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# The Level of Natural Anticoagulants in Transfusion Dependent Thalassemia Patients in Kelantan, Northeastern Malaysia

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## Abstract

The association of the severe form of thalassemia with complications of blood transfusion, iron overload, bony deformity and gall stone is well described. Currently, due to improvement in the way of managing thalassemia, most of thalassemia patients survive longer. However more new complications had been observed.

Increased incidence of thromboembolism among thalassemia patients had triggered various studies done to determine hypercoagulable state among thalassemia patients. Several factors for the hypercoagulable state had been identified such as RBC membrane disruption, chronic platelet activation and defect in coagulation pathway. This study was carried out to determine the level of Protein C, protein S, free protein S and antithrombin among thalassemia patients and to compare the level of Protein C, protein S, free Protein S and antithrombin level between thalassemia patients and healthy control. This was a case control study done at Hospital Universiti Sains Malaysia. Thirty six thalassemia patients who came for regular blood transfusion and 20 healthy blood donors for normal control were recruited. Blood samples were collected and analyzed for Protein C, protein S, free protein S and antithrombin using ACL Elite Pro. The result showed mean Protein C and free protein S levels were significantly lower (54.8 ± 13.2% and 70.0 ± 12.1% respectively) in thalassemia patients compared to age-matched normal control (94.1 ± 16.3% and 99.8 ± 17.3% respectively), whereas mean total protein S (54.8 ± 13.2% and 94.1 ± 16.3%; respectively) and antithrombin (70.0 ± 12.1% and 99.8 ± 17.3%; respectively) level were similar. In conclusion there was a significantly decreased Protein C and free protein S in thalassemia patients which might suggests hypercoagulable state in thalassemia patients. Since there are a lot of similarities in finding from other studies, we believe that many more study to look for other parameters contributing to hypercoagulable state in thalassemia patients is needed.

**Keywords:** Hypercoagulable state; Thalassemia patient; Protein C; Protein S; Free Protein S and Antithrombin

## Introduction

Thalassemia is a common hereditary blood disorders that results from genetic defects causing deficient synthesis of hemoglobin polypeptide chains [1]. The association of the severe form of the disease with complication of blood transfusion, iron overload, bony deformity and gall stone is well described.

In recent years, there has been increasing reports of haemostatic derangement complication which significantly influence the morbidity and mortality of this disease [2-4]. Wide spectrum of haemostatic abnormalities ranges from subclinical derangement of hemostatic parameters to various thromboembolic events such as pulmonary embolism, deep vein thrombosis and portal vein thrombosis have been reported [4]. The phenomenon was seen more common in thalassemia intermedia patients than in regularly transfused thalassemia major patients. Among thalassemia major patients, the prevalence is higher in splenectomized patients [4,5].

The mechanism of the thromboembolic event in thalassemia has not been fully elucidated. There are diverse factors contributing to its etio-pathogenesis. Among factors that have been actively investigated are increased platelet activation, endothelial activation, thalassemic red blood cell membrane abnormalities leading to activation of the coagulation cascade and changes in coagulation protein level [4,6,7].

A significant reduction of the Protein C, protein S and antithrombin level were revealed by many studies in the past [8,4]. It had been said as main contributing factors in various thromboembolic events in thalassemia patients. Pertaining to those data, the study was conducted to look for any changes in the haemostatic parameters among transfusion dependent thalassemia patients in our centre.

## Patients and Methods

In this case-control study we included thirty six transfusion dependent thalassemia patients and twenty healthy blood donors matched by sex and age, as a control group. None of our subjects had a history of thrombosis in the past.

Five ml of blood were collected into EDTA container and another 2.7 ml into trisodium citrate container. The blood collection was taken immediately from patients and control groups before blood transfusion and donation respectively. Collected blood samples were analysed for the WBC, platelet, reticulocyte and NRBC count using Sysmex XE 5000 analyzer and for Protein C, protein S, free protein S and antithrombin using ACL Elite Pro coagulometer.

## Result

Thirty six transfusion dependent thalassemia patient and 20 healthy control subject who fulfilled inclusion and exclusion criteria were recruited. Thalassemia group consist of 33 (91.7%) HbE/β thalassemia patients, 2 (5.6%) β thalassemia major and 1(2.8%) HbH disease patients. Among them, 13 (36.1%) had done splenectomy while 23 (63.9%) are non-splenectomized patient. There was no documented incidence of embolism in all thalassemia patients.

