Levels of Serum Interleukin-6 and Tumor Necrosis Factor in Postsplenectomized Thalassemic Patients

SUPORN CHUNCHARUNEE, M.D.*,
PHONGJAN HATHIRAT, M.D.***,
NAPAPORN ARCHARARIT, M.Sc.**,
UMAPORN UDOMSUBPAYAKUL, M.Sc.**,
VICHAI ATICHARTAKARN, M.D.*

Abstract

Levels of serum interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) were studied in 34 nonsplenectomized thalassemic patients (Thal/nonsplenec), 43 postsplenectomized thalassemic patients (Thal/postsplenec), 13 splenectomized non-thalassemic patients (nonThal/postsplenec) and 18 normal control by enzyme linked immunosorbent assay method. Serum IL-6 concentration in Thal/postsplenec was significantly increased when compared with Thal/nonsplenec and normal volunteers (3.55±2.47 pg/ml *vs* 2.38±2.31 pg/ml, p=0.036 and 3.55±2.47 pg/ml *vs* 2.66±0.45 pg/ml, p=0.028, respectively). This study also demonstrated that TNF-α value in Thal/postsplenec was drastically increased above normal control level (15.8±4.86 pg/ml *vs* 9.16±2.18 pg/ml, p=0.001) and the level was statistically significantly higher than that in Thal/nonsplenec (15.5±4.86 pg/ml *vs* 9.96±5.19 pg/ml, p=0.001). There was a trend toward increasing of cytokine levels in Thal/postsplenec with higher platelet count although no correlation was observed. This study addresses the possible role of IL-6 and TNF-α in the pathogenesis of reactive thrombocytosis in Thal/postsplenec.

Interleukin-6 (IL-6) is a polypeptide synthesized primarily by endothelium, T lymphocytes and monocytes, this cytokine expresses a pleiotropic function. Elevated levels of serum IL-6 was previously observed during the acute-phase response and in chronic conditions associated with infection, inflammation, or malignancy(1). IL-6 plays an important role in megakaryocytopoiesis and platelet production(1). Previous studies revealed an association between high plasma IL-6 and reactive thrombocytosis but a small group of patients with splenectomy and thalassemia was enrolled(3,4). Although thrombocytosis occurs in infection and certain inflammatory states may be mediated through an increase in IL-6 level, other inflammatory cytokines including tumor necrosis

* Department of Medicine,
** Research Center,
*** Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand.

factor alpha (TNF-α) and interleukin-1 may also exert their effects on platelet production too.

Previous studies showed that patients with thalassemia major (majorThal) who underwent splenectomy invariably developed thrombocytosis and some of them subsequently had pulmonary thromboembolism[5,6]. The mechanism of thrombocytosis and its role in the pathogenesis of thrombosis is still not clear[7,8]. A recent study showed increased serum levels of TNF-α and macrophage-colony-stimulating-factor (M-CSF) in patients with ß-thalassemia/HbE (ß-Thal/HbE). This was shown to be related to the activation of mononuclear phagocytes[9,10]. The role of IL-6 in thalassemic patients with thrombocytosis has not been extensively studied.

To further ascertain the role of IL-6 and TNF-α in thrombocytosis associated with thalasemic patients who underwent splenectomy (Thal/postsplenec). Serum IL-6 and serum TNF-α were determined in nonsplenectomized thalassemic patients (Thal/nonsplenec), Thal/postsplenec, and non-thalassemic subjects who had been previously splenectomized for other causes (nonThal/postsplenec). The results were compared and evaluated in this study.

MATERIAL AND METHOD

The 77 thalassemic patients included 34 Thal/nonsplenec aged between 7-48 years (14±7.7 yrs) and 43 Thal/postsplenec aged between 6-38 years (15±6 yrs) and 13 nonThal/postsplenec aged between 17-59 years (34±11.3 yrs) and 18 normal control aged between 6-45 years (25.9±10 yrs) were studied. The average of platelet counts in those four groups was $302x10^9$/L, $714x10^9$/L, $416x10^9$/L and $310x10^9$/L, respectively. The patients' characteristics are demonstrated in Table 1. The age range and subtype of thalassemia in both groups were comparable. Splenectomy in nonThal/postsplenec was due to immune thrombocytopenic purpura, trauma or other types of hemolytic anemia.

