human Hemoglobin	3
2.2 Thalassemia	4
2.2.1 Definition and Classification	4
2.2.1.1 Beta-thalassemia	7
2.2.1.2 Alpha-thalassemia	7
2.2.2 Pathophysiologic Changes in Thalassemia	8
2.2.2.1 Oxidative Damage and Hemolysis of Thalassemic Erythrocytes	10
2.2.2.2 Iron Overload and Superoxide Formation	11
2.2.3 Treatment of Thalassemia	14
2.2.3.1 Blood Transfusion	14
2.2.3.2 Splenectomy	15
2.2.3.3 Iron Chelating Agents	16
2.3 Selenium	16
2.3.1 Chemistry	16
2.3.2 Physiological Roles of Selenium	17

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Page

CHAPTER

2.3.3 Human Selenium Metabolism 21
2.3.3.1 Absorption 22
2.3.3.2 Transport and Distribution 23
2.3.3.3 Excretion 24
2.3.3.4 Retention 25
2.3.3.5 Metabolic Transformation 26
2.3.4 Selenium in Human Foods and Dietary Intake 28
2.3.5 Human Requirement for Selenium 29
2.3.6 Selenium Functions and Related Diseases 32
2.3.7 Assessment of Human Selenium Status 34
2.4 Vitamin E (tocopherol) 39
2.4.1 Chemistry 39
2.4.2 Physiological Roles of Vitamin E 39
2.4.3 Metabolism of Vitamin E 41
2.4.4 Food Sources and Dietary Requirement of Vitamin E 42
2.4.5 Deficiency of Vitamin E in Human 42
2.5 Selenium and Vitamin E Status in Thalassemias and Their Roles in Thalassemic Erythrocyte Membranes 44

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Page

CHAPTER

III MATERIALS AND METHODS 46

3.1 Subjects 46

3.1.1 Thalassemic Patients 46

3.1.2 Non Thalassemic Children 46

3.2 Sample Collection 47

3.2.1 Preparation of Glasswares and Plastic Tubes 47

3.2.2 Blood Collection 47

3.3 Biochemical Assessments 48

3.3.1 Determination of Hemoglobin and Hematocrit 48

3.3.2 Determination of Hemolysis 49

3.3.2.1 Principle 49

3.3.2.2 Reagents 49

3.3.2.3 Procedure 49

3.3.2.4 Calculation 50

3.3.3 Determination of Erythrocyte GSH-Px Activity 50

3.3.3.1 Principle 50

3.3.3.2 Reagents 51

3.3.3.3 Procedure 52

3.3.3.4 Calculation 52

3.3.4 Determination of Erythrocyte Selenium 53

3.3.4.1 Principle 53

3.3.4.2 Reagents 53

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CHAPTER		Page
	3.3.4.3 Procedure	54
	3.3.4.4 Calculation	56
	3.3.5 Determination of Plasma Vitamin E	56
	3.3.6 Determination of Erythrocyte Deformability	56
	3.4 Statistical Analysis	56
IV	RESULTS	57
	4.1 Clinical Profiles of Thalassemic Patients	57
	4.2 Hematological Data of Normal Subjects and Thalassemic Patients	60
	4.3 Selenium and Vitamin E Status of Normal Subjects and Thalassemic Patients	62
	4.4 Relationships Between Erythrocyte Selenium, Erythrocyte GSH-Px and Plasma Vitamin E of Normal Subjects and Thalassemic Patients	67
	4.5 The Effects of Vitamin E Supplementation on Plasma Vitamin E and Erythrocyte GSH-Px of Thalassemic Patients	70
V	DISCUSSION	74
	5.1 Selenium and Vitamin E Status	74

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Page

CHAPTER

5.2 The Relationship Between Erythrocyte Selenium Level and Erythrocyte GSH-Px Activity 78

5.3 The Relationship Between Plasma Vitamin E Level and Erythrocyte GSH-Px Activity 79

5.4 The Effects of Vitamin E Supplementation on Plasma Vitamin E Level and Erythrocyte GSH-Px Activity 80

VI SUMMARY 82

VII SUGGESTIONS FOR FURTHER STUDY 86

BIBLIOGRAPHY 87

APPENDIX 112

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LIST OF TABLES

Table		Page
1	A summary of some characteristics of selenium	18
2	Daily selenium intake in different population groups	30
3	Estimated safe and adequate daily dietary intake of selenium	31
4	Postulated biological function of selenium	32
5	Diseases which have been linked with a low selenium status	33
6	Techniques for assessment of selenium status	35
7	Blood selenium levels in human populations worldwide	37
8	Clinical profiles of 10 Hb H disease patients	58
9	Clinical profiles of 30 β-thalassemia/Hb E patients	59
10	Clinical profiles of 2 β-thalassemia major patients	59
11	Mean±SD of ages and hematological values of normal subjects and thalassemic patients	61
12	Selenium and vitamin E status of normal subjects and thalassemic patients	63
13	Mean ± SD of plasma vitamin E and erythrocyte GSH-Px activity of 5 splenectomized thalassemic patients before and after 6-8 weeks vitamin E supplementation	71

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Page

Table

14 General, hematological and biochemical data of 14 normal subjects (APPENDIX 2) 115

15 General, hematological and biochemical data of 10 Hb H disease patients (APPENDIX 3) 116

16 General, hematological and biochemical data of 30 β-thalassemia/Hb E patients (APPENDIX 4) 117

17 General, hematological and biochemical data of 2 β-thalassemia major patients (APPENDIX 5) 118

18 Hematological and biochemical parameters of 5 splenectomized thalassemic patients before and after 6-8 weeks vitamin E supplementation (200 mg/day) 119

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LIST OF FIGURES

Figure		Page
1	Development of polypeptide and hemoglobin in gestation and infant age	5
2	Pathology in the erythrocytes of thalassemia	6
3	Pathophysiological changes in thalassemia	9
4	The production and reduction of activated oxygen is linked to the glycolysis and pentose phosphate pathways	12
5	The process of the heme iron loses on electron, resulting in the formation of ferric methemoglobin and release of superoxide	13
6	Scheme showing the postulated mechanisms of interaction between Se and vitamin E in preventing oxidant damage	20
7	Metabolic interrelationships between organic and inorganic forms of selenium	27
8	Vitamin E acts as a protector against oxidation	40
9	Mechanisms for hypovitaminosis E	43
10	Erythrocyte selenium concentrations of normal subjects and patients with Hb H disease and β-thalassemia/Hb E	64

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Page

Figure

11 Erythrocyte GSH-Px activity of normal subjects and patients with Hb H disease and β-thalassemia/Hb E 65

12 Plasma vitamin E concentrations of normal subjects and patients with Hb H disease and β-thalassemia/Hb E 66

13 Relationship between erythrocyte selenium concentrations and erythrocyte GSH-Px activities of normal subjects and thalssemic patients 68

14 Relationship between plasma vitamin E concentrations and erythrocyte GSH-Px activities of normal subjects and thalassemic patients 69

15 The effect of vitamin E supplementation on plasma vitamin E in 5 thalassemic patients 72

16 The effect of vitamin E supplementation on erythrocyte GSH-Px activity in 5 thalassemic patients 73

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CHAPTER I

INTRODUCTION

Thalassemia syndrome, resulting from defective production of globin chains, is the most common genetic disorder in Thailand. Approximately 1% of population suffer from this disease. It is a public health problem with significant morbidity and mortality. In Thailand common types are β-thalassemia/Hb E, Hb H disease and β-thalassemia major, which exert various degree of clinical severity ranging from asymptomatic to lethal cases (1). Indicative factors of severity are still not clear. However, the disease is associated with varying degrees of anemia, jaundice, hepatosplenomegaly and abnormal red blood cells (RBC).

In thalassemia, erythrocyte survival is shortened because thalassemic RBC is more susceptible to autoxidation than normal RBC, leading to increased in RBC hemolysis. Furthermore, Stocks et al., (2) have found an increased susceptibility to exogenous peroxidant threat in thalassemic RBC.

Selenium, as a component of glutathione peroxidase (GSH-Px), protects erythrocytes against oxidation of hemoglobin and hemolysis (3,4). GSH-Px has been shown to be an important enzyme in destroying hydrogen peroxide and organic peroxides such as lipid peroxides (5,6). It therefore guards against oxidative damage to the cell membranes and other oxidant-sensitive sites in

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the cell (7). In addition to selenium, vitamin E is believed to protect cell membranes from oxidative damage by preventing the formation of lipid peroxides (8). Thus, selenium and vitamin E status in thalassemic patients deserve to be examined.

Objectives

The purpose of this study is to investigate selenium status and its relationship with vitamin E in various types of thalassemia. Thus, in the 3 main groups of thalassemic patients, namely, Hb H disease, β-thalassemia/Hb E, and β-thalassemia major, the specific objectives are to assess the followings:

1. selenium status by determining erythrocyte selenium concentration and GSH-Px activity;

2. the relationships between erythrocyte selenium and GSH-Px as well as between plasma vitamin E and erythrocyte GSH-Px;

3. the effects of vitamin E supplementation on plasma vitamin E level and erythrocyte GSH-Px activity.

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CHAPTER II

LITERATURE REVIEW

2.1 Normal Human Hemoglobin

Hemoglobin (Hb) is a tetramer of four globin polypeptide chains, which have been named according to the Greek alphabets such as α-, β-, γ-, and δ-globin chains. Each of them is complexed to an oxygen binding heme moiety (9). Adult and fetal hemoglobins have α chains associated with β (Hb A, $\alpha_2\beta_2$), δ(Hb A$_2$, $\alpha_2\delta_2$) or γchains (Hb F, $\alpha_2\gamma_2$), whereas in the embryo ζ chains combine with γ (Hb Portland, $\zeta_2\gamma_2$) or ϵ chains (Hb Gower 1, $\zeta_2\epsilon_2$) and α and ϵ chains combine to form Hb Gower 2 ($\alpha_2\epsilon_2$) (10). The genetic information for every normal and abnormal globin chain is encoded in the nucleotide sequence of the DNA. These genes are located at specific loci on chromosome 11 and 16 (11).

Six different globin chains (α, β, γ, δ, ϵ, ζ) are known to occur in normal human hemoglobins at various stages of development. In early embryonic development, the primary Hb is Hb Gower 1, of which synthesis decreases at about 5 weeks of gestation, while synthesis of Hb F increases. Other embryonic hemoglobins, Gower 2 and Portland, are found before 8 weeks of gestation. At later stages of development, Gower hemoglobins and Portland Hb decline and eventually disappear by 10-12 weeks of gestation. At the same time, Hb F becomes the predominant Hb, making up over 90% of the total and

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maintains this level till just before term (12-30 weeks of gestation). The fetal Hb (Hb F) declines rapidly to 50-80% after birth and 10-15% by 4 months of age. In addition to Hb F, 5-10% of Hb A (adult Hb) is detected as early as 6 weeks of fetal life and remains constant up to 32 weeks of gestation, after which Hb A increases rapidly to 10-30% at term. The proportion remains relatively constant until the switch to adult Hb production occurs later in development. Normal adult has about 97% of Hb A, 2-3% of Hb A_2 and less than 1% of Hb F (Figure 1) (10, 12). The replacement of fetal Hb (Hb F) by adult Hb (Hb A) results in decreased oxygen affinity and greater sensitivity to the modulating influence of 2,3-diphosphoglycerate needed for optimal oxygen utilization in life ex-utero (10, 13).

2.2 Thalassemia

2.2.1 Definition and Classification

The thalassemias refer to a group of genetic disorders of hemoglobin synthesis, resulting from decreased or absent production of one or more of the normal globin chains (10). The imbalance in globin chain synthesis results in the presence of precipitated, uncombined polypeptide chains. Thalassemias are classified according to the affected type of globin chain of hemoglobin, into beta- and alpha-thalassemia (14). Both β- and α-thalassemias are common in Southeast Asia (Figure 2) (15).

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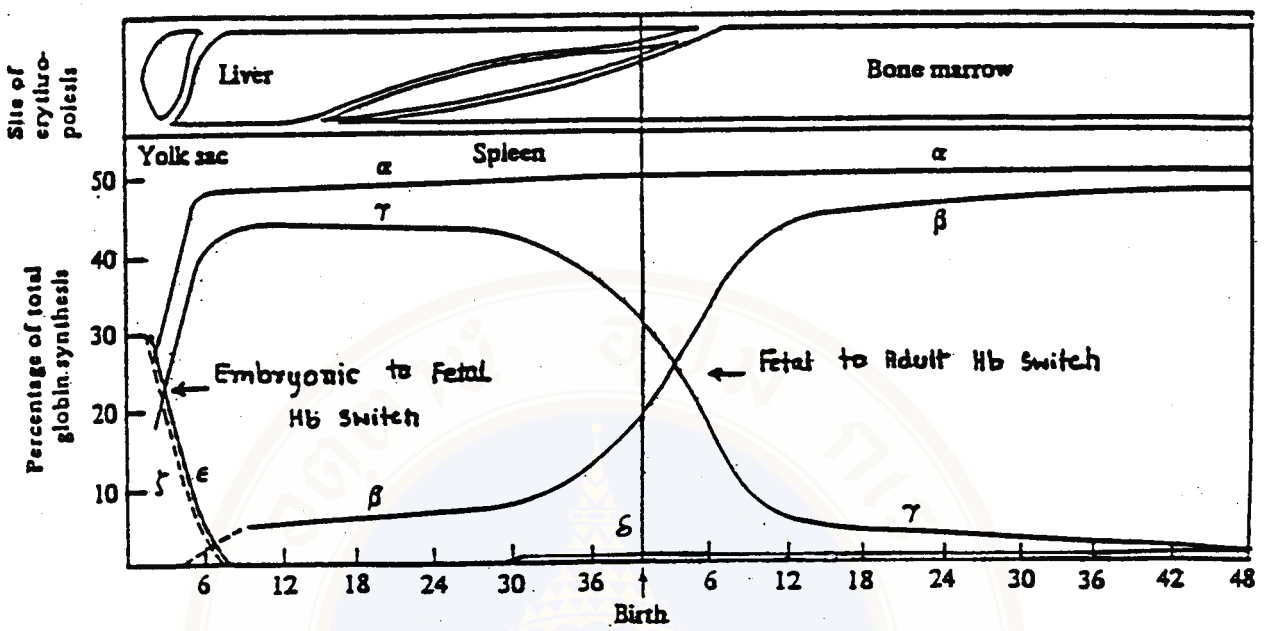

Post-conceptual age (weeks) Postnatal age (weeks)

	Embryo	Fetus-Newborn	Infant to 1 year
Hb Gower 1 ($\zeta_2\varepsilon_2$)	Present	0	0
Hb Gower 2 ($\alpha_2\varepsilon_2$)	Present	0	0
Hb F ($\alpha_2\gamma_2$)	Few	60-80%	1-2%
Hb A ($\alpha_2\beta_2$)	-	40-20%	96-98%
Hb A$_2$ ($\alpha_2\delta_2$)	-	2%	1%
Hb Portland ($\zeta_2\gamma_2$)	Few	Few	-

Figure 1 Development of polypeptide and hemoglobin in gestation and infant age (12).

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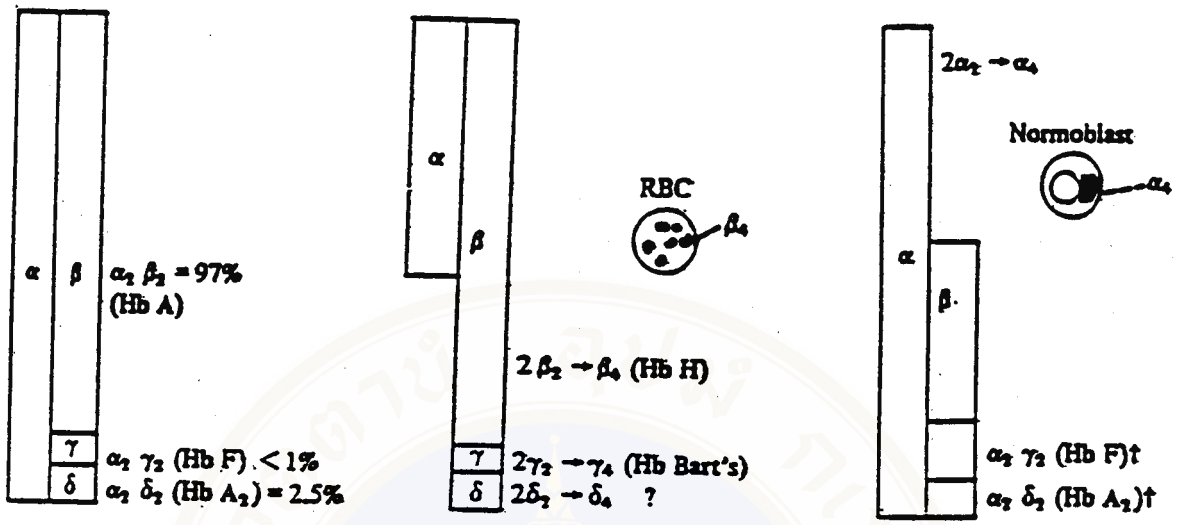

Figure 2 Pathology in the erythrocytes of thalassemia. Normally globin chain synthesis is balance without excess of any chains. In α-thalassemia, α-chain is inadequate leading to an excess of the other chains which polymerize to β_4, γ_4 and (?). In contrast, in β-thalassemia, β-chains are inadequate leading to an excess of the α-chains which polymerize to α_4; the γ-chains and δ-chains may be compensatingly increased resulting in elevation of Hb F ($\alpha_2\gamma_2$) and Hb A ($\alpha_2\delta_2$). Thus, Hb H (β_4) and Hb Bart's (γ_4) occur as a result of α-thalassemia. Elevation of Hb F and Hb A_2 is associated with β-thalassemia (15).

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2.2.1.1 Beta-thalassemia

The β-thalassemias are heterogenous group of genetic disorders characterized by reduced β-chain synthesis (β^{+}-thalassemia) or total absence of β-chain production (β^{o}-thalassemia) (10). They can be divided into β^{o}, β^{+}, δβ and silent β-thalassemia. In β^{o}-thalassemia, the β-DNA sequence or genes are present, but defects in processing, transport and stability of β-mRNA may occur, leading to no synthesis of β-mRNA or inactive β-mRNA. Most β^{+}-thalassemia genes lead to decrease β-mRNA resulting in low levels of β-globin chain produced. Silent β-thalassemia has normal hematologic findings including Hb A_2 and Hb F in the heterozygotes. In δβ-thalassemia, both δ- and β-chains are suppressed.

In addition to β-thalassemia, Hb E is common in Thailand, so interaction between these two genes can occur and results in a severe thalassemia disease similar to Cooley's anemia. Thus, it represents a serious public health problem in the country (16).