## Laboratory Parameters

There was significant difference in the WBC, platelet, reticulocyte and NRBC count between splenectomy and non-splenectomy patients. Splenectomy patients have higher WBC and platelet count compared to non-splenectomy patients. Reticulocyte count and NRBC were also elevated in splenectomy patients compared to non-splenectomy patients. Other parameters showed no significant difference between the two groups. Table 1.

There were significant differences of mean Protein C in thalassemia patients compared to normal control (54.8 ± 13.2% and 94.1 ± 16.3%; respectively). There were also significant differences of mean free protein S in thalassemia patients compared to normal control. (70.0 ± 12.1% and 99.8 ± 17.3%; respectively). Mean protein S in thalassemia patients also showed significant difference compared to normal control (94.3 ± 18.4% and 105.1 ± 16.7%; respectively). However there were no significant difference of mean antithrombin in thalassemia patients compared to normal control (115.4± 27.3% and 124.4 ± 12.5%; respectively). Refer to table 2.

Age adjusted comparison of Protein C, protein S, free protein S, antithrombin level between thalassemia patient and normal control also revealed similar result as shown in Table 3.

As shown in Table 4, a one-way ANOVA was used to test for Protein C, protein S, free protein S and antrithrombin level differences among three groups, splenectomised, non-splenectomized and control. There was a significant difference of mean Protein C between the groups, p <0.001. Scheffe post-hoc test indicated there were significantly lower mean (SD) Protein C in splenectomized patients [54.00(14.36)] and non-splenectomized [54.84 (12.86)] than control [94.14 (16.34)]. However the difference between splenectomised and non-splenectomized was not statistically significant.

For free protein S level, there was a significant difference of mean between groups F (2,53) =30.56, p <0.001. Post hoc Dunnet C procedure was applied because assumption for homogeneity of variances was violated (based on Levene test). Multiple comparisons indicate that mean (SD) free protein S level of splenectomised patients [75.70 (16.02)] and non-splenectomized patients [66.93 (8.51)] significantly lower than control group [99.79 (17.33)].There was no significant difference of mean free protein S between splenectomized and non-splenectomized patients.

On the other hand, one-way ANOVA test showed there was no significant difference of mean protein S and antithrombin between all the 3 groups [F (2,53) = 2.41, p=0.099 and F (2,53) = 0.842, p=0.436] respectively.

| | Splenectomy Mean (SD) | Nonsplenectomy Mean (SD) | 95% Confidence Interval | P value |
|---|---|---|---|---|
| Haemoglobin(g/dl) | | | -0.27 (-0.97,0.43) | 0.435 |
| WBC ( x 109/l) | | 7.73 (1.85) | 7.66 ( 5.14,10.17) | <0.001 |
| Platelet ( x 109/l) | | 213.83 (66.37) | 421.64 (262.64,580.62) | <0.001 |
| MCV (fl) | 76.34 (2.94) | 67.13 (15.33) | 9.20 (0.43,17.99) | 0.400 |
| MCH | 23.35 (1.83) | 22.61 (3.01) | 0.741 (-1.14,2.62) | 0.428 |
| MCHC | 30.60 (2.00) | 31.6 (1.77) | -1.00 (-1.07,0.07) | 0.129 |
| RDW % | 23.69 (4.70) | 25.26 (5.72) | -1.56 (-5.36,2.23) | 0.408 |
| NRBC | 31.83 (34.44) | 0.54 (0.46) | 31.29 (10.48,52.10) | 0.007 |
| Reticulocyte (%) | 12.67 (8.48) | 1.77 (1.30) | 10.89 (5.75,16.03) | 0.001 |
| Albumin | 44.00(4.26) | 44.00 (1.88) | 0.00 (-2.08,2.08) | >0.95 |
| Bilirubin | 48.54 (19.86) | 45.61 (15.56) | 2.93 (-9.20,15.06) | 0.627 |
| AST | 81.85 (39.95) | 61.48 (25.28) | 20.37 (-1.67,42.41) | 0.069 |
| ALT | 82.69 (32.43) | 61.70 (41.49) | 20.99 (-6.18,48.17) | 0.126 |
| Ferritin | 4182.62 (2623.15) | 3397.83 (1811.63) | 784.79 (-719.75,2289.33) | 0.297 |