Cytokine assay

Venous blood sample was taken from each patient for platelet count and cytokine (IL-6 and TNF-α) assay. Platelet count was performed on the same day of blood collection. Sera for IL-6 and TNF-α were kept at -70°C until used. The enzyme linked immunosorbent assay (ELISA) was used for the measurements of IL-6 and TNF-α using commercial kit from R&D system, Minneapolis, U.S.A.

Statistical Analysis

The 6.0 release of the SPSS was used for the analysis. All data were expressed as the mean±SD and analyzed by unpaired *t* test. Significant differences between groups were determined at $p < 0.05$.

Table 1. Clinical characteristic of the patients.

	Normal control n=18	Nonsplenectomized thalassemia n=34	Postsplenectomized thalassemia n=43	Postsplenectomized non-thalassemia n=13
Age , Mean ±SD (yrs)	25.9±10	14.6±7.7	15.5±6	34.5±11.3
Male/Female	8/10	18/16	23/20	4/9
β-thalassemia/HbE	-	26	32	-
β-thalassemia major	-	6	11	-
AE Bart's	-	2	-	-
Platelet count (mean±SD x 10^9/L)	310±115	302±169	714±350	416±479

Table 2. Serum IL-6 and serum TNF-α levels in nonsplenectomized thalassemia, postsplenectomized thalassemia, splenectomized non-thalassemia and normal control.

Subject	IL-6 levels (pg/ml)			TNF-α levels (pg/ml)		
	N	mean±S.D.	p-value	N	mean±S.D.	p-value
1. Normal control	17	2.66±0.45		18	9.16±2.18	
Thal/nonsplenec	34	2.38±2.31	0.495	26	9.69±5.19	0.645
2. Normal control	17	2.66±0.45		18	9.16±2.18	
Thal/postsplenec	43	3.55±2.47	0.028*	41	15.82±4.86	0.001*
3. Normal control	17	2.66±0.45		-	ND	
nonThal/postsplenec	13	3.23±1.03	0.002*	-	ND	
4. *Thal/nonsplenec*	34	2.38±2.31		26	9.69±5.19	
Thal/postsplenec	43	3.55±2.47	0.036*	41	15.82±4.86	0.001*
5. *Thal/nonsplenec*	34	2.38±2.31		-	ND	
nonThal/postsplenec	13	3.23±1.03	0.054	-	ND	
6. *nonThal/postsplenec*	13	3.23±1.03		-	ND	
Thal/postsplenec	43	3.55±2.47	0.541	-	ND	

* statistically significant

RESULT

The values of IL-6 and TNF-α among each group are shown in Table 2. IL-6 levels were similar in normal control and Thal/nonsplenec (2.66±0.45 pg/ml *vs* 2.38±2.31 pg/ml) whereas Thal/postsplenec had significantly higher levels of IL-6 than Thal/nonsplenec (3.55±2.47 pg/ml *vs* 2.38±2.31 pg/ml, p=0.036). In addition, IL-6 levels in Thal/postsplenec were slightly higher than nonThal/postsplenec without statistical significance (3.55±2.47 pg/ml *vs* 3.23±1.03 pg/ml). Significant differences were observed in both Thal/postsplenec and nonThal/postsplenec when compared with normal control (3.55±2.47 pg/ml *vs* 2.66±0.45 pg/ml, p=0.028 and 3.23±1.03 pg/ml *vs* 2.66±0.45 pg/ml, p=0.002, respectively). No statistically significant correlation between serum IL-6 levels and platelet counts was observed in Thal/postsplenec (r = 0.164). Similarly, TNF-α values in Thal/postsplenec were drastically increased above control values (15.8±4.86 pg/ml *vs* 9.16±2.18 pg/ml, p=0.001). Moreover, the same trend was observed when comparing this group with Thal/nonsplenec (15.8±4.86 pg/ml *vs* 9.69±5.19 pg/ml, p=0.001). No statistically significant correlation between serum TNF-α concentration and platelet counts was noted in Thal/postsplenec (r = -0.126).

Thal/nonsplenec, Thal/postsplenec and nonThal/postsplenec were further stratified in 4 groups according to the number of platelet count, i.e., low (<150 x 10^9 cells/L), normal (150-400 x 10^9 cells/L), high (400-1,000 x 10^9 cells/L) and extremely high (>1,000 x 10^9 cells/L). The serum IL-6 and serum TNF-α levels of each category is demonstrated in Table 3. Interestingly, there was a trend toward increasing of both cytokine levels in the patients with higher platelet counts.

