Prothrombotic markers in Thalassemia major patients: A paradigm shift

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ABSTRACT

Background: It is being increasingly recognised that thalassemia major patients, like intermedia, have increased propensity for thromboembolism. Deficiency of natural anticoagulants is more recently defined finding contributing to the hypercoagulable state. The aim this study is to determine natural anticoagulants levels and their correlation with maternal characteristics, haematological and biochemical markers.

Methods: This is a prospective case-control study. We registered 80 patients and 60 healthy controls from Jan 2009 to Dec 2013. Complete blood counts, prothrombin time, activated partial thromboplastin time, protein C, protein S, antithrombin, serum ferritin, liver enzymes; HbsAg and Anti-HCV were evaluated.

Result: There were 42 males and 38 females with mean age of 12.30±5.50 years. The mean protein C, protein S and antithrombin in patients and control were 58.25±22.5 versus 110.67±22.60, 67.90±19.58 versus 98.70±21.54 and 89.73±18.09 versus 104.0±10.98 (p<0.001) respectively. Protein C was predominantly deficient in 65% followed by protein S and antithrombin in 35% and 20% respectively. Protein C deficiency divulged positive correlation with protein S deficiency (p = 0.035) and antithrombin deficiency with hemoglobin of ≤8gm% (p<0.0025). No significant correlation of prothrombotic markers was established with maternal characteristics, hepatic dysfunction, hepatitis and serum ferritin.

Conclusion: Substantial decrement in prothrombotic markers, primarily protein C, may be implicated in elevated thrombosis; however follow-up data is required to establish definitive thromboembolic events.

KEY WORDS:
Thalassemia major, Hypercoagulopathy, Protein C, Protein S, Antithrombin

INTRODUCTION

Thalassemia major is a recessively inherited heterogeneous disorder, with more than 200 mutations recognised, consequential from inadequate beta globin chain synthesis.[1] It is the most frequent global genetic disorder with substantial morbidity and mortality.[2] Prevalence is primarily in the Mediterranean, Indian subcontinent, Middle East and central Asia vicinity.[3] The carrier rate is 5-7% in Pakistan. Though no registry is maintained, anticipated 5000-9000 neonates are born annually with thalassemia major in Pakistan.[3]

Most patients in under resource countries like Pakistan remain under transfused and iron overloaded. Extramedullary haematopoiesis with progressive hepatosplenomegaly and hypersplenism, substantially lead to worsening of the anaemia, often coupled with thrombocytopenia.[4] Interestingly these patients seem to suffer from bleeding complication as well as from thrombotic events.[5,6]

Amplified bleeding propensity is well recognised complication in these patients, accountable in 29.6%.6 Increased bleeding affinity is partly due to thrombocytopenia, accountable in 33.3% and partly due to acquired coagulation factor deficiencies.[6]

The survival expectations over the preceding few years have been distinctly better with conventional therapeutic options.[7] However certain new dilemmas are being increasingly recognised: thromboembolism, pulmonary hypertension, cerebral thrombosis, portal venous thrombosis and right sided heart failure-cor pulmonale.[7-11]

Thromboembolism, both arterial and venous, is a well depicted phenomenon in thalassemia major.[12] Various aspects are contributory; firstly amplified expression of thrombogenic phosphatidylserine and phosphotidylethanolamine in the membrane and oxidation of membrane phospholipids.[2,13,14] Secondly increased platelets aggregation and activation and lastly increased expression of endothelial adhesion proteins leads to thrombosis.[13,15] However DNA mutations are not significantly correlated in thrombotic pathogenesis.[13,16]

Chronic Hypercoagulopathy due to the deficiency of natural anticoagulants is one of the recently defined complication in thalassemics.[17,18] There are various possible explanations for decreased levels of inhibitors; liver impairment, chronic hepatitis, and vitamin K insufficiency and increased turnover.[7,19] Another explanation of protein C deficiency is its binding affinity with phosphatidylserine, abnormally exposed on erythrocyte membrane of thalassemia major resulting in more consumption.[7] Consequently prothrombotic

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haemostatic imbalances often exist in thalassemics even in early life, which culminate in an unavoidable clinical thrombosis.

