Serum C-Reactive Protein Level in Postsplenectomized Thalassemic Patients

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Abstract

C-reactive protein is an established marker for the detection of acute and chronic inflammatory processes. The most potent stimulator for the hepatic synthesis of this protein is interleukin 6. Previous studies have shown that inflammatory cells and inflammatory cytokines, such as interleukin 6, interferon gamma, etc were elevated in postsplenectomized thalassemic patients. The aim of this study was to determine serum C-reactive protein concentration in postsplenectomized ß thalassemic patients (ß thal/HbE postsplenec), and to compare them with those in nonsplenectomized ß thalassemic patients (ß thal/HbE), postsplenectomized non thalassemic patients (postsplenec), reactive thrombocytosis (RT), chronic myeloproliferative disorders (MPD) and normal adult volunteers. Serum C-reactive protein concentration as determined by an automatic Behring Nephelometer was carried out in 28 ß thal/HbE postsplenec, 22 ß thal/HbE, 12 postsplenec, 23 RT, 21 MPD, and 26 healthy adult volunteers. The values of CRP in ß thal/HbE postsplenec were significantly higher when compared with ß thal/HbE, and normal volunteers (4.1±0.7 *vs* 1.6±0.4 mg/L P = 0.006, and 4.1±0.7 *vs* 0.45±0.09 mg/L, P < 0.001). CRP levels in ß thal/HbE postsplenec were also higher than the postsplenec group (4.1±0.7 *vs* 0.19±0.7 mg/L P = 0.095). On the contrary, they were significantly lower than those in RT (4.1±0.7 *vs* 55.4±14.8 mg/L, P = 0.002). However, when compared to those with MPD, the values were not statistically different (4.1±0.7 *vs* 17.1±12.3 mg/L, P = 0.871). Interestingly, there was a trend towards increasing C-reactive protein levels in ß thal/HbE postsplenec patients with higher platelet count, although no correlation was observed. Besides the inflammatory process, platelet and/or factor(s) that control(s) thrombopoiesis seem(s) to play a role in the high serum C-reactive protein levels in the studied population.

Key word : C-reactive Protein, Thalassemia, Reactive Thrombocytosis, Chronic Myeloproliferative Disorders

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C-reactive protein (CRP) is a pentameric, discoid protein with five identical subunits with molecular weight of 110-140 KD. Many biological activities of CRP have been shown, including non-specific host defence against bacteria, parasites, immune complexes as well as activation of classical complement pathway. Moreover, CRP is an established marker for the detection of acute and chronic inflammatory processes. The most potent stimulator for the hepatic synthesis of this protein is interleukin-6 (IL-6)[1-4]. Both serum CRP and IL-6 are increased in inflammation and also in reactive thrombocytosis (RT)[5]. Causes of thrombocytosis are several, and can be grossly classified as secondary and primary or neoplastic[6-8]. Secondary can be reactive, which to us means "short" lived thrombocytosis, which will normalize after removal of the underlying causes. Thrombocytosis is a common feature in postsplenectomized, especially thalassemic patients. We, therefore would like to compare serum CRP in these various thrombocytotic conditions.

Subjects

The investigation was carried out in 28 postsplenectomized ß thalassemic patients (ß thal/HbE postsplenec), 22 nonsplenectomized ß thalassemic patients (ß thal/HbE), 12 postsplenectomized non thalassemic patients (postsplenec), 23 reactive thrombocytosis by causes other than ß thal/HbE postsplenec (RT), 21 chronic myeloproliferative disorders (MPD), and 26 healthy adult volunteers.

Methods

Serum was prepared from venous blood of each patient during a steady state, except those in the RT group. It was then stored at -70°C for later assay. C-reactive protein concentration was determined by an automatic Behring Nephelometer 100 (Dade Behring, Germany.) using N-Latex CRP mono reagent standard and control. The reagents were supplied by Dade Behring Diagnostic. Assay procedure was done according to the manufacturer's instructions. The detectable range of this assay was 0.175 - 1,100 mg/L.