DISCUSSION

IL-6 has been shown to induce megakaryocytopoiesis and increase platelet production both in vitro and *in vivo*[1]. IL-6 synthesis may be induced by other inflammatory cytokines such as TNF-α, interleukin-1 and interferon-gamma. Recent study

Table 3. Cytokine levels stratified by platelet counts in nonsplenectomized thalassemia, postsplenectomized thalassemia and splenectomized non-thalassemia.

Serum IL-6 Levels (Mean ± SD) in Each Subgroup

Platelet count ($x10^9$ cells/L)		Nonsplenectomized thalassemia (n=34)		Postsplenectomized thalassemia (n=43)		Splenectomized non-thalassemia (n=13)	
		No.	pg/ml	No.	pg/ml	No.	pg/ml
Low	< 150	6	0.89±1.13	1	3.39	1	3.47
Normal	150-400	17	2.71±2.68	4	4.95±3.99	7	3.36±1.39
High	400-1,000	11	2.64±1.79	36	3.10±1.54	4	3.42±0.48
Extremely high	> 1,000		-	2	4.25±5.41	1	3.27

Serum TNF-α Levels (Mean ± SD) in Each Subgroup

Platelets count ($x10^9$ cells/L)		Nonsplenectomized thalassemia (n=26)		Postsplenectomized thalassemia (n=39)	
		No	pg/ml	No.	pg/ml
Low	< 150	6	6.61±6.69	1	12.9
Normal	150-400	13	10.92±4.05	4	14.56±2.17
High	400-1,000	7	10.06±5.41	33	16.56±4.79
Extremely high	> 1,000	0	-	1	6.4

has demonstrated an elevation of these inflammatory cytokine levels in majorThal which may be attributed to activation of mononuclear phagocytes for the clearance of abnormal erythroid cells[9-11].

In this study, Thal/postsplenec had increased in serum levels of IL-6 and TNF-α compared to Thal/nonsplenec and normal control. Serum IL-6 levels in Thal/postsplenec were higher than nonThal/postsplenec but it was not statistically significant.

No direct correlation between IL-6 levels or TNF-α levels and platelet counts was observed but there was a trend toward increasing of cytokine levels in patients with higher platelet counts regardless of splenectomy. It is probable that the increased platelet production in thalassemic patients is at least if not all, cytokine-driven and this study suggests that IL-6 and TNF-α may play a role. Previous studies have shown that TNF-α inhibited the growth of multipotential precursor cells, committed progenitor cells, erythroid and granulocyte-macrophage component. However, its effect on megakaryocytopoiesis had an enhanced effect on megakaryocytopoiesis and platelet production indirectly *via* IL-6 and other cytokines induced from accessory cells[12].

In conclusion, an increase of serum IL-6 and serum TNF-α levels were noticed in Thal/postsplenec. However, their precise role in the pathogenesis of thrombocytosis in these patients remains to be elucidated.

ACKNOWLEDGMENT

This work was supported by Mahidol University Research Grant 1997.

(Received for publication on July 11, 1997)

REFERENCES

1. Mayer P, Geissler K, Valent P, et al. Recombinant human interleukin 6 is a potent inducer of the acute phase response and elevates the blood platelets in nonhuman primates. Exp Hematol 1991; 19: 688-96.
2. Ishibashi T, Kimura H, Shikama Y, et al. Interleukin 6 is a potent thrombopoietic factor in vivo in mice. Blood 1989; 74: 1241-4.
3. Hollen CW, Henthorn J, Koziol JA, Burstein SA. Elevated serum interleukin-6 levels in patients with reactive thrombocytosis. Br J Haematol 1991; 79: 286-90.
4. Tefferi A, Ho TC, Ahmann GJ, Katzmann JA, Greipp PR. Plasma interleukin-6 and C-reactive protein levels in reactive versus clonal thrombocytosis. Am J Med 1994; 97: 374-8.
5. Hirsh J, Dacie JV. Persistent post splenectomy thromboembolism: A consequence of continuing anemia. Br J Haematol 1966; 122: 44-53.
6. Sonakul D, Pacharee P, Laohapand T, Fucharoen S, Wasi P. Pulmonary artery obstruction in thalassemia. Southeast Asian J Trop Med Pub Heth 1980; 11: 516-23.
7. Visudhiphan S, Ketsa-Ard K, Tumliang S, Piankijaguni A. Significance of blood coagulation and platelet profiles in relation to pulmonary thrombosis in ß-thalasemia/HbE. Southeast Asian J Trop Med Pub Heth 1994; 25: 449-55.
8. Chuansumrit A, Isarangkura P, Mahapan W, Hathirat P, Pintadit P. Thrombotic risk of children with thalassemia. J Med Assoc Thai 1993; 2: 80-2.
9. Wanachiwanawin W, Siripanyaphinyo U, Chinprasertsuk S, et al. Clinical significances of increased serum concentrations of IFN-γ and other cytokines in ß-Thal/HbE. In : VIII Congress Asian Pacific Division of International Society of Hematology 1995; Abstract no.312.
10. Wiener E, Wanachiwanawin W, Chinprasertsuk S, et al. Increased serum level of macrophage colony-stimulating factor (M-CSF) in alpha-and beta-thalassemia syndromes.correlation with anaemia and monocyte activation. Eur J Haematol 1996; 57: 364-69.
11. Orter W, Lindemann A, Horn S, Mertelsmann R, Herman F. Tumor necrosis factor (TNF)-alpha but not TNF-beta induces secretion of colony stimulating factor for macrophages (CSF-2) by human monocytes. Blood 1987; 70: 1700-3.
12. Dan K, Gomi S, Inokuch K, et al. Effects of interleukin-1 and tumor necrosis factor on megakaryocytopoiesis: mechanism of reactive thrombocytosis. Acta Haematol 1995; 93: 67-72.