2.2.1.2 Alpha-thalassemia

Alpha-thalassemia is characterized by a defect in α-globin chain synthesis resulting in an excess of β- and γ-chains. This leads to the precipitation of unpaired globin chains (β- or γ-globin chain precipitation) (10). Four main types of α-thalassemia can be defined depending on the number of α-globin gene deletion in the diploid genome. Thus, deletion of 1, 2, 3, and 4-globin genes

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results in α-thalassemia 2 (-α/αα), α-thalassemia 1 (--/αα), Hb H disease (--/-α) and Hb Bart's hydrops fetalis (--/--), respectively. Deletions of 1 or 2 α-genes do not give rise to clinical disease, whereas deletion of four α-genes leads to the most severe form of thalassemia, Hb Bart's hydrops fetalis, in which the fetus dies in utero or soon after birth. Two genotypes of Hb H disease are commonly found in Thailand: α-thalassemia 1/α-thalassemia 2 (--/-α) and α-thalassemia 1 / Hb Constant Spring (--/α^{cs} α). Rare Hb H diseases can occur from the interaction between α-thalassemia 1 and Hb Mahidol or Hb Suan-Dok(16).

2.2.2 Pathophysiologic Changes in Thalassemia

The basic defect in thalassemia is a partial or total reduction in the output of globin chain which leads to imbalanced production of the partner globin chain, and reduction proportionately in heme synthesis. The outcome is the decrease in the numbers of Hb molecules in the red cells leading to small size (microcytosis) and poor hemoglobinization (hypochromia and reduced mean cell Hb). Moreover, the aggregation and precipitation of the excess globin chain in the red cells as inclusion bodies lead to rigid membrane and short survival of red cells. The sequence of pathophysiologic changes is summarized in Figure 3 (15).

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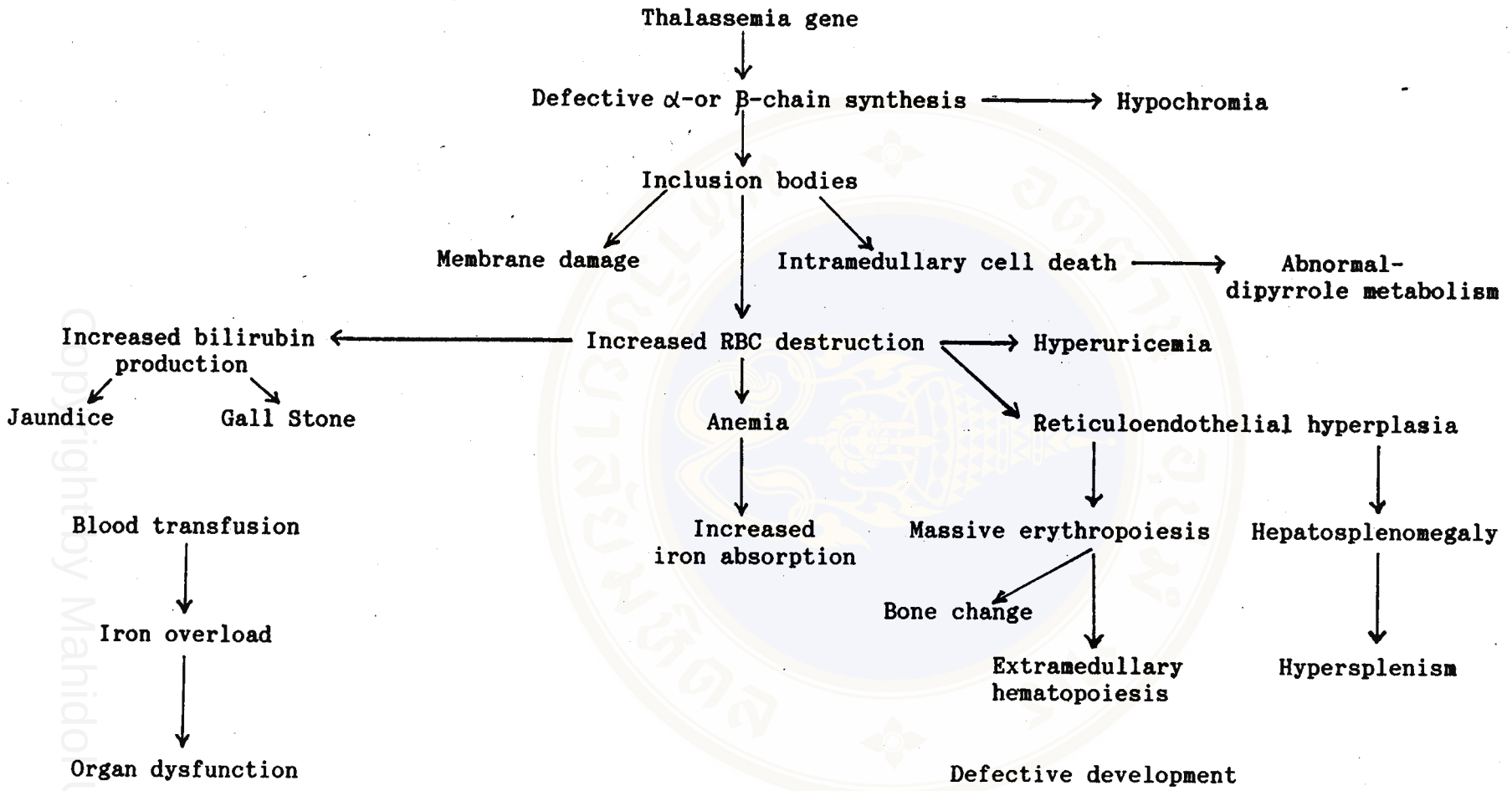

Figure 3 Pathophysiologic changes in thalassemia (15)

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2.2.2.1 Oxidative Damage and Hemolysis of Thalassemic Erythrocytes

Oxidation is a main contributor to the degenerative processes leading to cellular breakdown (17). The direct oxidative threat is not posed by oxygen itself but rather by a series of high energy derivatives of oxygen includes superoxide (O_2^-), peroxide (O_2), the hydroxyl radical (OH·) and singlet oxygen (18).

Thalassemic erythrocytes are particularly sensitive to oxidant stress or activated oxygen since excess hemoglobin subunits are being autoxidized to precipitated hemichromes (19). The unstable hemoglobins and isolated hemoglobin chains in thalassemias are also associated with increased rates of methemoglobin formation (18). Furthermore, an increased lipid membrane peroxidation has been demonstrated in thalassemic erythrocytes (20). On the other hand, the low amount of hemoglobin is not adequate to act as a 'sump' for the dangerous free oxygen radicals (20). Therefore, the thalassemic erythrocytes are susceptible to autoxidation and hemolysis leading to a shortened erythrocyte survival.

Defenses Against Oxidative Threats

Several enzymes in the erythrocyte are involved in the protection of the major cell components from oxidative threats such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px). Superoxide dismutase converts two molecules of superoxide into oxygen and

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hydrogen peroxide (21). Catalase causes direct breakdown of hydrogen peroxide to oxygen and water, but probably the more important mechanism is the reduction of hydrogen peroxide by reduced glutathione (GSH), catalysed by GSH-Px. The resulting oxidized glutathione (GSSH) is reduced in turn by NADPH from the pentose phosphate pathway. The overall result is a linked metabolic pathway as shown in Figure 4, in which increased turnover of methemoglobin will result in both increased activity of the glycolytic pathway and of the pentose phosphate shunt.

GSH-Px was found to have increased activity in Hb H disease and in mild forms of β-thalassemia major (22, 23) as well as catalase and SOD.

The final defense against oxidation is the presence of antioxidants in the cell membrane, of which the most important is probably vitamin E (24). This has been shown in the erythrocyte to provide protection against superoxide as well as hydrogen peroxide (25).

2.2.2.2 Iron Overload and Superoxide Formation

The excessive accumulation of iron in thalassemia is not just the effect of the increased erythropoiesis on the increase in iron absorption but also the result of packed red cell transfusion (26). The accumulation of excessive globin chains (α or β chains) and iron in thalassemic erythrocytes leads to much more formation of superoxides than that found in the normal erythrocytes.

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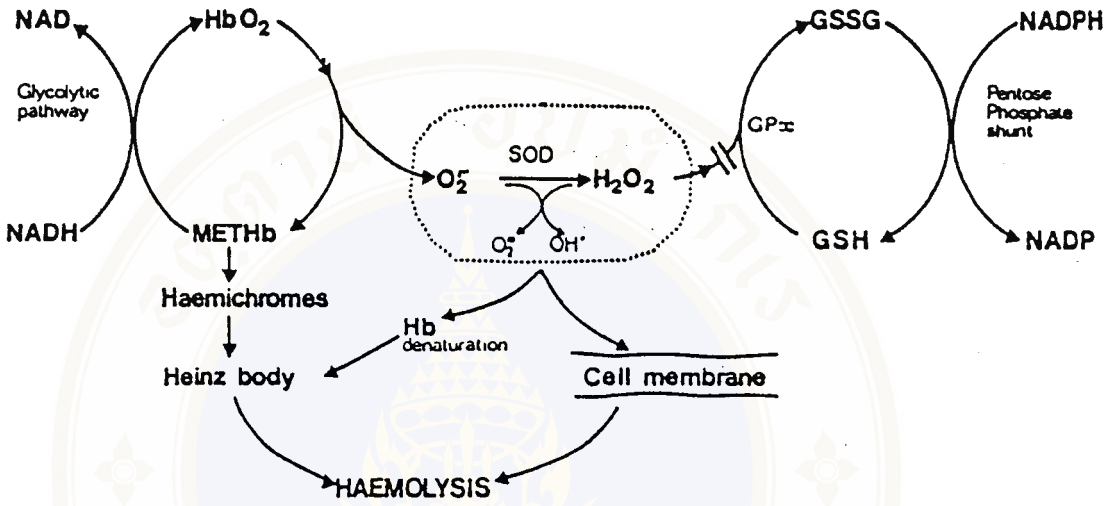

Figure 4 The production and reduction of activated oxygen (stippled area) is linked to the glycolytic and pentose phosphate pathways. The processes on the left, as well as giving rise to activated oxygen, will also give haemichrome and Heinz body formation. The accumulation of activated oxygen can potentially cause hemolysis in two ways. It can directly produce cell membrane oxidation but will also give hemoglobin denaturation with Heinz body formation(18).

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The possibility that the autoxidation of oxyhemoglobin to methemoglobin involves the displacement of a superoxide radical (18,27). The purposed mechanism is that in oxyhemoglobin there is a polarization of an electron from the heme iron to the bound oxygen. Normally this shared eletron is returned to the iron when oxygen is released from hemoglobin, and the iron retains in ferrous (Fe^{++}) state. This return of the electron to the iron is dependent on the absence of any displacing anions in the heme pocket. Although the heme pocket is hydrophobic, the entry of water or other small anions will result in the displacement of oxygen with an extra electron, i.e. superoxide. In the process the heme iron loses an electron, resulting in the formation of ferric (Fe^{+++}) methemoglobin, as shown in Figure 5 (18).

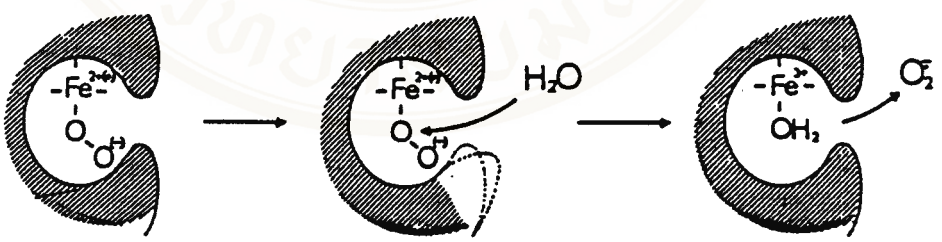

Figure 5 The heme pocket of oxyhemoglobin is represented on the left. Small fluctuations of the heme pocket may allow access of water to the heme (centre). This will result in the formation of methemoglobin and release of superoxide (right).

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Normally about 3% of the total body hemoglobin is converted to methemoglobin each day. In thalassemic erythrocyte, the unstable hemoglobins and isolated hemoglobin chains are associated with increased rates of methemoglobin formation and then increased releases of superoxide.

2.2.3 Treatment of Thalassemia

Thalassemia is a severe chronic hemolytic anemia due to an inherited defect of globin chain synthesis. The clinical symptoms of the untreated case are extreme anemia, hepatosplenomegaly, thalassemic face, bone age retardation, severe osteoporosis with spontaneous fractures, growth retardation, malnutrition, and increased susceptibility to infections.

The treatment of thalassemia includes genetic counselling and prenatal diagnosis; packed red cell transfusion; folate supplementation; avoiding and being alert to infection, particularly in splenectomized cases; consuming nutritious diets; splenectomy in the cases with hypersplenism, massive splenomegaly, severe Hb H disease; multiple transfusions; iron-chelating agents; bone marrow transplantation and others.

2.2.3.1 Blood Transfusion

Blood transfusion is the main basis treatment of symptomatic thalassemia for maintaining Hb level between 9 and 14 g/dl (10). A transfusion is commonly given either when the patient becomes symptomatic, or when a low Hb

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level is reached, usually 4-6 g/dl. Two types of transfusion programs have been used, 1) moderate transfusions to maintain Hb levels at 7-9 g/dl, and 2) hypertransfusion programs to maintain the Hb level above 10 g/dl in an attempt to decrease the effects of anemia and prevent development of orthodontic problems and other bone abnormalities. Both approaches have resulted in more normal growth and development, fewer pathologic fractures of bone, and increased psychologic well-being (28).

2.2.3.2 Splenectomy

Enlargement of the spleen, result of extramedullary hematopoiesis and the increased destruction of abnormal erythrocytes, is a general finding in patients with thalassemia major (29). Splenectomy, is usually reserved for those patients who develop an increasing transfusion requirement due to antibody-mediated hemolysis as a result of multiple transfusion (30). Moreover, splenectomy may be performed as well for hypersplenism. After splenectomy those patients resume good condition and show a reduction in transfusion requirement (31). However, in the absence of the spleen, iron may distribute preferentially to organs such as the liver and the heart, in which iron-induced dysfunction may cause substantial morbidity and death (32). Thus, these cases should receive regular chelation therapy with desferrioxamine. Furthermore, the major long-term complication after splenectomy is bacterial infection.

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2.2.3.3 Iron Chelating Agents

Because thalassemic patients with severe anemia require lifelong transfusional support, nonchelated patients with severe β-thalassemia die from iron overload between the age of 15 and 25 years (33). The administration of chelating agents is attempted to deal with the problem of iron overload from regular blood transfusion. The roles of iron chelating agents in the treatment of transfusional iron overload have recently been reviewed (34). Desferrioxamine (Desferal(R)) is the only useful iron chelating agent for the prevention or treatment of iron overload in thalassemia. This drug is effective in inducing iron excretion and is relatively nontoxic. However, the prevention of cardiac damage by desferrioxamine has not been demonstrated (35). At the present time, despite the expense and cumbersome nature of administration, the regular use of desferrioxamine is the only hope of forestalling the consequences of iron overload in regularly transfused thalassemic patients.

2.3 Selenium

2.3.1 Chemistry

Selenium appears in a group VI element of the periodic table. Its atomic number is 34 and atomic weight is 78.96 . Although sulfur and selenium show similar chemical properties, there are distinctions between them that have important biological and metabolic consequences (36). Selenium exists in the following oxidation states:

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-2, 0, +2, +4, and +6: all except the +2 state are generally found in nature (37).

Several inorganic and organic forms of selenium in human nutrition and metabolism are listed in Table 1 (38). Furthermore, there are naturally occurring stable isotopes of the element; ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se, and ^{82}Se. A radioisotope of selenium, ^{75}Se, a gamma emitter with a half life of 122 days, has been used extensively for studying selenium metabolism in bacteria and animals.

2.3.2 Physiological Roles of Selenium

The physiological role of selenium was first appreciated following the demonstration that the element is an essential component of a number of enzymes (39-41). In mammalian nutrition, a selenoenzyme of particular interest is glutathione peroxidase (GSH-Px: EC 1.11.1.9) (4, 42) but other selenium-dependent enzymes have been identified in bacterial systems, including glycine reductase, formate dehydrogenase, nicotinic acid hydroxylase, xanthine dehydrogenase and thiolase (39-41).

Recently, the structure, synthesis and function of GSH-Px has been reviewed (43, 44). This enzyme (Molecular Weight about 80,000) consists of four equal subunits each containing one atom of selenium which is essential for enzymatic activity (45-47). The selenium in GSH-Px is specific in the form of selenocysteine (44, 46). It is widely distributed in mammalian tissues and liver is one of the richest sources. In normal human and rat liver,

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Table 1 A summary of some characteristics of selenium (38)

A. Selenium (Se): group VI element, at. no. = 34

Resembles S in physical and chemical properties.

B. Inorganic forms: H_2Se, H_2SeO_3, H_2SeO_4 also inorganic selenides (e.g., CdSe)

^{75}Se: γ-emitter; t 1/2 = 122 days.

C. Organic forms:

1) Selenoamino acids:

Nutritional significant: Se-methionine
Se-cystine
Se-cysteine

Others: e.g., Se-cystathionine
Se-methylselenocysteine

2) Metabolic intermediates:

Selenodiglutathione (GSSeSG)

Selenopersulfides (e.g., GSSeH)

Dimethylselenide

Trimethylselenonium

3) Selenoproteins:

Glutathione peroxidase; formate reductase; glycine reductase

4) Others: e.g., 6-selenopurine, Se-prolyl-tRNA

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this selenoenzyme is about 0.25% of soluble protein of the organ (39).

GSH-Px appears to function in animal cells as a protective enzyme, localized in the cytosol and mitochondrial matrix space, that guards against peroxidative damage of the tissues (8, 43). The enzyme serves to catalyze the conversion of lipid peroxides (and H_2O_2) to hydroxy acids and water according to the following general reaction:

$$ROOH + 2GSH \xrightarrow{GSH\text{-}Px} ROH + GSSG + H_2O$$

This action against lipid peroxides is particularly important in protecting cellular and subcellular membranes from oxidative damage.

GSH-Px may be an adaptive enzyme increasing in response to oxidant stress (43), aging (48, 49), in some patients with hemolytic diseases such as α- and β-thalassemia (22, 23, 50) and glucose-6-phosphate dehydrogenase (G-6-PD) deficiency (51).

Because of the antioxidant nature of vitamin E and the need of glutathione (GSH) in the above enzyme reaction, biochemical schemes to explain the nutritional relationships between vitamin E, selenium and sulfur-containing amino acids have been proposed (8, 52), as shown in Figure 6.