**Table 1:** Comparison of Full blood count, liver function test and serum ferritin level between splenectomy and non-splenectomy patients

| Variables | Control[a] | Thalassemia Patient[a] | Mean Difference (95% CI) | P value[b] |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Protein C | 94.14 ± 16.34 | 54.54 ± 13.22 | 39.60 (31.55,47.65) | <0.001 |
| Protein S | 105.12 ± 16.79 | 94.41 ± 18.73 | 10.71 (0.60,20.81) | 0.038 |
| Free Protein S | 99.79 ± 17.33 | 70.09 ± 12.32 | 29.69 (21.7,37.68) | <0.001 |
| Antithrombin | 124.36 ± 12.49 | 116.09 ± 27.28 | 8.27 (-4.69,21.24) | 0.210 |

**Table 2:** Comparison of Protein C, protein S, free protein S, antithrombin level between thalassemia patients and healthy control

[a] Mean (SD) [b] Independent t-test applied

| Variables | Normal Control | Thalassemia Patients | Mean Difference ( 95% CI ) [a] | P value [b] |
|---|---|---|---|---|
| Protein C | 90.42 (74.38,106.47) | 54.30 (48.98,59.63) | -36.118 (- 53.02, - 19.21) | <0.001 |
| ProteinS | 117.39 ( 97.55, 137.25) | 94.56 (87.97, 101.15) | -22.84 ( -43.75, -1.924 ) | 0.030 |
| Free Protein S | 98.53 (91.16,105.91) | 70.79 (65.59, 75.99) | -27.74 (-37.53, -17.95) | <0.001 |
| Antithrombin | 127.36 (115.45,139.27) | 114.42 (106.03,122.81) | -12.94 ( -28.74,2.87) | 0.110 |

**Table 3:** Age adjusted comparison of Protein C, protein S, free protein S, antithrombin level between thalassemia patients and normal control.

[a] Adjusted mean difference ( 95% confidence interval ) Bonferroni adjustment applied [b] ANCOVA applied (adjusted for age)

| | Frequency(%) | ProteinC Mean (SD) | ProteinS Mean (SD) | Free ProteinS Mean (SD) | Anti Thrombin |
|---|---|---|---|---|---|
| Splenectomized | 13 | 54(14.36) | 96.86(21.18) | 75.70(16.02) | 114.65(32.23) |
| Non-Splenectomized | 23 | 54.84(12.86) | 93.03(17.55) | 66.93(8.51) | 116.90(24.82) |
| Control | 20 | 94.14(16.34) | 105.12(16.80) | 99.79(17.33) | 124.36(12.50) |

**Table 4:** Comparison of Protein C, protein S, free protein S and antithrombin level between splenectomised thalassemia patient,nonsplenectomized and normal control, aOne way ANOVA test.

## Discussion

Hypercoagulable state in thalassemia becoming important aspect to be explored since many earlier studies had revealed their strong association. In our study, we have revealed a significantly decreased Protein C and protein S in thalassemia major patients which might suggests hypercoagulable state in this group. The result is consistent with other studies that had been done before by several investigators.

Study by Eldor et al. [9] on 62 β thalassemia major patients revealed similar findings of significantly low level of Protein C and protein S while level of antithrombin was not impaired [9]. Another study done on thalassemia major patient also showed reduction in Protein C and protein S level in 29.4% and 38.2% of the patients respectively while antithrombin level only showed reduction in 2.9% of the patients [10]. However study done among haemobglobin E/ β thalassemia patients showed significantly low levels of antithrombin together with significant lower Protein C and protein S. This study however did not specify whether the patients were thalassemia intermedia or thalassemia major [11].

Capellini et al. [8] suggested that low level of naturally occurring anticoagulant proteins such as Protein C found in their study are possible due to hepatic dysfunction as Protein C, protein S and antithrombin are synthesized in the liver and are very sensitive to defect in synthetic function of the liver even at mild degree [12]. In our study, serum albumin level which reflects synthetic function of the liver is within the normal range. Therefore liver function impairment alone cannot explain the significantly low level of Protein C and protein S found in our study subject.