Assessment of hematological parameters.

Complete blood count was done in EDTA-blood using automated cell counter (Coulter JT3, Bedfordshire, England). Hemoglobin typing was analyzed by HPLC technique (Hemoglobin testing system, Variant, Bio-rad, California, U.S.A.).

Statistical Analysis

All data were expressed as mean ± the standard error of mean (mean±SEM). Difference of means between the two groups was determined by unpaired students (t) test, and Mann-Whitney U test. P value of less than 0.05 was considered significant. The Pearson product monent coefficient of correlation and Spearman rank correlation coefficient were used to measure relationship between the parameters.

RESULTS

Clinical characteristics of patients and controls are summarized in Table 1. As can be seen, age and sex distribution of ß thal/HbE postsplenec, ß thal/HbE, and normal controls were almost the same. However, the mean age of RT, MPD and postsplenec was respectively 57.7, 48.4 and 37.6 years old. Seven patients in the ß thal/HbE postsplenec group received blood transfusion within 3 months prior to the study. Platelet counts in the ß thal/HbE postsplenec group were significantly higher than those in the ß thal/HbE, postsplenec, and normal control groups; but were significantly lower than those in the RT, and MPD groups.

Figure 1 shows serum CRP levels in the various groups. Results of the ß thal/HbE postsplenec group were compared with others, and statistically analysed. All except the two, which paired with MPD and postsplenec showed statistically significant difference.

Platelet counts of ß thal/HbE, ß thal/HbE postsplenec, and postsplenec were stratified into 4 groups according to their numbers i.e. low (<150x 10^9 cells/L), normal (150-400x10^9 cells/L), high (400-1,000x10^9 cells/L), and extremely high (>1,000x10^9 cells/L). Interestingly, there was a trend towards increasing serum CRP levels in the ß thal/HbE postsplenec group with higher platelet counts (Fig. 2). In those with high and extremely high platelet counts, their CRP concentration were respectively 3.6±0.8 mg/L and 6.86±2.2 mg/L. Table 2 shows the number of patients with different serum CRP levels.

DISCUSSION

C- reactive protein (CRP) is nonspecifically increased in various pathologic conditions, including inflammation and reactive thrombocytosis. The present report determined the levels of serum CRP in various conditions with normal

Table 1. Clinical characteristics of the patients.

	Normal	ß thal/Hb E	ß thal/Hb E postsplenec	Postsplenec	RT	MPD
N	26	22	28	12	23	21
Age (years)	26.1±1.5	24.6±1.4	25.5±1.9	37.6±3.6	48.4±3.9	57.7±3.9
Male/Female	13/13	10/12	14/14	3/9	10/13	14/7
Recent blood transfusion	-	-	7	-	-	-
Hb (g/DL)	14±0.5	7.3±0.4	6.7±0.2	6.6±0.4	13.5±0.6	11.1±0.7
Hct (vol%)	43±1.5	24.4±1.1	22.4±0.45	21.7±1.0	40.4±1.6	35.8±1.8
WBC (x10^9 cells/L)	7±0.2	7.7±0.4	11.8±1.7	8.96±0.65	8.96±0.65	67.3±23.5
Platelet count (x10^9 cells/L)	282±36	240±27	747±1.3*	322±36	1,363±105	1,337±82

Mean ± SEM

* $P < 0.05$ significantly different from normal control, ß thal/HbE, postsplenec, RT, and MPD