The role of vitamin E is to prevent lipid peroxide (ROOH) formation which results in lipid peroxidation and cell damage. Selenium, as a component of GSH-Px, acts to remove H_2O_2 and so diminishes free radical formation and

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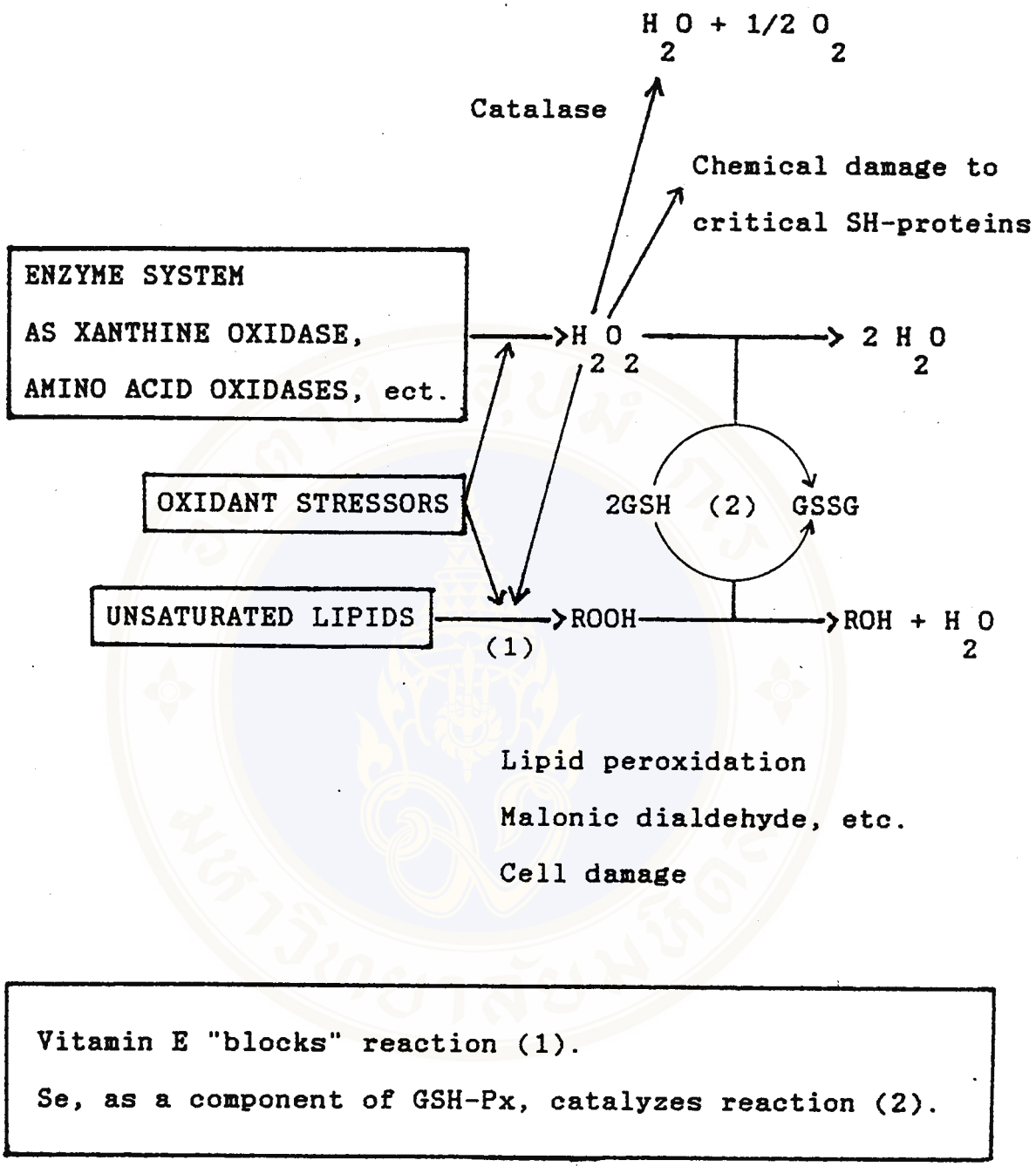

Vitamin E "blocks" reaction (1).

Se, as a component of GSH-Px, catalyzes reaction (2).

Figure 6 Scheme showing the postulated mechanisms of interaction between Se and vitamin E in preventing oxidant damage (8).

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also remove lipid peroxides, as mentioned above (8, 53). Sulfur amino acids serve to maintain GSH levels to support the enzyme reaction (53).

The relationship between selenium and GSH-Px in preventing lipid peroxidation is complicated by the discovery of non-selenium dependent GSH-Px activity in many tissues (54-57), now known to be due to one or more glutathione-S-transferases (58) which can use organic peroxides, but not H_2O_2 as substrates. This selenium independent activity may be in part account for the variation in response of different tissues and species to selenium and vitamin E deficiencies.

A high correlation has been observed between tissue selenium concentration and GSH-Px activity (59-61). It was also found that there was a low selenium and GSH-Px activity in plasma cord blood and red cells than that in either pregnant or non-pregnant female adults. It was suggested that low enzyme activity in the fetus might represent a phase of development adapted to the low intrauterine oxygen environment (60).

2.3.3 Human Selenium Metabolism

Selenium metabolism is highly dependent on the chemical form, amount of the element ingested and on the presence or absence of numerous interacting dietary factors (62).

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2.3.3.1 Absorption

Numerous balance studies conducted in humans have shown that the apparent absorption of dietary selenium ranges between 55 and 70 % (63-65). The ingested selenium is absorbed in the duodenal region of small intestine.

The forms of selenium presented to the intestinal mucosa are not known, but presumably there are organic forms such as selenomethionine and other sulfur analogues from digestion of food, and also inorganic forms such as selenite, selenate and possibly selenide (66). The studies carried out in young New Zealand women (67, 68) using the radio isotope ^{75}Se demonstrated that selenomethionine was better absorbed than selenite-selenium (95 - 97 % versus 44 - 70 %), and this has been confirmed in later studies with human subjects (69, 70).

Generally, it appears that selenomethionine and selenium from foods have a higher value for absorption in humans than selenite, with the exception of fish meal where selenium absorption is affected by the presence of other metals. The quantity of selenium administered may also influence the degree of selenium absorption (71). A sudden decrement in selenium intake reduced apparent absorption, presumably because of a continued relatively high rate of endogenous fecal loss (64).

Humans appear to have little or no homeostatic control over the absorption of selenium compounds from the gastrointestinal tract, as shown by a study where no difference was found in selenium absorption between

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periods of selenium depletion and repletion (64).

Greger and Marcus (72) showed that selenium was better absorbed (72%) from a high protein than from a low protein diet (44%), the protein intakes (150 g/day and 50 g/day) being supplied mainly as bread. Their findings are difficult to interpret due to the different forms of selenium ingested in the high and low protein groups.

2.3.3.2 Transport and Distribution

Several reports have suggested the existence of possible selenium transport proteins in blood plasma of animals (73-75). However, no selenium transport protein has been identified in humans.

Information is rare about the form of selenium in erythrocytes, platelets, leucocytes and plasma. Plasma selenium is mainly protein bound and probably associated with α_2 and β globulins and also with lipoproteins (53, 76, 77). Selenium concentration of plasma usually less than that of the erythrocytes and it rises and falls more readily in response to changes in selenium intake than erythrocyte selenium (78). The close association between blood selenium and dietary intake has been reviewed elsewhere (79).

Tissues distribution of selenium were found (80) in autopsy samples from North Americans: kidneys > liver > spleen > pancreas > testes > heart > muscle > lungs > brain. The total body selenium content of North Americans was calculated to be 14.6 mg (range 13.0 - 20.3 mg),

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whereas that of New Zealanders was estimated to be only 3.0 - 6.1 mg (80, 65). This geographical difference is thought to reflect differences in the dietary selenium intake between the two countries.

Casey et al., (81) compared selenium contents of various adult tissues. The New Zealand values were similar to those reported in other low selenium areas, and were lower than the values obtained in `selenium-adequate´ areas; except for the kidney cortex which contained high levels, but not as high as those in U.S.A. (82).

2.3.3.3 Excretion

Urine is the main route of excretion of selenium followed by feces with trivial respiratory and dermal losses (68, 64), except where the intake is toxic (77). Daily urinary excretion of selenium is closely associated with plasma selenium (70, 83), dietary intake (79), and with drinking water where the selenium concentration is substantial (84). The importance of the kidneys in the homeostasis of selenium in human was emphasized by work from New Zealand which showed that persons of low selenium status had low renal plasma clearances of selenium (85).

Human balance studies indicated that fecal excretion accounts for a relatively constant fraction of the total excretory output over a wide range of dietary intakes (86, 64). For example, Chinese men residing in a Keshan disease area and consuming 8.8 mcg/day in their diet excreted 3.4 mcg/day or 48 % of their total

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excretory output in their feces (86), whereas young North American males adjusted to a dietary selenium intake of 226 mcg/day excreted 86 mcg/day or 43 % of their total excretory output in their feces (64).

Negligible losses of selenium via the pulmonary or dermal routes were observed when humans took microgram oral doses of selenite or selenomethionine (67, 68) and no pulmonary excretion was detected in a subject given 1 mg of selenium as sodium selenite (87). Human sweat from North Americans was found to contain an average of 1.3 mcg Se/liter (64). Thus, sweat losses would represent a minor pathway of selenium excretion in them but might constitute a more important excretory route in persons with low dietary intakes, if indeed the selenium concentration in their sweat were similar.

2.3.3.4 Retention

The selenium status of subjects plays an important role in determining the level of reserve selenium in the body. Most observations strongly suggest that selenium is stored in the body and under conditions of low selenium intake, the body tends to retain more effectively than when selenium intakes are generous (88,64).

Selenium homeostasis is maintained by urinary excretion of selenium metabolites (53). Under selenium excess condition, trimethylselenonium was rapidly excreted along with urinary selenium metabolites that was suggested for humans (53).

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The whole body turnover of selenium in humans can also be expressed by a three-term exponential curve (67). It was found that after administration of Se-methionine, the terminal half-life was longer than with selenite (234 days versus 103 days). Striking differences in the retention of selenium as influenced by the form administered have also been observed in later studies with human subjects. Selenium as selenomethionine was much more effective in raising blood selenium levels in New Zealanders of low selenium status than as the sodium selenite form (83). Supplementation with selenium-rich wheat or yeast raised plasma and red cell levels in Finnish men with low initial plasma level more efficiently than supplementation with sodium selenite (89).

2.3.3.5 Metabolic Transformation

The metabolism of selenium in animals and men has attracted increased attention since the discovery that selenium is an integral component of the enzyme GSH-Px. Subsequently, the form of selenium in GSH-Px was identified as selenocysteine (45, 46).

The possible metabolic pathway relating organic and inorganic forms of selenium in the human have been reviewed, as shown in figure 7 (38, 44, 90). The metabolic transformations of selenium are complex and depend on the chemical form supplied to the body. Briefly, selenite (SeO_3^{2-}) or selenoamino acids, such as selenomethionine, are metabolized and converted via enzymatic and

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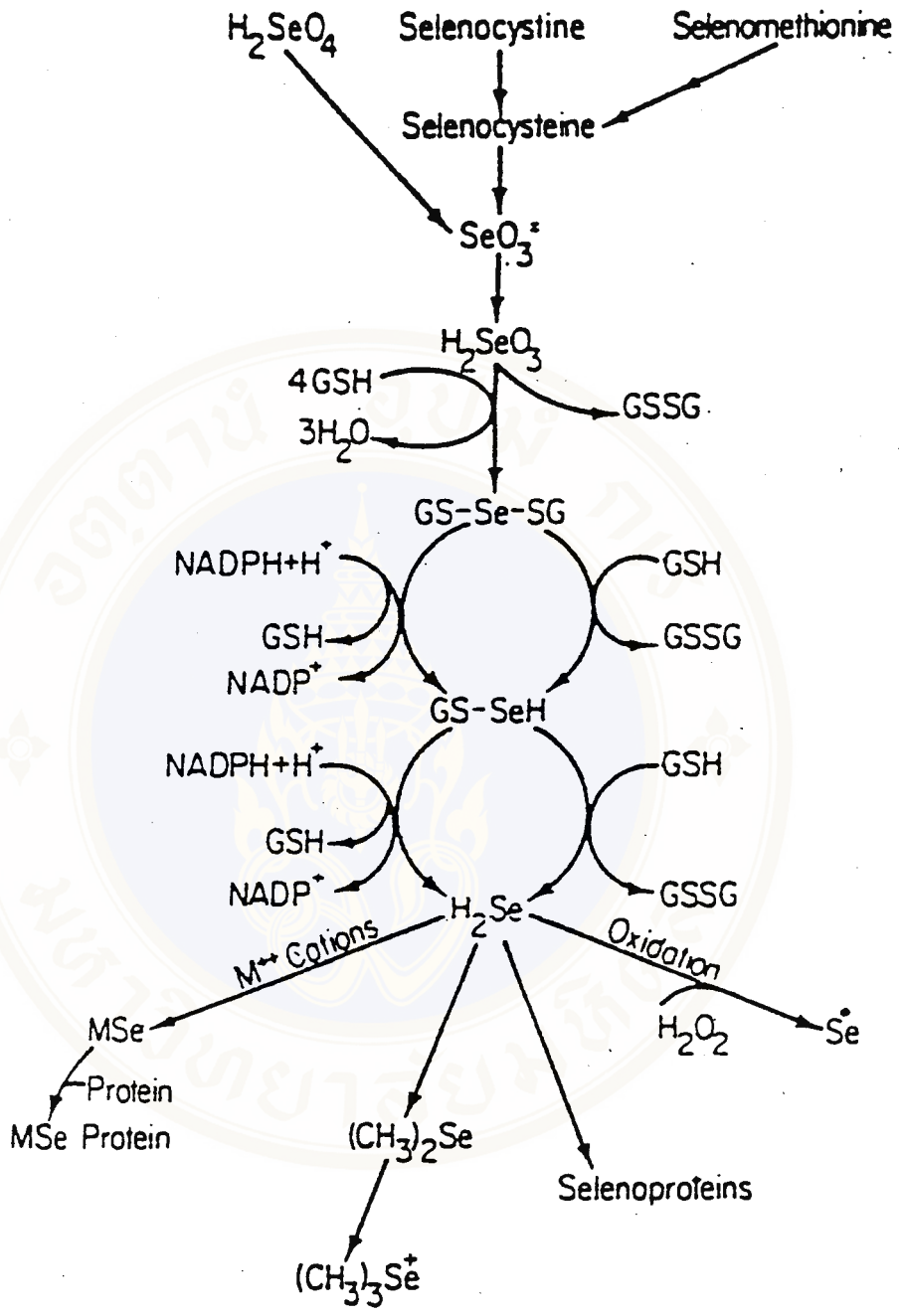

Figure 7 Metabolic interrelationships between organic and inorganic forms of selenium (38). Base on Magos and Webb (90).

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nonenzymatic steps to selenide (H_2Se) which then may be oxidized to elemental selenium, interact with plasma or cellular proteins. Selenium as selenide can also be metabolized to dimethylselenide ion, which is excreted via the lungs or trimethylselenonium ion, which is excreted via the kidneys. The latter is a normal excretory product of selenium under physiological conditions whereas dimethylselenide may be exhaled in appreciable quantities under conditions of high or toxic levels of selenium intakes (91, 92). However, the later work suggests that trimethylselenonium ion also becomes important under conditions of high exposure (93).

2.3.4 Selenium in Human Foods and Dietary Intake

The selenium content of human foods is influenced by a number of factors including the class of food itself, the geographical origin of the food, and the extent and type of processing and cooking.

The level of food selenium is related to the protein content of the food as well as to the geographical origin of the food. Thus, the selenium content of plant and animal products corresponds generally to the level of selenium in the soil (94, 95). Seafoods, organ meats and muscle meats are commonly good sources of selenium, containing more than 0.2 mcg Se/g (96). Grains and cereal products contain variable amounts of selenium depending on where they are grown. Fruit and vegetables are generally poor sources, containing about 0.01 mcg Se/g or less (96).

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Cooking appears to result in little significant loss of selenium in most foods, but dry heating of cereals may result in significant reductions in their selenium content (97). Furthermore, the selenium in vegetables may be lost into the cooking water (97). The element is also lost in the refining of sugar, as reported by Morris and Levander (96). The level of selenium in brown sugar and white sugar approximates 0.011 and 0.003 mcg Se/g, respectively.

Total daily dietary selenium intakes by humans vary greatly with the source of the foods consumed and to some extent with the choice of foods. Significantly lower selenium intakes could be expected in people eating little meat and obtaining their food supply from low-selenium areas.

Selenium concentration in human tissue tends to be related to natural dietary selenium concentration (98, 67). Average selenium intake of the population living in different parts of the world are investigated (98-108) as shown in Table 2.

2.3.5 Human Requirement for Selenium

Because there has been no description of a pathological condition in man that can be attributed to selenium deficiency alone, it is not possible to precisely define the dietary selenium requirement for human subjects.

Although requirements for selenium have not been

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Table 2 Daily selenium intake in different population

Country	Se Intake (mcg/day)	Reference
Canada	98.3-224.2	(99)
China, Keshan disease area	11	(100)
China, Moderate-Se area	116	(100)
Egypt	21	(101)
Finland	30	(102)
Japan	88.3	(103)
New Zealand	28	(79)
New Zealand	56.2	(104)
U.K., Britain	60	(105)
U.S.A.	132	(104)
U.S.A., Washington, DC	71	(106)
U.S.A., South Dakota	216.4	(107)
Venezuela	325.8	(108)

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established, the United States Food and Nutrition Board (109) has concluded that for healthy adults daily intakes of selenium approximating 50 to 200 mcg represents a safe and adequate intake range, with correspondingly lower intakes suggested for younger subjects (Table 3). This range was calculated from experiments with animals since little information is available from human studies.

Table 3 Estimated safe and adequate daily dietary intake of selenium (RDA,1980) (109)

Group		Se Intake (mcg/day)
Infants	(0 - 0.5 yr)	10 - 40
	(0.5 - 1 yr)	20 - 60
Children	(1 - 3 yr)	20 - 80
	(4 - 6 yr)	30 - 120
Adolescents	(7 - 10 yr)	50 - 200
	(11 + yr)	50 - 200
Adults		50 - 200

Any daily intake within this range is considered to be safe and adequate but it should be noted that the recommendation do not imply that the upper limit of the range is more desirable or beneficial than the lower limit (110).

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2.3.6 Selenium Functions and Related Diseases

The postulated functions of selenium in biological systems are shown in Table 4 (111). The only clearly established physiological role of selenium in mammalian systems is as a component of the enzyme GSH-Px. However, recent studies suggest that selenium may also function in electron-transfer mechanisms and may involve nonheme iron proteins (36).

Table 4 Postulated biological function of selenium (111)

1. Enzyme function
2. Oxidation - reduction
3. Protection of membranes - oxidant damage
4. Essential nutrient, growth
5. Reproduction
6. Regulatory role, initiation of protein synthesis
7. Immunological function, antibody formation
8. Detoxification reaction

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Selenium seems to play many roles in human body, and the reports of the diseases in man linked with a low selenium status have been summarized by Robinson (112) in Table 5.