Studies have revealed more venous events in thalassemia intermedia, in disparity thalassemia major patients reveal arterial incidents predominantly, particularly anaemic patients.[1] Incidence of stroke varies from 2% to 20% in thalassemia major patients.[8] It is also well established that thalassemia major patients often have cardiac dysfunction due to iron overload which will further augment the risk of stroke and thrombosis.[8,13]

The objective of our study is to evaluate levels of naturally occurring anticoagulants in transfusion dependent thalassemia major patients. Moreover, secondary objective is to correlate inhibitors deficiency with maternal characteristics, haematological parameters, coagulation markers, hepatic function, hepatitis markers and serum ferritin levels.

MATERIALS AND METHODS

Patients and controls:

This is a prospective case-control study, in which 80 patients with β-thalassemia major were enrolled. The patients were registered from Jan 2009 to Dec 2013. Sixty age matched healthy controls were also registered. An informed consent was attained from the patients aged ≥18 years and from parents/guardians if the patient aged <18 years.

All registered patients were on regular blood transfusion and parenteral iron chelation, as per requirement. Patients on oral or intravenous anticoagulants, chronic hepatitis (child score 3), and splenectomised patients were excluded. Pregnant patients, those suffering from nephrotic syndrome and malabsorption syndromes were also excluded from the study. Neither of the patient had any thromboembolic or bleeding manifestations prior to the study. Ethical approval for the study was granted by Ethical and Research Committee Liaquat National Hospital, prior to the study (App # 0040-2011 LNH/ERC).

Method:

All blood samples were collected under aseptic conditions from patients and controls. Blood samples for coagulation studies were collected in sodium citrate 3.2% tubes and centrifuged at 4000 rpm for 15 minutes to separate plasma and processed immediately for Partial thromboplastin time (PTT) and activated partial thromboplastin time (aPTT). Thereafter plasma was stored at -20°C for two weeks, till processed for special coagulation assays in batch. Complete blood counts were measured by sysmex analyzer XT 2000 (Sysmex, Japan). Prothrombin time, activated partial thromboplastin time and protein C were explored by coagulation assay whereas protein S and antithrombin through chromogenic assay on Sysmex CA-1500 (Sysmex, Japan). Deficient levels for protein C and protein S were defined as <70iu/dl while deficiency for antithrombin III was detected at level <80%.[6] Serum ferritin and liver enzymes were detected by HITACHI 912 by photometric assay. Hepatitis B and hepatitis C were established by chemiluminescence method (Abbott Axsym, USA).

Statistical analysis:

Data was entered and analysed using SPSS version 17. The results were expressed as mean±standard deviation (SD) for quantitative variables and qualitative variables are presented as frequency and percentages. Student's 't' test was applied for the comparison of mean. Data were considered statistically significant at p value <0.05.

We also computed spearman correlation at 5% level of significance to identify relationship between the deficiency of protein C, protein S and antithrombin with maternal characteristics (age and gender), haematological parameters, biochemical and coagulations markers. Chi-square test was applied for correlation of prothrombotic markers with hepatitis B and C.

RESULTS

Of the 80 thalassemia major patients, 42 (52.5%) were males and 38 (47.5%) females. The mean age of patients was 12.30±5.5 (range 3 to 24) years. In control group, 32 (53.3%) were male and 28 (46.6%) were female with the mean age of 13.39±4.5 (range 9-22) years.

Protein C, protein S and antithrombin were deficient in 65%, 35% and 20% respectively. Mean levels of all prothrombotic markers were decreased significantly in patient group compared to controls (P<0.001) as shown in Fig-1. The haematological and biochemical parameters of analysed patients and control groups are shown in table-1. Serum ferritin, alanine aminotransferase (ALT) and aspartate transaminase (AST) were significantly higher in patient group versus control group (p<0.001). HbsAg was reactive in 2.5% (2 patients) while Anti HCV was detected in 35% (28 patients) of patients. However, no seropositivity for HbsAg and anti-HCV were seen in control group.

With respect to correlation assessment, protein C deficiency illustrated positive correlation (p=0.035) with protein S insufficiency and antithrombin deficiency demonstrated positive correlation with the low haemoglobin ≤8gm% (p=0.025). There was no statistically significant correlation detected between low levels of protein C, protein S and antithrombin with maternal characteristic (age and gender), hepatic dysfunction, hepatitis reactivity and haematological markers (including platelets count, total lymphocyte count (TLC) count, prothrombin time and activated partial thromboplastin time) (p>0.05).

High serum ferritin showed positive correlation with elevated ALT but no significant correlation was detected with any of the prothrombotic markers studied (p>0.05). In our observation none of our patient exhibited clinical or radiological evidence of venous or arterial thromboembolism during the follow up.