Besides, low Protein C, protein S level can be due to inherited deficiency of Protein C and protein S. However it is very unlikely because even the prevalence of more common congenital

thrombophilic mutations like factor V Leiden, MTHFR C677T, and prothrombin G20210A mutations are not increase in thalassemia patient [13].

Another possible cause of the low level of Protein C and protein S level is increased in consumption following the chronic activation of coagulation system. This possibility is supported by previous studies showed significant elevation of thrombin-antithrombin (TAT) complexes level found in thalassemia major patients compared to normal [9,11]. The findings might suggest the presence of continuous thrombin generation in thalassemia patients. However this explanation could not explain the normal antithrombin level found in our thalassemia major patient.

Normal antithrombin level found in our study could be because of regular transfusion every 2-3 weeks given to our thalassemia major patient rendered a protective effect and caused less marked effect on antithrombin reduction. A study done on anticoagulant proteins in patient thalassemia intermedia and major showed a significant decreases antithrombin level in thalassemia intermedia patient, both splenectomised and non- splenectomised patient whereas in thalassemia major patients, antithrombin showed a significant reduction only among splenectomised group. There is no significant difference of antithrombin level in non-splenectomized thalassemia major patients compared to normal control [12].

Beside the laboratory evidence pointing towards a higher coagulable state in thalassemia intermedia patients compared to thalassemia major, epidemiological and clinical study also pointed the same findings. Incidence of thromboembolic event is more in thalassemia intermedia compared to thalassemia major patients. A study had shown that there was a larger prevalence of venous thromboembolic events in transfusion-independent patients with thalassaemia intermedia (29%) than in regularly transfused patients with thalassaemia major (2%) [12]. Another study also showed similar result. Out of 2190 patients with thalassemia intermedia, 4% experienced a thrombotic event while for thalassemia major patient, only 0.9% out of 6670 patients develop a thrombotic event. Incidence of pulmonary hypertension also more pronounced in thalassemia intermedia patients compared to thalassemia major [14].

More transfusion given can reduce the hypercoagulability in thalassemia patient because frequent transfusion will reduce the circulating abnormal thalassemic red blood cell and therefore reduce the effect of procoagulant activity of the abnormal red blood cell. It is proven that thalassemic red blood cells have procoagulant activity [15]. Furthermore, a case study done on a splenectomised haemoglobin E/β thalassemia woman with pulmonary arterial hypertension showed normalization of thrombin-antithrombin level and reduce of pulmonary vascular resistance occur after chronic transfusion of packed red blood cells was given in addition to other ongoing treatment with warfarin, acetyl salicylic acid, desferrioxamine, and other supportive measures [16].

Besides, our splenectomized patients have a significantly high level of platelet count compared to non-splenectomized patient. Other study also showed a same result [17]. White blood cell, nucleated red blood cell and reticulocyte count also showed a significantly high level in splenectomised patients. This result is consistent findings seen in post splenectomy patients. High platelet level was associated with increased risk of thrombosis in splenectomy patients. However no significant difference for Protein C, protein S and antithrombin level found between splenectomy and nonsplenectomy patient. These findings could be due to a small sample size for both groups of patients.

By history taking, none of our patient has any thromboembolic event. However this does not mean they do not have hypercoagulable state. Hypercoagulable state in thalassemia patient can occur in early childhood while the manifestation of thromboembolic event can occur in later age. Besides, several studies done showed there was increase incident of silent ischemic lesions in patients with β-thalassemia intermedia especially in splenectomised adults who are transfusion-independent and those with elevated platelet counts detected by several brain magnetic resonance imaging studies. The prevalence is as high as 60% of the population studied having silent ischaemic infarct Musallam et al. [3].

## Conclusion

There is a significantly decreased Protein C, protein S and free Protein S in thalassemia patients which might suggests hypercoagulable state in this group. Since the findings are similar with other studies, we believe that many more studies to look for other parameters contributing to hypercoagulable state in thalassemia patients is needed.

The measurement of these proteins was relied on mainly on their activities. By having said that, other factors may influence the results; such as: liver disease, iron toxicity, chelating agents, associated infection or intercurrent diseases.

Perhaps this study may provide beneficial contribution for future study in predicting the etiology of thromboembolic event in thalassemia patients and thus directing us to re-assess the management of thalassemia patients in future.

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