Table 5 Diseases which have been linked with a low selenium status (112)

protein energy malnutrition	cancer
hemolytic anemia	muscular complaints
sickle cell anemia	arthritis
Keshan disease	muscular dystrophy
hypertension	multiple sclerosis
ischemic heart disease	aging
alcoholic cardiomyopathy	cataract
alcoholic cirrhosis	Werner's syndrome
pancreatitis	muscular degeneration
cystic fibrosis	diabetic retinopathy
infertility	neuronal ceroid lipofuscinosis

The hematological, selenium and vitamin E status of the preterm infant is of continuing interest (113, 114), as well as oxidative hemolysis in protein malnutrition (115).

Evidence linking selenium and cancer is controversial and is frequently reviewed (116-119). There are doubts about extrapolating findings from animal

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13966

studies to human cancers, but with the great progress being made at the cell and molecular levels, some clues should emerge about the importance of selenium for prevention and treatment of human cancers (120).

Nutritional deficiency of selenium has been firmly linked to cardiomyopathy of children and young women in China known as Keshan disease, although other factors may be involved (121).

Recently, biochemical evidence of selenium deficiency has been found in some total parenteral nutrition patients not supplemented with selenium (122, 123). A few patients have had muscle weakness or pain which responded to selenium administration. In addition, several cases of cardiomyopathy have been reported in patients receiving total parenteral nutrition (124). It is possible that selenium deficiency played a role in these cases.

2.3.7 Assessment of Human Selenium Status

The importance of selenium in human nutrition was established many years ago, but development of methods for accurately assessing their stores in the body and their status in humans has proved difficult. However, suitable methods of assessing selenium status in humans are needed for population surveys and to diagnose selenium deficiency and toxicity. Thus, various indices of selenium status has been recently reviewed (125), including analytical approaches (dietary intakes, tissue levels, and urinary

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levels); functional biochemical measurements (GSH-Px activity); interacting substances. Robinson and Thomson commented that no single technique for assessing selenium status is satisfactory and that different methods provide different information (66). Thus, many laboratories use several methods (Table 6).

Table 6 Techniques for assessment of selenium status (66)

Measurement of:	Sample	
1 Selenium	blood	hair
	plasma	toenails
	erythrocytes	fingernails
	platelets	autopsy tissues
2 Enzyme activity		
GSH-Px	blood	
GSH-S-transferase	erythrocytes	leucocytes
catalase	plasma	neutrophils
superoxide dismutase	platelets	lymphocytes
3 Interacting substances		
vitamin E, C, A	blood	hair
protein	plasma	toenails
Zn, Cu, Fe	erythrocytes	fingernails
Cd, Hg, Pb	platelets	autopsy tissues
	leucocytes	urine

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Blood selenium levels have been widely used as an index of selenium status since the development of fluorimeter assays. Selenium concentrations in human whole blood are variable, depending on the selenium content of foods consumed and the regional availability of the element (126, 127). Selenium contents in blood of normal subjects have been reported by several investigators in different geographic areas of the world as shown in Table 7. Studies in human beings suggest that erythrocyte selenium levels are more suitable than plasma or serum levels for monitoring long-term status because of the relatively slow turnover of the erythrocyte (an average life span = 120 days) (78, 136). However, plasma selenium levels can indicate the current selenium uptake (short-term status) (70).

The form of selenium seems to affect the level of selenium in erythrocyte and plasma of human subjects (83). The finding showed that the erythrocyte and plasma selenium tended to plateau when the subjects were given selenite but continued to rise in subjects given selenomethionine as long as dosing continued.

In addition, 24 hour urines are often used with occasionally toenails, fingernails and skin in surveys in which the subjects might have been exposed to high selenium intakes as from drinking water (137, 138). Dietary selenium intake has a marked influence on urinary selenium levels. Thus, this parameter has been used for assessment of current selenium intake (53, 136).

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Table 7 Blood Se levels in human populations worldwide

Country	Biological Matrix	Se Content (mcg/ml)	Reference
Canada: Ontario	Whole Blood	0.182	(128)
	Plasma	0.144	(128)
	Red Cell	0.236	(128)
China			
Low-Se area with Keshan disease	Whole Blood	0.012	(129)
Moderate-Se area (Beijing)	Whole Blood	0.095	(129)
High-Se area without toxicity	Whole Blood	0.440	(129)
High-Se area with acute selenosis	Whole Blood	3.200	(129)
Egypt	Whole Blood	0.068	(130)
Finland			
Lappeenranta	Whole Blood	0.056	(131)
Helsinki	Whole Blood	0.081	(131)
New Zealand			
Tapanui	Whole Blood	0.060	(79)
Auckland	Whole Blood	0.083	(79)
U.K.	Whole Blood	0.120	(132)
	Whole Blood	0.320	(133)
U.S.A.			
Ohio	Whole Blood	0.157	(134)
South Dakota	Whole Blood	0.265	(134)
Venezuela			
Caracas	Whole Blood	0.355	(135)
Seleniferous area (Villa Bruzual)	Whole Blood	0.813	(135)

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Hair selenium is not feasible in many Western countries where selenium-containing shampoos might be used, but their use seems not to affect blood, plasma or urine selenium (139, 140). Recently, toenails have been suggested as a useful indicator of human selenium status (141). However, the precise relationship between overall body selenium status and the selenium content of nails is yet to be established, and various factors that might influence nail growth (and thereby affect selenium content) need to be determined.

Erythrocyte GSH-Px activity may be the most sensitive and useful indicator of human selenium status. The selenium in GSH-Px is biologically active and does not represent inert selenium complexed with heavy metals or nonspecifically bound to proteins. Moreover, the enzyme activity appears to plateau in certain tissues at a level that might be considered a nutritional requirement (78). The assay is not complicated by selenium contamination but the enzyme loses activity in stored samples. However, Schrauzer and White (127) reported no response of GSH-Px activity to selenium supplementation and no correlation of this enzyme with blood selenium in human subjects. GSH-Px activity in platelet has been suggested by Levander et al. (142) for measurement of selenium nutrition, especially for the immediate availability and also for testing the tissue store.

Other factors must also be considered when GSH-Px activity is used to assess selenium status because the

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activity of the enzyme can be influenced by many additional variables such as age, sex, exposure to preoxidants, toxicants or heavy metals and deficiencies of iron or vitamin B-12 (43).

From this brief review, blood selenium level seems to be the best index for selenium deficiency and toxicity and urinary selenium reflects the current dietary intake of selenium.

2.4 Vitamin E (tocopherol)

2.4.1 Chemistry

Vitamin E designates the group of compounds (toco and tocotrienol derivatives) which exhibit in four major compounds: α-, β-, γ-, δ-tocopherol, each of which can exist in various stereoisomeric forms. The biological activity of α-tocopherol is more qualitative than the other forms (143).

α-Tocopherol is yellow oil, insoluble in water and soluble in organic solvents. High temperature and acids do not affect the stability of vitamin E, but it is readily oxidized in the presence of iron salts or in rancid fats. The acetate ester form of α-tocopherol is more stable.

2.4.2 Physiological Roles of Vitamin E

Vitamin E acts as a lipid antioxidant, diminishing the peroxidation of polyunsaturated lipids by scavenging free radicals generated during the univalent reduction of

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molecular oxygen (144) and during the normal activity of oxidative enzymes (145). Lipid peroxidation is the oxidative deterioration of polyunsaturated lipid (146, 147). The breakdown of these cellular constituents leads to cell damage (Figure 8) (149). Vitamin E can react with free radicals by converting them into a less reactive and nonharmful forms, and possibly react with other oxidizing intermediates (148, 149), thus, preventing the chain of events.

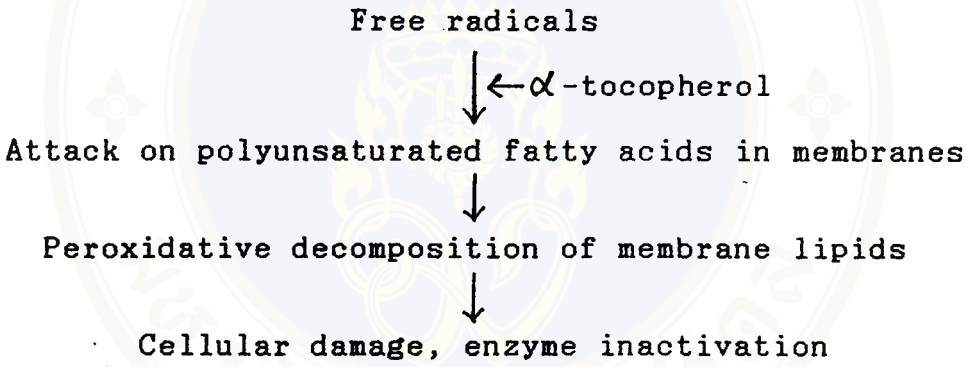

Figure 8 Vitamin E acts as a protector against oxidation (149)

A protector against oxidation, vitamin E interacts with wide variety of nutrients: vitamin A, selenium, the sulfur amino acids methionine and cysteine / cystine, polyunsaturated fatty acids and vitamin C (146, 147).

In addition, vitamin E function occurs in the membranous parts of cells. Lucy (150) has proposed that vitamin E is an important structural component of biological membranes, interacting with phospholipids.

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Here it interdigitates with phospholipids, cholesterol, and triglycerides, the three main structural elements of membranes. Other physiological functions probably include participation in nucleic acid metabolism, and it also appears that the tocopherols may be a component of the cytochrome reductase segment of the terminal respiratory chain in intermediary metabolism. In general, it appears that vitamin E plays an important role in ensuring the stability and integrity of cellular membranes: thus far in man, the only such demonstrated effect is on the erythrocyte.

2.4.3 Metabolism of Vitamin E

The absorption of vitamin E is dependent on the person's ability to digest and absorb fat. Bile is essential for its absorption, and the predominant route of absorption is via gastrointestinal lymphatics. For maximal absorption, in corporation of the vitamin into mixed micelles is necessary (151).

When taken up from the intestine into lymph, vitamin E is transported to the circulation via chylomicrons. It does not appear to have a specific lipoprotein carrier, and circulating vitamin E is primary associated with the low-density lipoproteins in man. It has been shown that the level of plasma vitamin E is highly correlated with total plasma lipids and also with total plasma cholesterol, as well as with low-density lipoprotein (152).

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Circulating vitamin E is accumulated slowly by tissues. Adipose tissue continually accumulates vitamin E (153). High vitamin levels have been found in adipose tissue, liver, heart and adrenal cortex (143). The remaining tissues have levels similar to those of the plasma.

2.4.4 Food Sources and Dietary Requirement of Vitamin E

Vitamin E is ubiquitous in its distribution and is found particularly in vegetable fats and oils, dairy products and meat, eggs, cereals, nuts, and leafy green and yellow vegetables. The richest sources is vegetable oils (soybean, corn, cottonseed, and safflower oil) and the products made from them.

According to the Food and Nutrition Board of the National Academy of Sciences, the recommended dietary allowances for vitamin E are set at 4-5 IU for infants, 7-12 IU for children and 12-15 IU for adults (109).

2.4.5 Deficiency of Vitamin E in Human

Deficiency disease in an otherwise normal human has not been reported but the general causes of a deficiency are shown in Figure 9 (143). Some evidence of deficiency has been demonstrated in human subjects, especially in premature and newborn infants, and infants with steatorrhea (fat absorption defects). Fat absorption defects in adults also cause biochemical evidence of low

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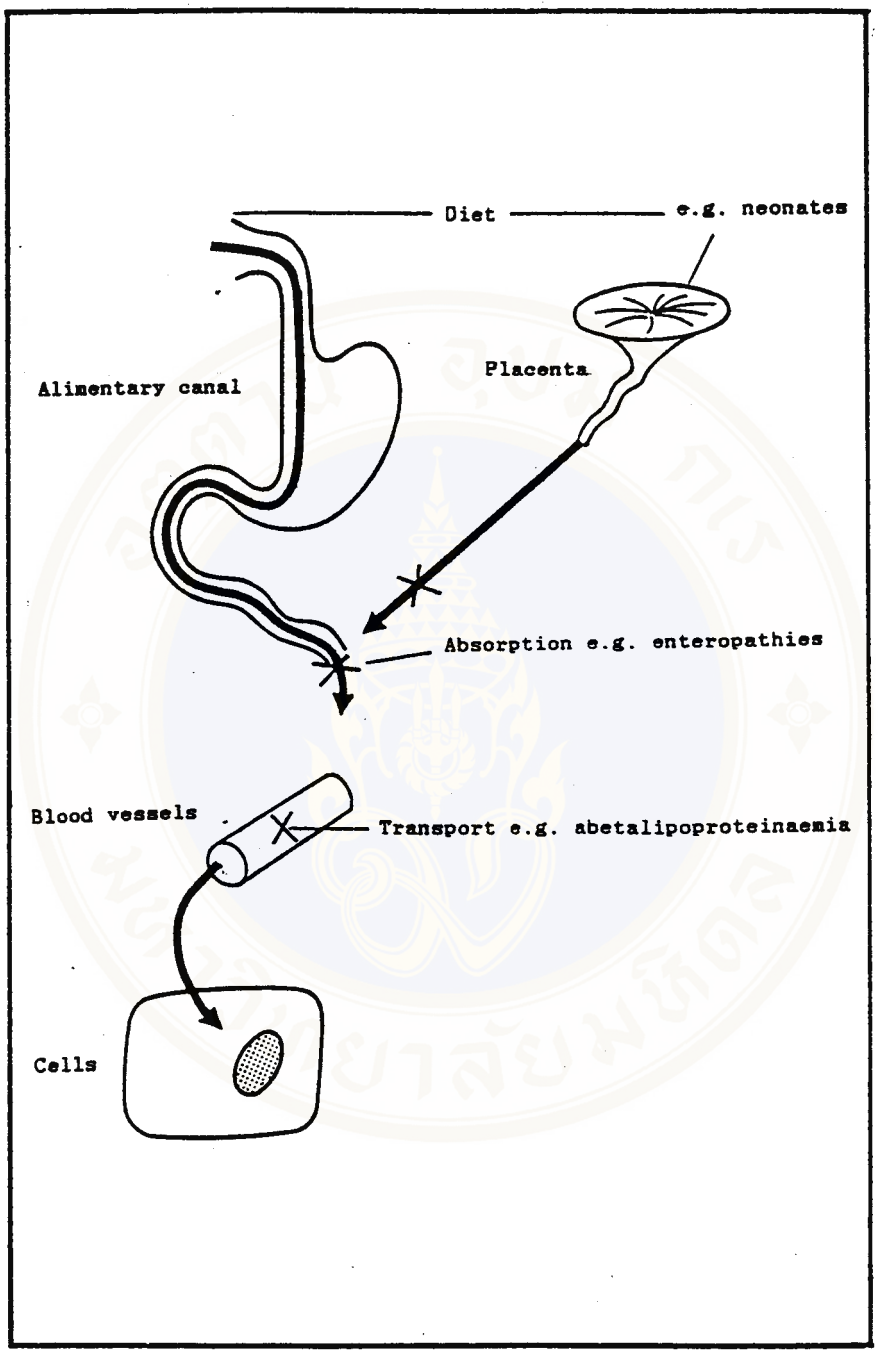

Figure 9 Mechanisms for hypovitaminosis E (143).

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tocopherol levels.

Vitamin E deficiency further occurs in congenital hemolytic anemias such as β-thalassemia (20), sickle cell anemia (50) and glucose - 6 - phosphate dehydrogenase deficiency (154). In sickle cell anemia, low plasma and erythrocyte levels of vitamin E were found, whereas erythrocyte GSH-Px activity was increased and inversely correlated with cellular vitamin E content (50).

Moreover, human vitamin E deficiency has been produced by artificial diets high in unsaturated fatty acids (143).

2.5 Selenium and Vitamin E Status in Thalassemias and Their Roles in Thalassemic Erythrocyte membranes

The thalassemic erythrocyte membranes are severely damaged by the excess precipitating hemoglobin chains (155). Furthermore, the lipid contents of erythrocyte membrane particularly phospholipids and cholesterol are increased in patients with Hb H disease (156) and β-thalassemia (20, 157). Thus, the thalassemic erythrocytes are more susceptible to autoxidation than normal erythrocytes (2). GSH-Px (a selenoenzyme) and vitamin E have been established as antioxidant against oxidative damage. Therefore, selenium and vitamin E status in thalassemic patients were concerned by several investigators.

Activity of erythrocyte GSH-Px was increased in thalassemias both Hb H disease and β-thalassemias (22,23),

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whereas low plasma vitamin E concentration was found in β-thalassemias both heterozygotes (158, 159) and homozygotes (160). In view of the data indicating lipid membrane peroxidation in erythrocytes of β-thalassemia major patients, it has been postulated that the low plasma vitamin E levels may be due to a secondary consumption of the antioxidant consequent to the membrane oxidation rather than a primary cause in its metabolism and absorption (20).

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CHAPTER III

MATERIALS AND METHODS

3.1 Subjects

3.1.1 Thalassemic Patients

The study was carried out in 3 groups of thalassemic patients. The first group was Hb H disease consisting of 6 males and 4 females with the age ranging from 5 to 13 years. The second group was β-thalassemia/ Hb E patients consisting of 15 males and 15 females aged between 5 to 17 years. The third group was β-thalassemia major consisting of 2 males of 6 and 14 years of age. They were recruited from Hematology Clinic, Department of Pediatrics, Ramathibodi Hospital. They were informed about the objective and design of the study. In all cases the diagnosis of thalassemia was established by clinical, hematological, electrophoretic and genetic work up. The history of blood transfusion and splenectomy in each patient was recorded. All except one case (β-thalassemia major) did not receive iron chelating agent (desferal).

3.1.2 Non Thalassemic Children

A control group of non thalassemic children, 14 children with the same age range, were concomitantly studied. They exhibited normal nutritional status without hematological disease.

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3.2 Sample Collection

In this study only blood analysis was performed. All samples were collected between March and November 1988.

3.2.1 Preparation of Glasswares and Plastic Tubes

All glasswares and plastic tubes used were washed with detergent in tap water, and allowed to drain dry at room temperature. They were then immersed in 30 % nitric acid for 48 hours, rinsed with distilled water and then immersed in deionized water for 24 hours, and oven dried at 110 ^{o}C for glasswares and 40 ^{o}C for plastic tubes. To protect it from air contamination, the glasswares were covered with parafilm until used.

Deionized water was used for the preparation of all reagents in order to avoid the contamination of selenium.

3.2.2 Blood Collection

Twenty - two milliliters of fasting blood was collected by venous puncture, using steriled disposable polypropylene syringe. Sixteen milliliters of the blood was placed in acid-washed test tube containing EDTA (1 mg EDTA / ml blood) for the determinations of hemoglobin, hematocrit, hemolysis, erythrocyte GSH-Px activity, erythrocyte selenium and plasma vitamin E. Two milliliters of blood was placed in test tube containing heparin solution (20 units / ml blood) for the determination of erythrocyte deformability. Four

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milliliters of the blood was placed in test tube without anticoagulant and the serum was separated for other biochemical parameters. All samples were transported immediately in ice box to the laboratory. Hematocrit (Hct) were measured with a hematocrit centrifuge.