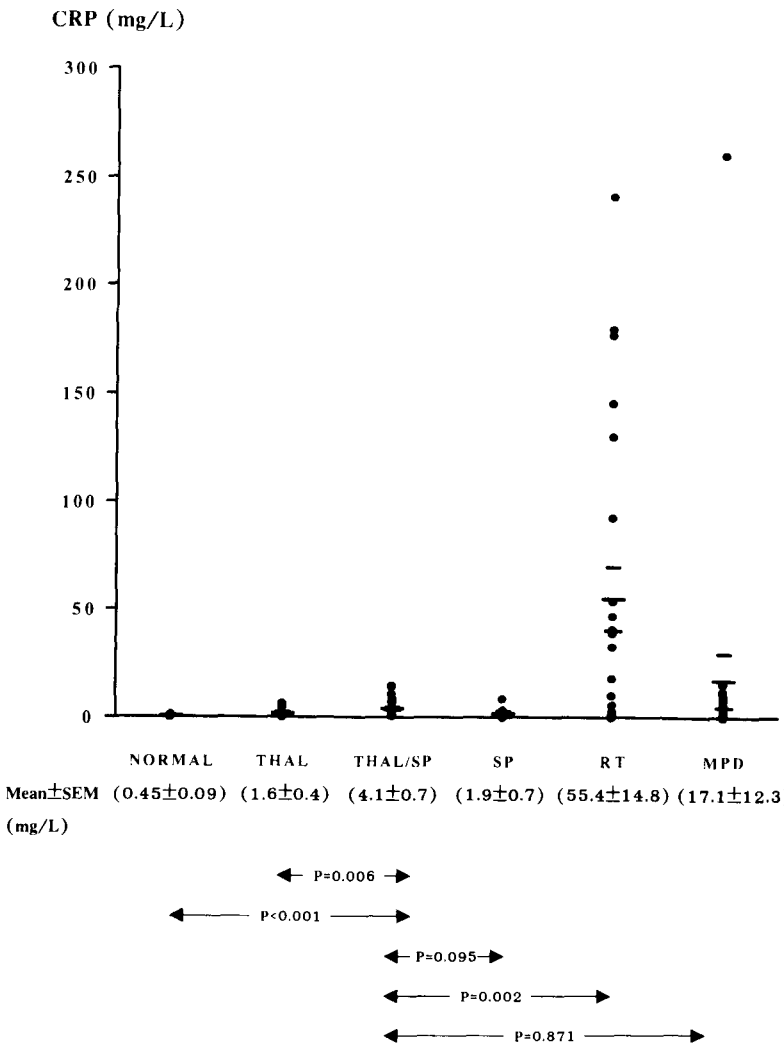

Fig. 1. CRP levels in nonsplenectomized ß thalassemia/Hb E disease (THAL), postsplenectomized ß thalassemia/Hb E disease (THAL/SP), postsplenectomized non thalassemia (SP), reactive thrombocytosis (RT), chronic myeloproliferative disorders (MPD), and normal controls.

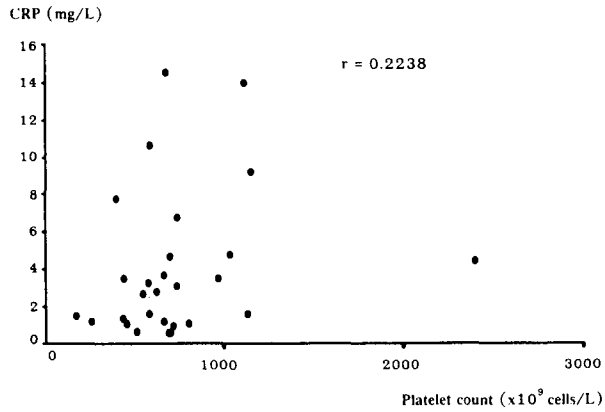

Fig. 2. The correlation between CRP and platelet count in postsplenectomized ß thalassemic patients

Table 2. Number of subjects stratified by CRP concentrations.