ระดับซีรั่ม Interleukin-6 และ Tumor Necrosis Factor และความสัมพันธ์ระหว่างระดับ cytokine ทั้งสองชนิดนี้ต่อภาวะเกร็ดเลือดสูงในผู้ป่วยธาลัสซีเมียหลังตัดม้าม†

สุภร จันท์จารุณี, พ.บ., นภาพร อัจฉราฤทธิ์, วท.ม.**,*
*พงษ์จันทร์ หัตถีรัตน์, พ.บ.***, อุมาพร อุดมทรัพยากุล, วท.ม.**,*
*วิชัย อติชาตการ, พ.บ.**

การศึกษาระดับของ interleukin 6 (IL-6) และ tumor necrosis factor alpha (TNF-α) ในซีรั่มผู้ป่วยธาลัสซีเมียที่ไม่ตัดม้าม 34 ราย ผู้ป่วยธาลัสซีเมียหลังตัดม้าม 43 ราย ผู้ป่วยที่ตัดม้ามด้วยสาเหตุต่างๆ 13 รายและในคนปกติ 18 รายด้วยวิธี ELISA พบว่า ระดับซีรั่ม IL-6 ในผู้ป่วยธาลัสซีเมียหลังตัดม้ามสูงกว่าผู้ป่วยธาลัสซีเมียที่ไม่ตัดม้ามและคนปกติอย่างมีนัยสำคัญทางสถิติ (3.55±2.47 *vs* 2.38±2.31 พิโครกรัมต่อมิลลิลิตร, p=0.036 และ 3.55±2.47 *vs* 2.66±0.45 พิโครกรัมต่อมิลลิลิตร, p=0.028)

สำหรับระดับซีรั่ม TNF-α ในผู้ป่วยธาลัสซีเมียหลังตัดม้ามสูงกว่าผู้ป่วยธาลัสซีเมียไม่ตัดม้ามและระดับ TNF-α ในคนปกติอย่างชัดเจน (15.8±4.86 *vs* 9.69±5.19 พิโครกรัมต่อมิลลิลิตร, p=0.001 และ 15.8±4.86 *vs* 9.16±2.18 พิโครกรัมต่อมิลลิลิตร, p=0.001) การศึกษานี้แม้จะไม่พบความสัมพันธ์อย่างมีนัยสำคัญทางสถิติระหว่างระดับ cytokine ในซีรั่มผู้ป่วยธาลัสซีเมียหลังตัดม้ามกับจำนวนเกร็ดเลือด แต่พบว่าผู้ป่วยธาลัสซีเมียหลังตัดม้ามที่มีเกร็ดเลือดสูงจะมีระดับ cytokine ในซีรั่มเพิ่มขึ้น ดังนั้น IL-6 และ TNF-α น่าจะมีบทบาทสำคัญในการเกิดภาวะเกร็ดเลือดสูงในผู้ป่วยดังกล่าว

* ภาควิชาอายุรศาสตร์,
** สำนักงานวิจัย,
*** ภาควิชากุมารเวชศาสตร์, คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี, มหาวิทยาลัยมหิดล, กรุงเทพฯ 10400.
† งานวิจัยนี้ได้รับเงินสนับสนุนจากงบประมาณแผ่นดินประจำปี 2540