EDTA blood was carefully mixed by inversion of the tube capped with parafilm and was centrifuged at 1500 rpm for 10 minutes. The plasma was then transferred to another tube for the determination of plasma vitamin E. Erythrocytes were washed twice with 0.9 % saline solution, then analyzed immediately for hemolysis of erythrocytes by using hydrogen peroxide hemolysis test. The remaining of erythrocytes was diluted 1:1 with 0.9 % saline solution, and stored at -20 °C to 4 °C for subsequent determination of erythrocyte GSH-Px activity and erythrocyte selenium concentration.

3.3 Biochemical Assessments

3.3.1 Determination of Hemoglobin and Hematocrit

Venous blood was anticoagulated with EDTA, 1 mg / ml blood. Hemoglobin concentration is determined by the conventional cyanmethemoglobin method (161) and hematocrit is determined by the conventional centrifugation method (161).

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3.3.2 Determination of Hemolysis

3.3.2.1 Principle

The susceptibility of erythrocyte to hemolysis in the presence of hydrogen peroxide (H_2O_2) was determined using modified Gordon's method (162). The hemolysis test was performed with fresh, saline-washed erythrocytes incubated as 5% suspension in 2.4% H_2O_2. After 3 hours incubation at 37°C, the samples were centrifuged and the absorbance were read at 540 nm to determine the extent of hemoglobin released.

3.3.2.2 Reagents

- 2.4% H_2O_2 (Merck)
- 0.9% NaCl (Merck)
- Saline Phosphate Buffer (PBS), pH 7.4

  (0.15 M $NaH_2PO_4.2H_2O$, 0.15 M Na_2HPO_4, 0.9% NaCl, Merck)

3.3.2.3 Procedure

- Wash erythrocytes twice with 0.9% NaCl then centrifuge at 1,500 rpm for 10 minutes.
- Add 3.9 ml of 37°C PBS, pH 7.4 in 0.1 ml of saline-washed erythrocytes (2.5% suspension).
- Mix, centrifuge and remove the supernatant.
- Resuspend the erythrocytes in 2.0 ml of 0.9% NaCl (5% suspension).
- Add the following reagents into five test tubes.

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	Tube 1 (ml)	Tube 2 (ml)	Tube 3 (ml)	Tube 4 (100%) (ml)	Tube 5 (blank) (ml)
RBC, 5% suspension	0.25	0.25	0.25	0.25	0.25
2.4% H_2O_2	0.25	0.25	0.25	0.25	-
PBS, pH 7.4	-	-	-	-	0.25
	incubate at 37°C for 3 hr				
PBS, pH 7.4	4.50	4.50	4.50	-	4.50
Distilled Water	-	-	-	4.50	-

- Mix by inversion, then centrifuge at 1,500 rpm for 10 minutes.

- Read the absorbance of supernatant at 540 nm against the control supernate (Tube 5).

3.3.2.4 Calculation

$$\% \text{ hemolysis} = \frac{\text{OD unknown}}{\text{OD 100\% hemolysis}} \times 100$$

OD unknown = mean OD of tube 1,2,3

OD 100% hemolysis = OD of tube 4

3.3.3 Determination of Erythrocyte GSH-Px Activity

Enzyme GSH-PX activity was determined by the method of Beutler (163).

3.3.3.1 Principle

Glutathione peroxidase (GSH-Px) catalyzes the oxidation of GSH to GSSH by hydrogen peroxide :

$$2\ GSH + ROOH \xrightarrow{GSH\text{-}Px} GSSG + H_2O + ROH$$

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where ROOH is a peroxide. t-Butyl hydroperoxide is the most suitable substrate for assay of the enzyme. The rate of formation of GSSH is measured by means of the glutathione reductase (GR) reaction:

$$GSSH + NADPH + H \xrightarrow{GR} 2\ GSH + NADP$$

The oxidation of NADPH is followed at 340 nm. One unit of GSH-Px activity is defined as the amount of enzyme that catalyzed the transformation of one mole of NADPH per minute under the assay conditions.

3.3.3.2 Reagents

- Tris-HCl, 1 M, EDTA, 5 M, pH 8.0
- GSH, 0.1 M
  (Glutathione, reduced form (GSH), Sigma)
- GR, 10 U/ml
  (Glutathione Reductase (GR), Type IV, Sigma)
- NADPH, 2 mM
  (β-Nicotinamide dinucleotide phosphate, reduced form (NADPH), Type III, enzymatical reduced, Sigma)
- t-Butyl hydroperoxide, 7 mM
  (tert-Butyl hydroperoxide [$(CH_3)_3COOH$], Fluka)
- 2-Mercaptoethanol-EDTA
  (2-Hydroxyethylmercaptan, Ethylenediaminetetraaetic acid (EDTA), Disodium Salt Dihydrate, Sigma)

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3.3.3.3 Procedure

The following reagents were added to cuvettes.

	Blank(µl)	System(µl)
Tris-HCL,1M,EDTA,5mM,pH 8.0	100	100
GSH, 0.1 M	20	20
GR, 10 U/ml	100	100
NADPH, 2 mM	100	100
1:20 hemolysate	10	10
H_2O	670	660
Preincubate at 37 °C for 10 minutes		
t-Butyl hydroperoxide, 7 mM	-	10

The decrease of optical density (OD) of the system was measured against that of the blank at 340 nm.

An additional blank assay prepared by substituting 2-mercaptoethanol-EDTA stabilizing solution for hemolysate were carried out in order to correct for nonenzymatic oxidation of GSH and NADPH by t-Butyl hydroperoxide.

3.3.3.4 Calculation

To calculate the enzyme activity (E), in international units per gram of hemoglobin,

$$E = \frac{\Delta OD}{6.22} \times \frac{V_C}{V_H} \times \frac{100}{Hb} \times \text{dilution}$$

where V_C is the cuvette volume (=1.00 in the systems), V_H is the volume of hemolysate in the reaction system, OD is the change per minute in the optical density at 340 nm, 6.22 is the optical density of a 1 mM solution of NADPH

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(one mole of NADPH is oxidized for each mole of t-butyl hydroperoxide reduced), and Hb is the concentration of hemoglobin in grams per 100 ml in the hemolysate.

3.3.4 Determination of Erythrocyte Selenium

3.3.4.1 Principle

The AOAC selenium determination by fluorometric method (164) is modified. First, sample was wet digested with concentrated nitric acid and concentrated perchloric acid. Then, 6 N hydrochloric acid was used to convert all Se (VI) (which occur during digestion with nitric acid and perchloric acid) to Se (IV) form.

The pH of solution was adjusted to pH 1.5 by ammonium hydroxide solution using cresol red as an indicator. Sample was incubated at 50°C for 20 minutes with 2,3-diaminonaphthalene (DAN) solution. The complex Se-DAN is then extracted by cyclohexane, and the fluorescense is measured.

3.3.4.2 Reagents

All of reagents used were analytic grade

- Conc nitric acid (Merck)
- Conc perchloric acid (Merck)
- 5 N Sulfuric acid (Merck)
- 6 N Hydrochloric acid (Merck)
- 6 N Ammonium hydroxide (Merck)
- 0.02 M Disodium EDTA (Sigma)
- 0.02% Cresol red (Merck)

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- Cyclohexane (Merck)
- Selenium standard solution (1.000 g ± 0.002 g of SeO_2), Merck)
- 0.1% DAN solution (1 mg/ml)
  (2,3 Diaminonapthalene, Fluka)

3.3.4.3 Procedure

Erythrocyte samples (dilution 1:1 with 0.9 % saline) were thawed at room temperature and thoroughly mixed by inversion. A control sample of pooled erythrocytes was similarly prepared. Duplicate measurements were made for each sample.

Each sample (1 ml) was pipetted into digestion tube with two glass beads and 5 ml conc HNO_3 was added. The sample was digested in heating block and temperature was increased slowly over period of 30 minutes to 130°C. Dense brown fume was formed. When the fume became light honey color (about 1 to 1 1/2 hr or solution was reduced to about 0.5 ml), remove the tube from the heating block and let it cool to room temperature. Add 2 ml conc $HClO_4$, re-digest in heating block and temperature was increased to 150°C. White wispy fume appeared. Continue heating until solution became clear or colorless (about 1 hr). Cool to room temperature, add 2 ml of 6N HCl and reheat at 130°C about 1 hr. Cool to room temperature. Transfer digested sample into glass stopper erlenmyer flask and rinse tube with deionized water three times to make sure that all of the digested sample are transferred. add 5

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ml of 0.02 N disodium EDTA while mixing. Adjust the volume of the digested sample to approximate 50 ml with deionized water. Add 5 drops of 0.02 % cresol red, mix, solution will be pink. Adjust pH of solution to pH 1.5 with 6 N NH_4OH and 1N HCl by pH meter (cresol red is used to easily observed the color change from pink to orange).

The next step has to be conducted in darkness. Add 2.5 ml of 0.1 % DAN solution, mix, incubate in water bath at 50°C for 20 minutes. Cool to room temperature. Transfer sample to separating funnel, add 3 ml cyclohexane to the sample and shake by hand for 1 minute (release gas during extraction) to extract Se-DAN complex. Let stand for 5 minutes, discard the water part (lower part) and transfer cyclohexane phase to the test tube (make sure there is no water contamination in cyclohexane phase which can be seen easily at the bottom of test tube). Read fluorescence at excitation wave-length of 380 nm and emission wavelength of 520 nm by spectrofluorometer (Shimadzu Spectrofluorometer, Model RF 154). The accuracy of the method was tested using Bovine Serum Pool 7292 Reference Material (National Bureau of Standard Reference Material, RM # 8419). Day to day variability in the technique is assessed by monitoring the values obtains for control sample (pooled erythrocytes). The selenium concentration obtained for the reference standard was 15.6 mcg/L compared with the certified value of 16.2 $\pm$ 2 mcg/L.

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3.3.4.4 Calculation

The concentration of erythrocyte selenium is calculated by conversion from the standard selenium and expressed in mcg/ml erythrocyte.

3.3.5 Determination of Plasma Vitamin E

The plasma vitamin E was analyzed by Pediatrics Hematology Laboratory, Ramathibodi Hospital using spectrophotometric method (Appendix 1) (165).

3.3.6 Determination of Erythrocyte Deformability

The erythrocyte deformability was performed by Hematology Laboratory of Ramathibodi Hospital using the laser diffractometric method (174).

3.4 Statistical Analysis

Conventional methods were used for the calculation of mean and standard deviation. Student's t-test (2 tailed) and paired t-test were used for comparision of parameters between two groups. Moreover, relationships between parameters by correlation analysis and linear regression analysis were made in the study.

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CHAPTER IV

RESULTS

4.1 Clinical Profiles of Thalassemic Patients

All patients received no blood transfusion for at least 3 months prior to this study. Their clinical profiles are shown in Table 8, 9 and 10.

Table 8 shows clinical profiles of 10 patients with Hb H disease, consisting of 6 males and 4 females aged between 5 to 13 years (mean ± SD = 9.3 ± 2.7). They were non-splenectomized patients. All except one did not receive any blood transfusion prior to the study. The remaining one female received blood transfusion more than 3 months before this study.

Clinical profiles of 15 male and 15 female β-thalassemia/Hb E patients are shown in Table 9, with their ages ranging from 5 to 17 years (mean ± SD = 11.4 ± 3.6). Twelve of them were splenectomized patients, who received blood transfusion more than 3 months before participating in this study. The remaining eighteen cases were non-splenectomized cases; of these, nine received no blood transfusion, and nine underwent blood transfusion more than 3 months.

Table 10 presents clinical profiles of two male patients with β-thalassemia major aged 6 and 14 years respectively (mean±SD = 10±5.6). Both were splenectomized patients, and were given blood transfusion more than 3 months prior to the study.

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Table 8 Clinical profiles of 10 Hb H disease patients

Patient	Sex	Age (yr)	Splenectomy	Blood Transfusion	
				no	> 3 mo
Yt.C.	M	5.3	-	+	
Yw.C.	F	7	-	+	
R.K.	F	7	-		+
T.P.	M	7	-	+	
S.S.	F	9	-	+	
M.S.	M	9	-	+	
W.K.	M	11	-	+	
P.W.	M	11.6	-	+	
O.P.	F	13	-	+	
J.K.	M	13	-	+	

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Table 9 Clinical profiles of 30 β-thalassemia/Hb E patients

Patient	Sex	Age (yr)	Splenectomy	Blood Transfusion	
				no	> 3 mo
K.P.	F	5	-	+	
A.O.	M	5	-	+	
S.K.	F	6.5	-		+
N.S.	M	7	+		+
C.S.	F	7.5	-	+	
A.D.	M	8	-	+	
T.S.	M	8	-		+
D.K.	M	8.8	+		+
C.K.	F	9	-		+
P.K.	F	9.8	-	+	
J.K.	M	10	-	+	
W.M.	M	10	-	+	
Su.S.	M	10	-	+	
B.S.	F	10.2	+		+
S.P.	M	11	+		+
B.K.	F	11	-		+
A.R.	M	12	+		+
Sr.S.	F	12	-	+	
K.W.	M	13	+		+
O.R.	F	13	-		+
D.N.	F	13.5	+		+
M.A.	M	14	+		+
J.S.	F	14	-		+
P.B.	M	15	+		+
J.T.	F	16	+		+
S.A.	F	16	-		+
L.M.	F	16.4	-		+
A.K.	M	17	+		+
J.N.	F	17	+		+
C.P.	M	17	-		+

Table 10 Clinical profiles of 2 β-thalassemia major patients

Patient	Sex	Age (yr)	Splenectomy	Blood Transfusion	
				no	> 3 mo
N.Su.	M	6	+		+
K.P.	M	14	+		+

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Weight for age and height for age of our thalassemic patients were lower than those of normal controls, details as shown in Appendix 2, 3, 4 and 5.

4.2 Hematological Data of Normal Subjects and Thalassemic Patients

Mean ± SD of ages and hematological parameters (Hb, Hct) of normal subjects and thalassemic patients are shown in Table 11. Patients with Hb H disease and β-thalassemia/Hb E and normal controls are about the same ages. Hb and Hct concentrations of patients with Hb H disease and β-thalassemia/Hb E were significantly lower than those of normal subjects. β-Thalassemia/Hb E patients showed significantly lower Hb and Hct than the values of Hb H disease patients. Based on WHO's criteria, all of the thalassemic patients were anemic. Case History of all patients are described in Appendix 3, 4 and 5.

The percentage of hydrogen peroxide hemolysis in the erythrocytes of normal and thalassemic subjects are summarized in Appendix 2, 3, 4 and 5. No hemolysis was found in normal control erythrocytes. Patients with β-thalassemia/Hb E showed a wide range of hemolysis from 0% to 59.2%, whereas Hb H disease patients presented a narrower range from 0% to 1.7%. Approximately 70% of β-thalassemia/Hb E patients showed more than 2% of hemolysis. Of interest, the cases with higher hemolysis also showed plasma vitamin E lower than 0.5 mg/dl.

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Table 11 Mean ± SD of ages and hematological values (Hb, Hct) in normal subjects and thalassemic patients*

Parameter	Mean ± SD			
	Normal n=14	Hb H dis n=10	β-thal/HbE n=30	β-thal major** n=2
Age (yr)	10.6±3.4[a]	9.3±2.7[a]	11.4±3.6[a]	10.0±5.6
Hb (g/dl)	12.9±1.2[a]	9.2±0.8[b]	8.1±1.4[c]	10.6±0.3
Hct (%)	39.5±3.6[a]	31.7±1.8[b]	24.2±3.0[c]	27.8±2.5

* Means in a row not sharing a common superscript are significantly different ($P<0.025$).

** No statistical analysis due to small n

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Moreover, the number of deformed erythrocytes were lower in thalassemic patients than those of normal controls (Appendix 2, 3, 4, 5). The lowest degree of erythrocyte deformability was found in the splenectomized thalassemic patients both of β-thalassemia/Hb E and β-thalassemia major. Erythrocytes from Hb H disease patients showed slightly lower deformability from the normals.

4.3 Selenium and Vitamin E Status of Normal Subjects and Thalassemic Patients

Mean values of erythrocyte selenium concentration, erythrocyte GSH-Px activity, and plasma vitamin E level of normal controls and those of the 3 groups of thalassemia are presented in Table 12 (Figure 10, 11, 12). When comparisons were made between each group of thalassemic patients (except β-thalassemia major group due to small sample size) and normal subjects, the mean values of erythrocyte selenium of Hb H disease and β-thalassemia/Hb E patients were not significantly different from the normal levels. However, significantly higher erythrocyte GSH-Px activity and significantly lower plasma vitamin E concentrations were observed in the patients with Hb H disease and β-thalassemia/Hb E in comparison to the normal controls. Furthermore, β-thalassemia/Hb E patients showed lower erythrocyte GSH-Px activity ($P<0.05$) and plasma vitamin E concentrations ($P<0.05$) than those of Hb H disease patients.