Subject	N	CRP (mg/L)									
		0-1.3		>1.3-5		>5-10		>10-20		>20-270	
		No.	%	No.	%	No.	%	No.	%	No.	%
Normal	26	26	100	-		-		-		-	
ß thal/HbE	22	**13**	**59.1**	7	31.8	2	9.1	-		-	
ß thal/HbE postsplenec	28	8	28.6	**14**	**50**	3	10.7	3	10.7	-	
Postsplenec	12	**7**	**58.3**	4	33.3	1	8.4	-		-	
RT	23	6	26.1	2	8.7	1	4.3	2	8.7	**12**	**52.2**
MPD	21	**8**	**38.1**	4	19.05	4	19.05	4	19.05	1	4.8

platelet count and thrombocytosis. It is interesting to note that normal healthy adults have a CRP level less than 1.3 mg/L, while 60-70 per cent of patients with thrombocytosis have a level more than 1.3 mg/L. As expected, the highest CRP level was found in patients with RT. Significantly high CRP levels in RT and MPD patients were consistent with the previous studies[1,5]. Markedly increased CRP levels with a broad distribution among individuals with RT were probably due to various accompanying stimuli, e.g. inflammation, infection, and malignancy. A previous report showed high correlated levels of serum IL-6 and CRP in RT[5].

Slightly increased CRP levels in nonsplenectomized thalassemic patients might be due to recurrent infection, immune system abnormality, such as less effective CMIR in response to antigen, and impairment of neutrophil phagocytic and killing functions[9,10].

Although increased levels of CRP were observed in patients with thrombocytosis, most of the CRP values did not exceed 5 mg/L. Marked increase in CRP levels in reactive thrombocytosis could be attributed to inflammatory cytokine with moderate thrombopoietic activity. Wide distribution of CRP levels in the MPD group suggested autonomous mechanism of thrombocytosis irrespective of inflammatory stimulation effect.

This study demonstrated an increase in CRP levels and a moderately broad distribution of CRP concentration among individuals in ß thal/HbE postsplenec. The protein concentration corresponded to the platelet count. Persistent thrombocytosis has been reported in postsplenectomized thalassemic patients[11]. These findings are in accordance with the increased serum level of interleukin-1, interleukin-6, and tumor necrosis factor alpha in these patients. Moreover, increased

platelet- derived growth factor, M-CSF and INF-γ were also observed in ß thalassemia[12-16]. In the previous study, the increased serum INF-γ was one of the factors responsible for severity of anemia. in these patients. IL-6 has been shown to induce the acute phase response and to have thrombopoietic activity[3,14,17]. Elevation of inflammatory cytokines in these patients may stimulate CRP production and thrombocytosis in ß thal/HbE postsplenec patients. The elevation of serum CRP level may not be directly caused by splenectomy alone. Since higher CRP levels were observed in ß thal/HbE in postsplenec than in postsplenectomized non thalassemic patients. Therefore, synergistic effects of splenectomy and cytokine stimulation may result in an increase of CRP concentration in postsplenectomized thalassemic patients. In addition, increased CRP levels and IL-6 levels in ß thal/HbE postsplenec may be attributed to a low grade chronic inflammatory process. The cause of this process remains to be studied. Our study demonstrated lower levels of CRP in the ß thal/HbE postsplenec group when compared to other subgroups of RT. This may be due to the different pathophysiologic conditions of each subgroup of RT and the variation among each subject.

SUMMARY

C-reactive protein was moderately increased in ß thal/HbE postsplenec. The protein value was significantly lower than other subgroups of reactive thrombocytosis, but it was not significantly different from patients with myeloproliferative disorders. The role of CRP in the pathogenesis of thrombosis in these patients remains to be evaluated.