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Table 12 Selenium and vitamin E status of normal subjects and thalassemic patients*

Parameter	Age (yr)	Mean ± SD			
		Normal	Hb H disease	β-thal/Hb E	β-thal major**
RBC Se (mcg/ml)	5-10	0.238±0.024(6)^{a}	0.232±0.023(6)^{a}	0.273±0.036(13)^{b}	0.191(1)
	>10	0.271±0.032(8)^{c}	0.243±0.030(4)^{ac}	0.257±0.042(17)^{bc}	0.303(1)
RBC GSH-Px (U/g Hb)	5-10	26.93±2.21 (6)^{a}	85.67±22.81(6)^{b}	57.93±10.74(13)^{c}	60.43(1)
	>10	32.77±3.01 (8)^{d}	75.68±10.63(4)^{be}	53.46± 9.68(17)^{cf}	49.99(1)
Plasma vit E (mg/dl)	5-17	0.76 ±0.18(14)^{a}	0.60 ±0.21(10)^{b}	0.34 ± 0.12(30)^{c}	0.26±0.06(2)

* Means in a column or a row not sharing a common superscript are significantly different ($P<0.05$)

** No statistical analysis due to small n

(n) Members of each group

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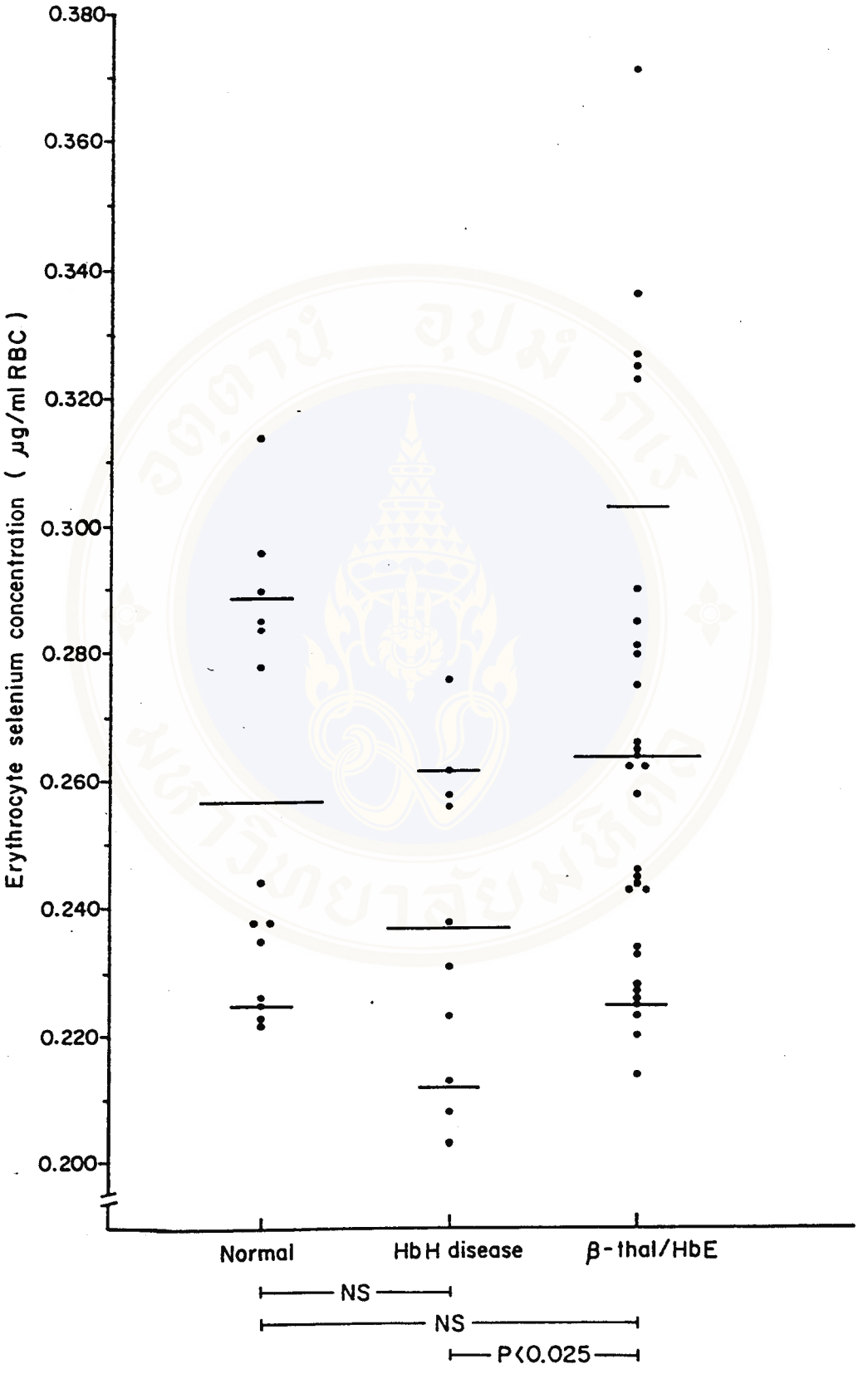

Figure 10 Erythrocyte selenium concentrations of normal subjects and patients with Hb H disease and β-thalassemia/Hb E

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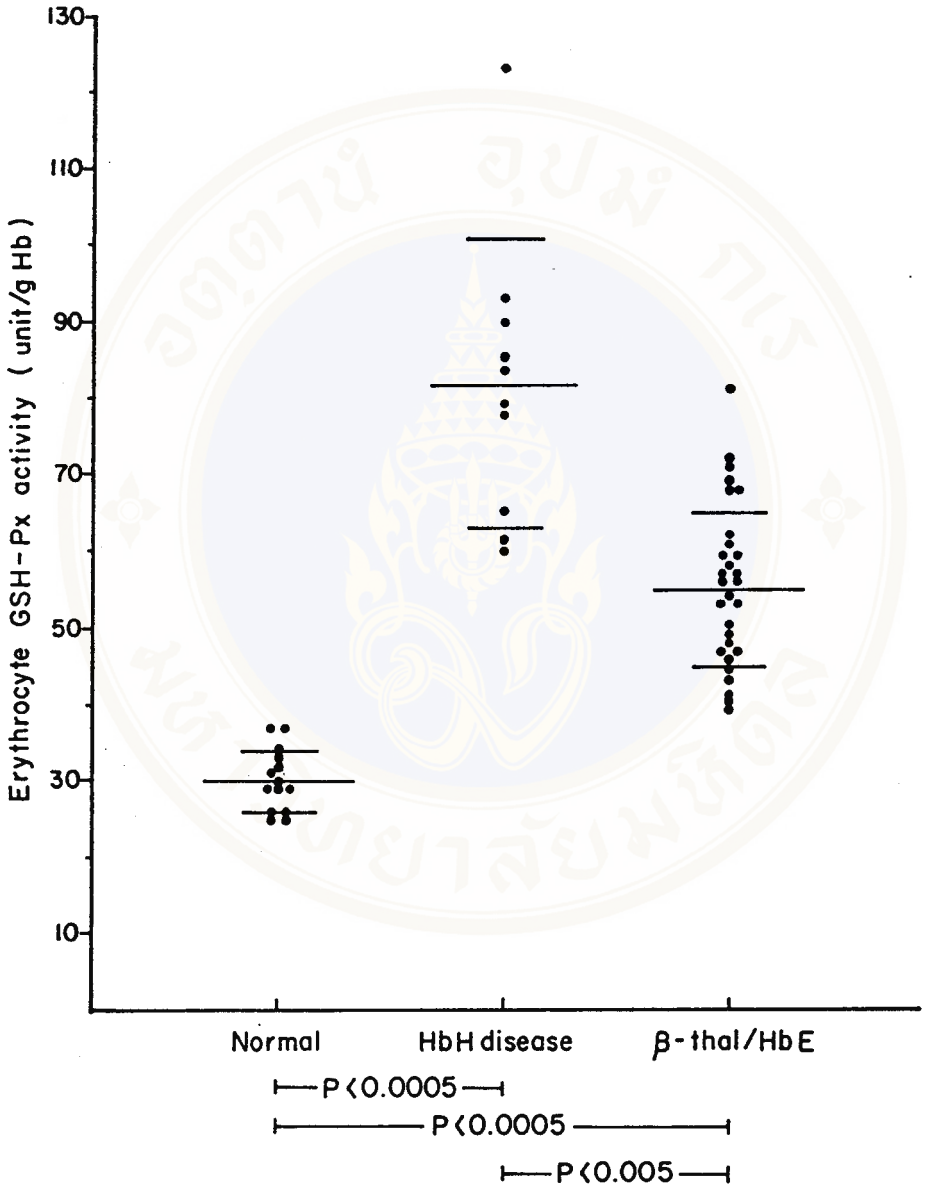

Figure 11 Erythrocyte GSH-Px activities of normal subjects and patients with Hb H disease and β-thalassemia/HbE

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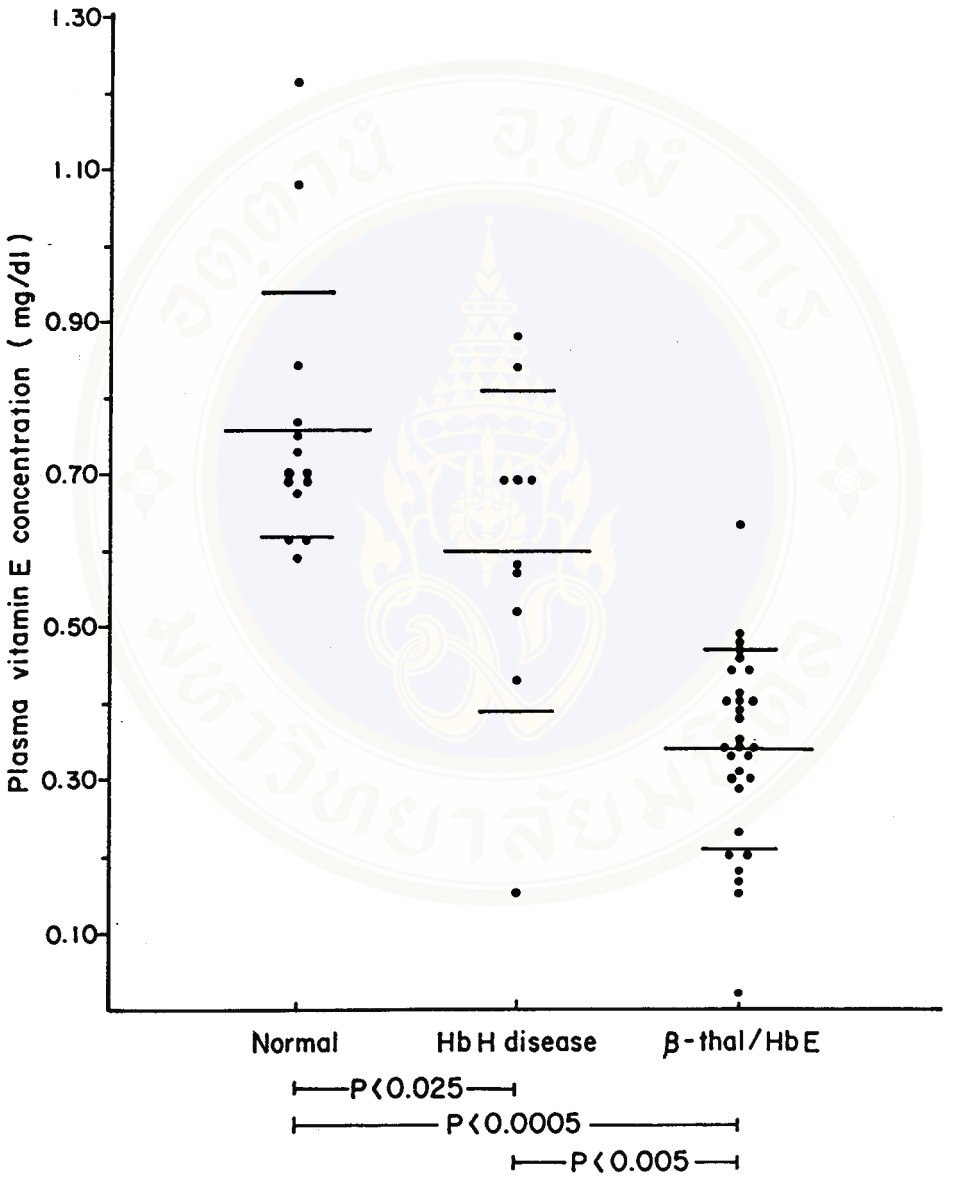

Figure 12 Plasma vitamin E concentrations of normal subjects and patients with Hb H disease and β-thalassemia/HbE

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4.4 Relationships Between Erythrocyte Selenium, Erythrocyte GSH-Px and Plasma Vitamin E of Normal Subjects and Thalassemic Patients

Figure 13 shows the relationship between erythrocyte selenium level and erythrocyte GSH-Px activity of normal subjects and thalassemic patients. A slight positive correlation was observed between erythrocyte selenium and GSH-Px of the β-thalassemia/Hb E patients ($r = 0.359$, $P<0.05$) as well as those of the Hb H disease cases ($r = 0.383$, NS). However, only β-thalassemia/Hb E data showed a significant correlation. In normal subjects, there was no correlation ($r = 0.181$) between erythrocyte selenium concentration and erythrocyte GSH-Px activity.

The relationship between plasma vitamin E and erythrocyte GSH-Px in normal controls and thalassemic patients are presented in Figure 14. A significant negative correlation ($r = -0.784$, $P<0.01$) was found for the values of the Hb H disease patients. The regression equation was $Y = 103.91 - 44.25X$; where X was plasma vitamin E concentration (mg/dl) and Y was erythrocyte GSH-Px activity (unit/g Hb). Normal subjects also showed a slight negative correlation ($r = -0.487$, $P<0.05$, $Y = 38.54 - 10.87X$), whereas no correlation ($r = -0.131$) was observed for the β-thalassemia/Hb E values.

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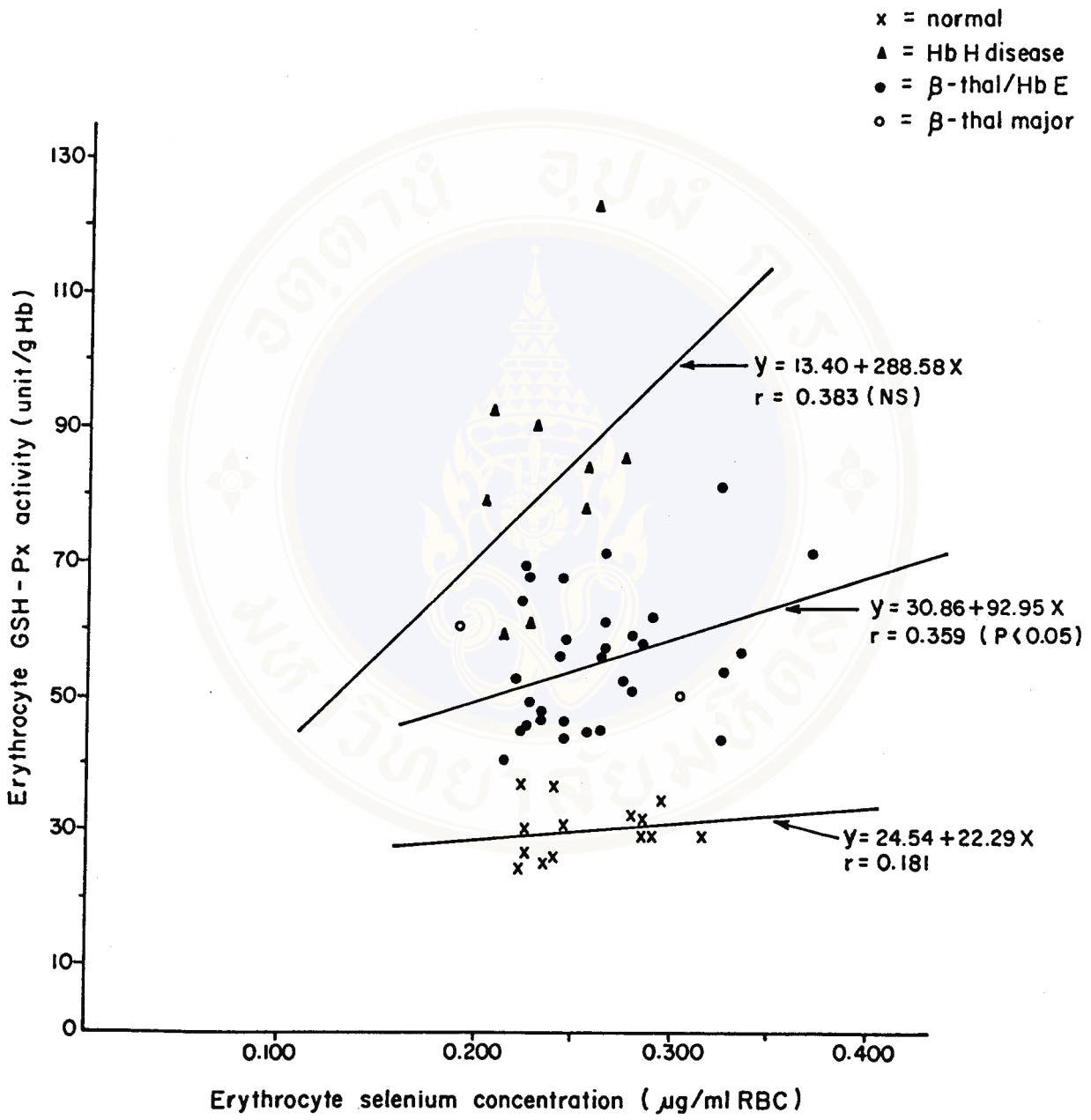

Figure 13 Relationship between erythrocyte selenium concentrations and erythrocyte GSH-Px activities of normal subjects and thalassemic patients.

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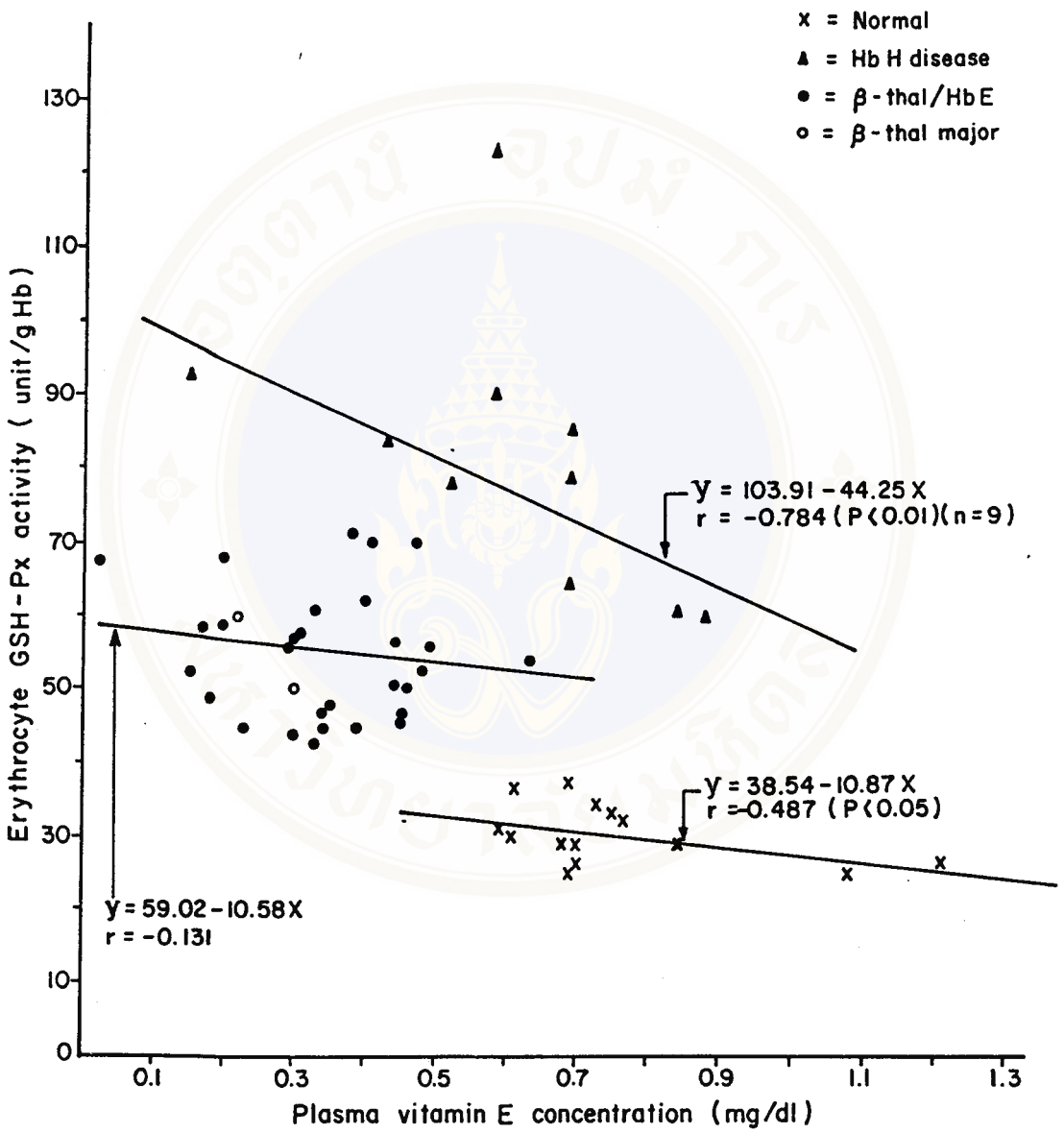

Figure 14 Relationship between plasma vitamin E concentrations and erythrocyte GSH-Px activities of normal subjects and thalassemic patients.

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4.5 The Effects of Vitamin E Supplementation on Plasma Vitamin E and Erythrocyte GSH-Px of Thalassemic Patients

Table 13 compares plasma vitamin E concentration and erythrocyte GSH-Px activity before and after 6-8 weeks of daily supplementation of 200 mg dl-alpha-tocopherol in five splenectomized thalassemic patients, whose plasma vitamin E were lower than 0.5 mg/dl along with severe hemolysis (ranging from 23-59%) by H_2O_2 hemolysis test. Individual data appears as Appendix 6. After supplementation, the mean plasma vitamin E significantly increased whereas the mean erythrocyte GSH-Px activity decreased remarkably from the baseline level (Figure 15, 16). No erythrocyte hemolysis due to hydrogen peroxide, improvement of skin lesion and better appetite were found in all patients after vitamin E supplementation. In addition, vitamin E supplementation exerts no effect on erythrocyte selenium concentration.

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Table 13 Mean ± SD of plasma vitamin E and erythrocyte GSH-Px activity in 5 splenectomized thalassemic patients before and after 6-8 weeks vitamin E supplementation (200 mg/day)*

Parameter	Mean ± SD	
	before	after
Plasma vitamin E (mg/dl)	0.19±0.10	1.76±0.37
RBC GSH-Px activity (U/g Hb)	55.84±9.06	45.46±2.54

* Means in a row are significantly different ($P<0.05$).

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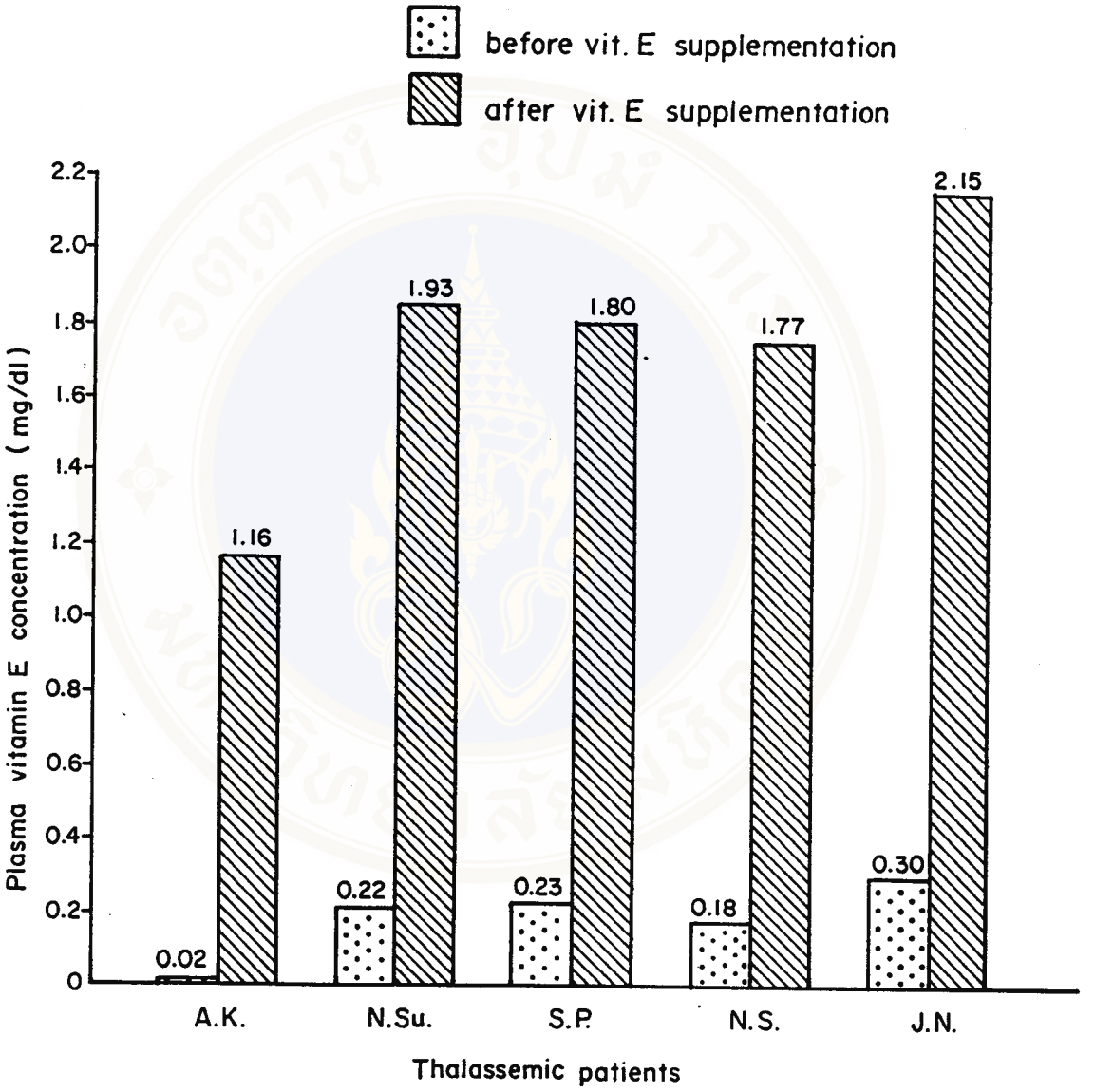

Figure 15 The effect of vitamin E supplementation on plasma vitamin E concentration in 5 thalassemic patients.

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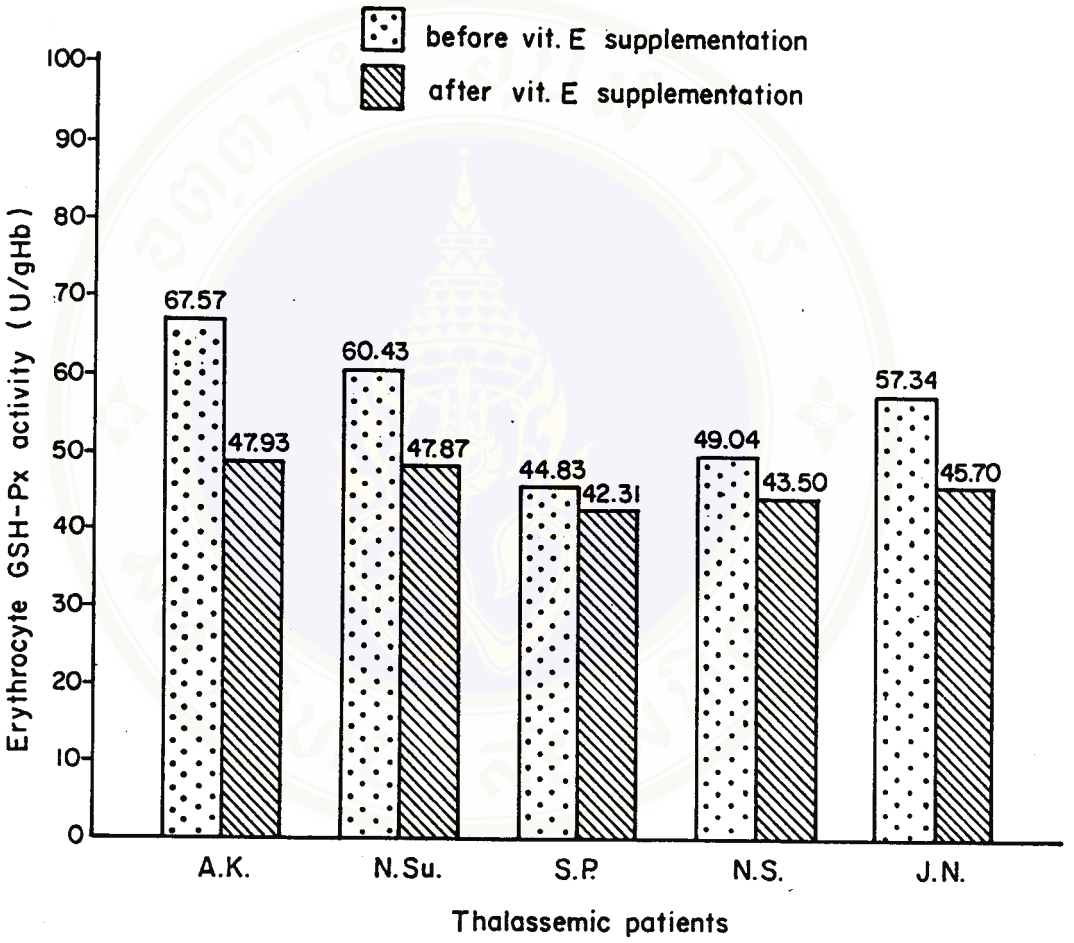

Figure 16 The effect of vitamin E supplementation on erythrocyte GSH-Px activity in 5 thalassemic patients.

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CHAPTER V

DISCUSSION

5.1 Selenium and Vitamin E Status

Data indicate that selenium status of the three-group thalassemic patients did not differ from normal controls since their erythrocyte selenium levels showed no significant differences from those of normal controls (Table 12). The erythrocyte selenium values are close to those reported previously for the Canadians (Table 7), with adequate dietary selenium intake and exhibiting normal selenium status (Table 2). At this point, it could be speculated that the thalassemic subjects in our study had adequate selenium intake and were in normal selenium status.

In addition to erythrocyte selenium content, the activity of erythrocyte selenoenzyme, GSH-Px, of thalassemic patients was determined in comparison to the normal value. In agreement with the results of previous reports (22, 23, 166), all thalassemic patients in our study, showed markedly increases in erythrocyte GSH-Px activity, approximately 1.5 - 3 times normal values (Table 12). Of interest, we found that erythrocyte GSH-Px activities of the Hb H disease patients was significantly higher than those of the β-thalassemia/Hb E patients.

The elevation of GSH-Px activity in thalassemic erythrocytes suggested that GSH-Px is needed in order to neutralize the augmented production of hydrogen peroxide

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(H_2O_2). The accumulation of H_2O_2 within thalassemic erythrocytes may result from the presence of free globin chains and unstable hemoglobin generating superoxide radicals which are decomposed by hydrogen peroxide dismutase (22, 167). Moreover, the increased susceptibility of erythrocyte membranes to lipid peroxidation in patients with β-thalassemia major have been demonstrated by Rachmilewitz et al. (20). Our results support the concept that GSH-Px may protect both erythrocyte membrane and hemoglobin from peroxidative damage. Thus, the increased GSH-Px activities in thalassemic erythrocytes allow us to speculate that these erythrocytes have been able to develop the defense mechanisms against oxidant threats.

Increased erythrocyte GSH-Px activities in our patients with Hb H disease confirm the findings in the previous reports (22, 166). Furthermore, we found that erythrocyte GSH-Px activities of the Hb H disease patients were significantly higher than those of the β-thalassemia/Hb E patients. It is likely that erythrocyte GSH-Px in Hb H disease patients may be more activated due to the increased amount of H_2O_2. Recently, Yenchitsomanus and Wasi (168) had measured erythrocyte superoxide dismutase (SOD) activities in patients with β-thalassemia/Hb E and Hb H disease. The SOD activities were significantly increased in both groups of patients with a strikingly higher activity noted in Hb H patients. This suggested that Hb H patients have increased remarkably, the

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production of H_2O_2 from the dismutation reaction and thus, demands greater erythrocyte GSH-Px activities.

In normal subjects, when erythrocyte GSH-Px activities were compared between two different aged groups (5.1-10.0 and 10.1-15.0 yr), the younger one showed significantly lower activity than those of the older aged group (Table 12). This finding agrees with the previous studies (43) which indicated the effect of age on erythrocyte GSH-Px activity in humans. In addition, the mean (±SD) erythrocyte GSH-Px activity of our normal subjects (30.27±3.96) is comparable to the value of normal Europeans (30.82±4.73) (163). Thus, this result suggests that our normal subjects have adequate selenium status.

Low plasma vitamin E levels were reported for patients with β-thalassemia both of heterozygote (158, 159) and homozygote (160) origins. Our results agree with their reports. Our thalassemic patients also exhibited significantly lower plasma vitamin E concentrations than the normal subjects in this study (Table 12). Previous studies (169, 170, 171) suggested that young children have lower blood vitamin E concentrations than adults. The expected range of plasma vitamin E values in children (1 - 12 yr) is 0.3 to 0.9 mg/dl (171), whereas in adults the expected range is 0.5 to 1.6 mg/dl (153). By using these criterias for the assessment of vitamin E status, all β-thalassemia major patients and approximately 60% of β-thalassemia/Hb E patients in this study are vitamin E deficient (Appendix 4, 5). In contrast, all except one of

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the patients with Hb H disease can not be classified as vitamin E deficient although they showed significantly lower mean plasma vitamin E level than their normal controls (Table 12, Appendix 3). This evidence supports our results of less hydrogen peroxide hemolysis in Hb H erythrocytes, whereas more hemolysis was observed among β-thalassemia/Hb E patients with depleted vitamin E status. Since our control subjects were selected with the same ethnic, social and economic backgrounds as the thalassemic patients, it is unlikely that the difference in vitamin E concentration is a reflection of the difference in dietary intake. The probable explanation is the elevated vitamin E utilization in the patients. Moreover, the increased susceptibility of thalassemic erythrocyte membrane to lipid peroxidation has been demonstrated in patients with β-thalassemia major (2, 20). So, it has been postulated that the cause of the deficiency is the increased vitamin E uptake into the pathological erythrocytes and other tissues in order to counteract the oxidative damage.

The elevated GSH-Px activity and low plasma vitamin E in these patients could be due to the increased lipid peroxidation in thalassemic erythrocyte membranes (2, 20), as a result of peroxide radicals released by an iron activated chain reaction (18, 27, 172). Our results seem to confirm the previous report that the lipid membrane of erythrocytes are protected against oxidant damage by vitamin E and GSH-Px (52).

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Nutritional status of most thalassemic patients were poorer than those of normal subjects (Appendix 2, 3, 4, 5), however, the selenium status of these patients were in the normal range. These data suggest that malnutrition status of these patients did not occur in parallel with that of selenium.

5.2 The Relationship Between Erythrocyte Selenium Level and Erythrocyte GSH-Px Activity

Data indicated that there was no correlation between erythrocyte selenium concentration and GSH-Px activity in normal subjects ($r = 0.181$) (Figure 13). The possible explanation is that each individual may have his optimal level of erythrocyte GSH-Px activity. This reason is supported by the studies of New Zealand subjects that GSH-Px activities in whole blood or erythrocytes varied directly with selenium concentrations whereas no relationship was obtained for oversea visitors with higher selenium status (78). On the other hand, there were slight correlations between erythrocyte selenium level and erythrocyte GSH-Px activity in thalassemic patients with Hb H disease ($r = 0.383$, NS) and β-thalassemia/Hb E ($r = 0.359$, $p < 0.05$), but only β-thalassemia/Hb E patients showed significant correlation. It is possible that these patients have higher requirement of GSH-Px due to the increase of lipid peroxidation in the erythrocyte membranes as well as the elevated H_2O_2 level within the erythrocytes. Thus, the erythrocytes may compensate by

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increasing tissue (RBC) selenium uptake for GSH-Px formation.

5.3 The Relationship Between Plasma Vitamin E Level and Erythrocyte GSH-Px Activity

The nutritional interrelationship of vitamin E and selenium has long been known in terms of their antioxidant roles. Vitamin E functions as membrane stabilizer by acting either as a component of membrane structure or via a free radical scavenging mechanism, while selenium display its role through the activity of GSH-Px (4, 8, 52). Thus, the relationship between plasma vitamin E concentration and erythrocyte GSH-Px activity in thalassemic patients was reported in this study (Figure 14).

A slight negative correlation ($r = -0.487$, $P<0.05$) and a moderate negative correlation ($r = -0.784$, $P<0.01$) between plasma vitamin E level and erythrocyte GSH-Px activity were observed among the normal subjects and Hb H patients, but no correlation ($r = -0.131$) was detected for the β-thalassemia/Hb E patients. The inverse correlation shown for the normal and Hb H disease groups supports the view that vitamin E can react with free radicals as well as preventing the peroxidation of polyunsaturated lipids (Figure 6). The decreased lipid peroxide (ROOH) formation may occur in the subjects with adequate vitamin E, thus resulting in diminishing GSH-Px activity needed to convert ROOH to the less damaging alcohol (ROH). On the contrary,

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with the condition of depleted vitamin E status, the activity of GSH-Px will be increased. However, there was no correlation between these parameters in β-thalassemia/Hb E patients. It is possible that since most β-thalassemia/Hb E showed markedly low vitamin E status, the GSH-Px might reach its maximum adaptive activity.

5.4 The Effects of Vitamin E Supplementation on Plasma Vitamin E and Erythrocyte GSH-Px

After 6-8 weeks of vitamin E supplementation (200 mg/day) in 5 splenectomized thalassemic patients, plasma vitamin E level and erythrocyte GSH-Px activity reponded while there was no effect on erythrocyte selenium concentration (Table 13, Appendix 6). The results indicated a significant increase of plasma vitamin E to normal level. At the same time, the erythrocyte GSH-Px activity was significantly decreased but did not reach normal level. The findings agree with the postulated machanism of vitamin E-selenium relationship in the prevention of peroxidative damage. However, GSH-Px protective system has the dual role of degrading H_2O_2 as well as the organic peroxides, which can not be duplicated by vitamin E. It is possible that oxidant stressors may cause the generation of increased quantities of H_2O_2 within erythrocytes, thus GSH-Px is still required as a major defense mechanism amidst the condition of vitamin E adequacy. Another possible explanation may be that erythrocyte membrane vitamin E content is still not fully

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repleted. Therefore, the elevation of erythrocyte GSH-Px activity from normal level still appear in these subjects.