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ระดับของ C-reactive Protein ในผู้ป่วยธาลัสซีเมียหลังตัดม้าม

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C-reactive protein (CRP) เป็นโปรตีนที่ตรวจพบได้ในภาวะที่ร่างกายมีอาการอักเสบเฉียบพลันหรือเรื้อรัง cytokine ที่มีความสามารถในการกระตุ้นให้ตับสร้างโปรตีนชนิดนี้ได้อย่างมีประสิทธิภาพ คือ interleukin-6 (IL-6) จากการศึกษาที่ผ่านมาในอดีตพบว่า ผู้ป่วยธาลัสซีเมียหลังได้รับการตัดม้ามจะมีจำนวน inflammatory cell และระดับ inflammatory cytokines เพิ่มมากขึ้น เช่น IL-6, interferon gamma เป็นต้น คณะผู้ทำการวิจัยจึงมีวัตถุประสงค์จะศึกษาระดับ CRP ในซีรั่มผู้ป่วยธาลัสซีเมียหลังตัดม้ามจำนวน 28 คน พร้อมกับเปรียบเทียบระดับของโปรตีนชนิดนี้ในซีรั่มของผู้ป่วยอื่น ๆ ได้แก่ ผู้ป่วยธาลัสซีเมียที่ไม่ได้ตัดม้าม 22 คน, ผู้ป่วยหลังตัดม้ามในโรคอื่น ๆ 12 คน ผู้ป่วยที่มีเกร็ดเลือดสูงจากสาเหตุต่าง ๆ ที่ไม่เกี่ยวกับความผิดปกติของไขกระดูก (RT) 23 คน ผู้ป่วยที่มีภาวะเกร็ดเลือดสูงจากความผิดปกติของไขกระดูก (MPD) 21 คน และคนปกติ 26 คน โดยวัดระดับ CRP ด้วยเครื่อง Behring Nephelometer ชนิดอัตโนมัติ ผลการวิจัยพบว่า ระดับ CRP ในผู้ป่วยธาลัสซีเมียหลังได้รับการตัดม้ามสูงกว่าผู้ป่วยธาลัสซีเมียที่ไม่ได้ตัดม้าม, และคนปกติอย่างมีนัยสำคัญทางสถิติ (4.1 ± 0.7 *vs* 1.6 ± 0.4 mg/L $p = 0.006$, 4.1 ± 0.7 *vs* 0.45 ± 0.09 mg/L $p < 0.001$) และสูงกว่าผู้ป่วยหลังตัดม้ามในโรคอื่น (4.1 ± 0.7 *vs* 1.9 ± 0.7 mg/L $p = 0.095$) แต่เมื่อเทียบระดับโปรตีนชนิดนี้กับผู้ป่วยกลุ่ม RT พบว่า ระดับโปรตีนต่ำกว่าอย่างชัดเจน (4.1 ± 0.7 *vs* 55.4 ± 14.8 mg/L $p = 0.002$) อย่างไรก็ตามพบว่าระดับ CRP ในผู้ป่วยธาลัสซีเมียหลังตัดม้ามไม่แตกต่างกับผู้ป่วยกลุ่ม MPD อย่างมีนัยสำคัญทางสถิติ (4.1 ± 0.7 *vs* 17.1 ± 12.3 mg/L, $p = 0.871$) สิ่งที่น่าสนใจที่พบคือ ระดับ CRP ในผู้ป่วยธาลัสซีเมียหลังตัดม้ามจะสูงขึ้นเมื่อจำนวนเกร็ดเลือดเพิ่มมากขึ้น ถึงแม้จะไม่พบความสัมพันธ์กันอย่างมีนัยสำคัญทางสถิติระหว่างระดับ CRP กับจำนวนเกร็ดเลือด

ผลการศึกษานี้แสดงให้เห็นว่า นอกจากการอักเสบแล้ว เกร็ดเลือดและหรือปัจจัยที่ควบคุมการสร้างเกร็ดเลือดดูเหมือนจะมีบทบาทต่อระดับ CRP ที่เพิ่มขึ้นในผู้ป่วยธาลัสซีเมียหลังตัดม้าม

คำสำคัญ : C-Reactive Protein, Thalassemia, Reactive Thrombocytosis, Chronic Myeloproliferative Disorders

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