Moreover, after vitamin E supplementation, the improvement of skin lesion, better appetite and negative red cell hemolysis (by H_2O_2 hemolysis test) were found in all 5 treated thalassemic patients. From the findings of this study suggest that the daily supplementation of 200 mg dl-alpha-tocopherol is beneficial for vitamin E deficient thalassemic patients.

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CHAPTER VI

SUMMARY

Selenium is an essential component of glutathione peroxidase (GSH-Px), one of the antioxidant enzymes that guards against peroxidative damage of erythrocytes as well as vitamin E. The objective of this study is to investigate selenium status, its relationship with vitamin E and the effect of vitamin E supplementation on erythrocyte GSH-Px in thalassemic patients. Forty-two thalassemic patients and 14 non thalassemic children participated in the study. The thalassemic patients consisted of 10 with Hb H disease, 30 with β-thalassemia/ Hb E and 2 with β-thalassemia major. They did not receive any blood transfusion within 3 months prior to this study. Selenium status was determined by erythrocyte selenium and GSH-Px activity, simultaneously vitamin E status was assessed by plasma vitamin E. The results are summarized below.

1. The mean ± SD of erythrocyte selenium in patients with Hb H disease, β-thalassemia / Hb E, β-thalassemia major and normal subjects were 0.237±0.025, 0.264±0.039, 0.247±0.079 and 0.257±0.032 mcg/ml RBC, respectively. There was no significant difference between erythrocyte selenium concentration of thalassemic patients and normal controls. This indicates that selenium status of thalassemic patients are within the normal range.

2. The mean±SD of erythrocyte GSH-Px activity in

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Hb H disease, β-thalassemia/Hb E, β-thalassemia major and normal subjects were 81.67±18.80, 55.40±10.22, 55.21±7.38 and 30.27±3.96 unit/g Hb, respectively. Erythrocyte GSH-Px activities of all thalassemic patients were significantly ($P<0.05$) higher than those of control subjects. The elevation of GSH-Px activity in thalassemic erythrocytes may result from an increased oxidative stress in the thalassemic erythrocytes and consequently, the adaptation of the erythrocytes' defense mechanism to prevent themselves from the oxidative damage.

3. Low plasma vitamin E level was found in thalassemic patients with Hb H disease, β-thalassemia/Hb E and β-thalassemia major. Their mean plasma vitamin E levels were 0.60±0.21, 0.34±0.13 and 0.26±0.06 mg/dl, respectively, while the value in normal controls was 0.76 ±0.18 mg/dl. The increased vitamin E uptake into the thalassemic erythrocytes in order to counteract the oxidative damage may be the plausible explanation.

4. There was no relationship between erythrocyte selenium and GSH-Px activity within the normal and Hb H disease groups ($r = 0.181$ and $r = 0.383$), whereas the relationship between these parameters in β-thalassemia/ Hb E patients was significant ($r = 0.359$, $P<0.05$). The possible explanation is that normal subjects may have their optimal level of erythrocyte GSH-Px activity, whereas thalassemic patients may have higher requirement of GSH-Px due to the increased oxidative stress in the thalassemic erythrocytes. Thus, the erythrocytes

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compensate by increasing tissue (RBC) selenium uptake for GSH-Px formation.

5. The relationship between plasma vitamin E and erythrocyte GSH-Px activity was evident among the normal controls and Hb H disease patients, whereas no relationship was found among those with β-thalassemia/Hb E patients. Data showed a slight negative correlation ($r = -0.487$, $P<0.05$) for the normals and a moderate negative correlation ($r = -0.784$, $P<0.01$) for those with Hb H patients. This indicates the adaptive physiological response of GSH-Px in the condition of low vitamin E status. No correlation of these parameters in β-thalassemia/Hb E might be due to markedly low vitamin E status as well as the possibility that GSH-Px might reach its maximum adaptive activity.

6. The effects of vitamin E supplementation on plasma vitamin E level and erythrocyte GSH-Px activity in 5 thalassemic patients with low vitamin E status were examined. After 6-8 weeks of daily supplementation of 200 mg dl-alpha-tocopherol, they exhibited significantly higher plasma vitamin E level along with significantly lower erythrocyte GSH-Px activity in comparison to the presupplementation values. The findings agree with the postulated mechanism of vitamin E-selenium relationship in the prevention of peroxidative damage.

7. After vitamin E supplementation, the improvement of skin lesion, better appetite and negative red cell hemolysis (by H_2O_2 hemolysis test) were found in

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all 5 treated thalassemic patients. The results of this preliminary study suggest that the daily supplementation of 200 mg dl-alpha-tocopherol is beneficial for vitamin E deficient thalassemic patients.

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CHAPTER VII

SUGGESTIONS FOR FURTHER STUDY

From the results of this study, additional works should be further explored as follows:

1. The effect of long term supplementation of vitamin E, selenium or combination of vitamin E and selenium on the level of erythrocyte vitamin E, selenium and GSH-Px activity in thalassemic patients is on area of interest.

2. Other parameters: SOD activity, catalase activity, malonyldialdehyde (MDA), red blood cell deformability and reduced glutathione (GSH) should be assessed in thalassemic patients because these parameters are associated with lysis of red cell.

3. The thalassemia trait and G-6-PD deficiency should be excluded from normal subjects. The following tests have to be performed: mean cell volume (MCV), G-6-PD activity and Hb typing. Screening for G-6-PD activity in thalassemic cases is also necessary since it link to GSH-Px system.

4. Finally, dietary selenium and vitamin E intakes of normal and thalassemic subjects should be assessed to confirm the adequacy of these nutrients.

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APPENDIX

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APPENDIX 1

Spectrophotometric Method for The Determination of Plasma Vitamin E

The Emmerie-Engel procedure (173) for measurement of vitamin E (tocopherol) in plasma has been made to improve the sensitivity, accuracy and reproducibility of the determination by Hashim and Schuttringer (165).

Principle

The plasma vitamin E was analyzed by spectrophotometric method according to the method of Hashim and Schuttringer (165) with slightly modification. After the plasma were precipitated by an equal volume of absolute ethanol, the whole mixture was subjected to extraction by an equal volume of n-heptane. To an aliquot of the upper layer, a bathophenanthroline reagent was added and the OD of β-carotene was measured at 460 nm. At the same time, the $FeCl_3$ reagent was added to produce a color. In order to suppress a background color due to excess Fe^{+++} ion, phosphoric acid is added to the system and the sample was read at 510 nm.

Reagents

- Absolute Ethanol (EtOH)
- n-heptane distilled (boiling point 98.43°C)
- Bathophenanthroline (4, 7 - diphenyl - 1, 10-phenanthroline, 0.147% in absolute ethanol)
- $FeCl_3 . 6H_2O$, 0.04% in absolute ethanol

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- Phosphoric acid (H_3PO_4), 6.23 ml diluted to 100 ml with absolute ethanol

Procedure

Into a screw-capped tube

1 ml plasma (EDTA) + 1 ml EtOH

↓

shake for 10 sec
(using a tube mixer)

↓ ← 2 ml Heptane

shake for 1 minute and centrifuge at 1,000 rpm for 5 minutes, 1 ml of heptane-extracted sample is used

↓ ← 200 µl Bathophenanthroline

cover with parafilm shake gently for 20 second,
read OD at 460 nm (β-carotene)

↓ ← 100 µl 0.04% $FeCl_3$

shake for 30 second

↓ ← 100 µl H_3PO_4

shake for 30 second,

read OD at 510 nm (α-tocopherol)

Calculation

$$\text{Vitamin E (mg/dl)} = 5.682 \, (OD_{510} - 0.4308 \times OD_{460})$$

(For Coleman Junior II spectrophotometer at Pediatric Hematology Laboratory of Ramathibodi Hospital)

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APPENDIX 2

Table 14 General, hematological and biochemical data of 14 normal subjects

Name	Sex	Age (yr)	Wt (kg)	Ht (cm)	Hct (%)	Hb (g/dl)	Plasma vit E (mg/dl)	Hemo-lysis (%)	RBC GSH-Px (U/g Hb)	RBC Se (mcg/ml)	RBC deformability		
											74	145	172*
S.T.	M	5.2	19.5	114	37	11.8	0.61	0	30.12	0.226	0.18	0.30	0.36
P.U.	F	6	21.8	114.5	35	11.7	0.70	0	25.98	0.238	-	-	-
A.J.	M	6	19.8	112	39.5	12.0	0.69	0	25.16	0.235	-	-	-
K.N.	M	7	25.1	118	35	13.4	0.84	0	29.17	0.285	0.11	0.20	0.28
D.H.	M	10	28.4	134	35	11.2	1.08	0	24.72	0.222	0.11	0.33	0.37
R.K.	M	10	31	135	39.5	11.6	1.21	0	26.45	0.225	-	-	-
Pa.N.	F	11	29	133.5	40	14.2	0.77	0	32.15	0.284	0.14	0.24	0.33
S.L.	M	11	31	138	37	12.5	0.73	0	34.46	0.296	0.15	0.28	0.33
K.S.	F	12	56	155	40	13.9	0.68	0	29.20	0.314	0.19	0.27	0.33
U.N.	F	13	36.8	146	41	13.8	0.75	0	32.64	0.278	0.18	0.33	0.38
F.T.	F	13.9	46.2	147.5	39	13.7	0.59	0	30.89	0.244	0.14	0.21	0.26
Pr.N.	M	14	48.6	160	43	15.0	0.70	0	29.32	0.290	0.17	0.24	0.31
K.P.	M	14	43.5	160	46	-	0.61	0	36.62	0.238	-	-	-
Ku.P.	M	15	48.5	162	46	-	0.69	0	36.89	0.223	-	-	-
Mean		10.6			39.5	12.9	0.76		30.27	0.257	0.15	0.27	0.33
SD		3.4			3.6	1.2	0.18		3.96	0.032	0.03	0.05	0.04

* dyn/cm^2

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APPENDIX 3

Table 15 General, hematological and biochemical data of 10 Hb H disease patients

Name	Sex	Age (yr)	Wt (kg)	Ht (cm)	Slenec-tomy	Hct (%)	Hb (g/dl)	Plasma vit E (mg/dl)	Hemo-lysis (%)	Ret (%)	IB/HB	RBC GSH-Px (U/g Hb)	RBC Se (mcg/ml)	RBC deformability		
														74	245	272*
Yt.C.	M	5.3	16.8	108.5	-	32	9.3	0.57	0	-	-	90.38	0.231	-	-	-
Yw.C.	F	7	17.3	110	-	30	8.7	0.43	0	-	-	83.83	0.258	-	-	-
R.K.	F	7	17.8	112	-	32	8.5	0.58	0	5.6	>50/neg	123.13	0.262	0.14	0.22	0.28
T.P.	M	7	22	126.5	-	31	9.4	0.88	0	1.0	>50/neg	59.66	0.213	0.11	0.19	0.27
S.S.	F	9	20.5	127.5	-	29.5	8.2	0.69	1.4	-	-	64.50	0.223	0.14	0.20	0.29
M.S.	M	9	29	130	-	33	8.1	0.15	0	10.3	>50/neg	92.54	0.208	0.10	0.20	0.25
W.K.	M	11	-	-	-	33.5	9.1	0.84	0	0.8	>50/neg	60.51	0.238	0.16	0.24	0.30
P.W.	M	11.6	24	130	-	30	9.7	0.69	0	0.9	>50/neg	85.33	0.274	0.12	0.19	0.27
O.P.	F	13	39	141	-	31	10.7	0.52	1.7	3.7	>50/neg	77.87	0.256	0.14	0.24	0.29
J.K.	M	13	28.5	140.5	-	35	9.8	0.69	0	1.1	>50/neg	78.99	0.203	0.16	0.22	0.28
Mean		9.3				31.7	9.2	0.60				81.67	0.237	0.13	0.21	0.28
SD		2.7				1.8	0.8	0.21				18.80	0.025	0.02	0.02	0.02

* dyn/cm^2

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APPENDIX 4

Table 16 General, hematological and biochemical data of 30 β-thalassemia/Hb E patients

Name	Sex	Age (yr)	Wt (kg)	Ht (cm)	Splenec-tomy	Hct (%)	Hb (g/dl)	Plasma vit E (mg/dl)	Hemo-lysis (%)	Ret (%)	RBC GSH-Px (U/g Hb)	RBC Se (mcg/ml)	RBC deformability		
													74	145	272*
K.P.	F	5	14.8	104	-	24	7.7	0.38	9.4	7.6	71.57	0.266	0.08	0.11	0.14
A.O.	M	5	16.3	100	-	25.5	8.1	0.33	4.7	3.0	61.13	0.265	0.04	0.11	0.15
S.K.	F	6.5	17.5	110	-	20	7.6	0.49	3.3	6.3	56.05	0.262	0.04	0.09	0.13
N.S.	M	7	17.5	111	+	23	7.8	0.18	59.2	8.4	49.04	0.228	0.06	0.09	0.12
C.S.	F	7.5	17.2	109	-	28.5	9.0	0.63	0	4.4	54.19	0.327	-	-	-
A.D.	M	8	21.0	118	-	23	6.7	0.46	0	5.5	50.42	0.280	-	-	-
T.S.	M	8	22.6	120.5	-	25	7.2	0.40	1.8	10.1	61.82	0.290	0.04	0.07	0.11
D.K.	M	8.8	23.5	122	+	27	7.1	0.48	0.2	>50	52.60	0.275	0.03	0.05	0.11
C.K.	F	9	21	120	-	18.5	6.7	0.33	19.5	17.5	42.98	0.325	0.06	0.11	0.15
P.K.	F	9.8	20.5	118.5	-	23	6.2	0.34	0	14.4	81.47	0.323	0.07	0.12	0.17
J.K.	M	10	-	-	-	29.5	9.7	0.29	4.4	4.4	55.82	0.243	0.06	0.10	0.14
W.M.	M	10	23.8	129	-	25	7.5	0.35	16.6	7.4	47.82	0.233	0.04	0.09	0.13
Su.S.	M	10	22.6	125	-	26.5	8.4	0.20	20.2	8.2	68.21	0.227	0.02	0.07	0.11
B.S.	F	10.2	17	113	+	25	9.6	0.17	6.6	>50	58.65	0.245	0.01	0.05	0.11
S.P.	M	11	23.4	118	+	25	10.9	0.23	23.6	>70	44.83	0.223	0.02	0.05	0.07
B.K.	F	11	21.5	120	-	19	5.8	0.44	6.7	9.3	40.59	0.214	0.02	0.10	0.15
A.R.	M	12	23.3	129	+	28	5.9	0.34	3.0	>50	46.70	0.234	0.02	0.05	0.08
Sr.S.	F	12	24	130	-	21	6.0	0.15	22.2	>50	52.58	0.220	0.02	0.09	0.12
K.W.	M	13	29.5	132	+	20	8.9	0.31	0	>50	57.81	0.285	0.02	0.05	0.06
O.R.	F	13	-	-	-	25	7.0	0.45	7.1	5.6	45.61	0.225	0.07	0.10	0.16
D.N.	F	13.5	29.3	130	+	27	10.1	0.45	1.0	>50	46.63	0.246	0.05	0.09	0.12
M.A.	M	14	31.5	143	+	25	9.7	0.30	10.6	>50	44.14	0.244	0.01	0.05	0.06
J.S.	F	14	44.5	156	-	22	7.6	0.41	3.0	9.1	71.00	0.371	0.05	0.07	0.11
P.B.	M	15	28.2	137	+	22	8.8	0.20	19.2	>50	59.08	0.281	0.05	0.06	0.10
J.T.	F	16	35.7	142	+	27	6.9	0.47	0.5	>50	69.70	0.226	0.02	0.05	0.09
S.A.	F	16	43.3	154	-	22	8.0	0.39	2.6	8.6	45.23	0.262	0.08	0.14	0.19
L.M.	F	16.4	52	156	-	30	9.2	0.44	4.8	5.1	56.74	0.336	0.04	0.08	0.14
A.K.	M	17	30	141	+	22	8.6	0.02	51.9	-	67.57	0.243	-0.01	-0.01	0.03
J.N.	F	17	31.8	136.5	+	23	10.8	0.30	22.7	>50	57.34	0.264	0.02	0.05	0.08
C.P.	M	17	51	170	-	25	9.3	0.34	0	6.0	44.68	0.258	0.06	0.12	0.15
Mean		11.4				24.2	8.1	0.34			55.40	0.264	0.04	0.08	0.12
SD		3.6				3.0	1.4	0.13			10.22	0.039	0.02	0.03	0.04

* dyn/cm^2

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APPENDIX 5

Table 17 General, hematological and biochemical data of 2 β-thalassemia major patients

Name	Sex	Age (yr)	Wt (kg)	Ht (cm)	Splenec-tomy	Hct (%)	Hb (g/dl)	Plasma vit E (mg/dl)	Hemo-lysis (%)	Ret (%)	RBC GSH-Px (U/g Hb)	RBC Se (mcg/ml)	RBC deformability		
													74	145	272*
N.Su.	M	6	15.5	102	+	29.5	10.8	0.22	58.5	-	60.43	0.191	0.01	0.06	0.07
K.P.	M	14	39.3	147	+	26	10.4	0.30	4.9	>50	49.99	0.303	0.05	0.10	0.12
Mean		10.0				27.8	10.6	0.26			55.21	0.247	0.03	0.08	0.10
SD		5.6				2.5	0.3	0.06			7.38	0.079	0.03	0.03	0.04

* dyn/cm^2

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APPENDIX 6

Table 18 Hematological and biochemical parameters of 5 thalassemic patients before and after 6-8 weeks vitamin E supplementation (200 mg/day)

Patient	Sex	Age (yr)	Type of thalassemia	Hemolysis (%)		Hb (g/dl)		Hct (%)		RBC Se (mcg/ml)		RBC GSH-Px (U/g Hb)		Plasma vit E (mg/dl)	
				before	after	before	after	before	after	before	after	before	after	before	after
A.K.	M	17	β-thal/Hb E	51.9	0	8.6	NA	22	23	0.243	0.258	67.57	47.93	0.02	1.1
N.Su.	M	6	β-thal major	58.5	0	10.8	NA	29.5	31	0.191	0.196	60.43	47.87	0.22	9
S.P.	M	11	β-thal/Hb E	23.6	0	10.9	11.0	25	25.5	0.223	0.228	44.83	42.31	0.23	1.80
N.S.	M	7	β-thal/Hb E	59.2	0	7.8	8.5	23	24	0.228	0.214	49.04	43.50	0.18	1.77
J.N.	F	17	β-thal/Hb E	22.7	0	10.8	11.1	23	25	0.264	0.261	57.34	45.70	0.30	2.15
Mean						9.8	10.2	24.5	25.7	0.230	0.231	55.84	45.46	0.19	1.76
SD						1.5	1.5	3.0	3.1	0.027	0.028	9.06	2.54	0.10	0.37
Paired t' test						NS		P<0.005		NS		P<0.025		P<0.0005	

13966

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