DISCUSSION

With ample blood transfusional support and judicious iron chelation, continued existence to fourth decade of life is not unusual in thalassemia major patients. Owing to promptly managed siderotic complications various non siderotic complications are being realized increasingly.

Table I: Comparison of haematological and biochemical parameters

Evaluated parameters	Patients Mean±SD	Control Mean±SD	P value
Age	12.30±5.5	13.39±4.5	0.291
Haemoglobin	8.30±1.62	12.72±0.94	0.001
Haematocrit	24.65±4.79	38.29±2.22	0.001
Platelets	146.57±92.17	246.50 ±64.80	0.001
Ferritin	4180.65±2485.30	93.9±43.46	0.011
AST	58.8±36.20	24.83±9.38	0.010
ALT	64.63±59.82	23±8.06	0.011
Protein C	58.25±22.5	110.67±22.60	0.001
Protein S	67.90±19.58	98.70 ±21.54	0.001
Antithrombin III	89.73±18.09	104.00±10.98	0.001
PT	12.23±1.65	10.93±0.86	0.012
aPTT	29.15±4.84	27.87±0.90	0.150

Correlation is significant at the P<0.05(2-tailed)

Table II: Frequency of natural anticoagulants deficiency in patients

Parameters	No of patients with deficiency	Deficient (%)
Protein C	52	65
Protein S	28	35
Antithrombin	16	20
Protein C & S	30	37.5
Protein S & Antithrombin	8	10
Protein C & Antithrombin	8	10
Protein C, Protein S & Antithrombin	6	7.5

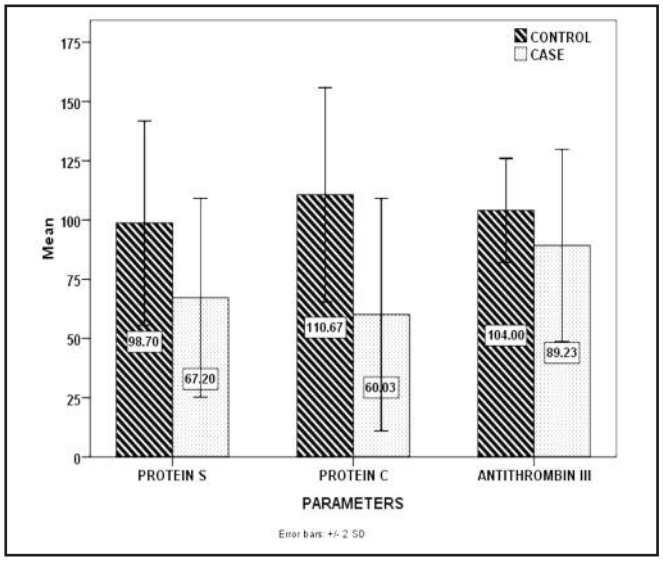

Fig. 1: Comparison between Protein C, protein S and antithrombin in patient and control groups (p<0.001).

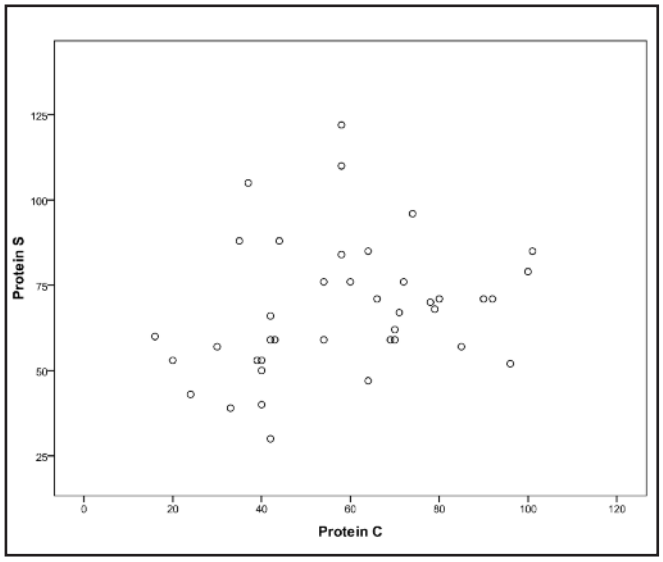

Fig. 2: Positive correlation of protein C and protein S (p=0.035).

In the emerging literature, there is strong evidence that altered levels of coagulation inhibitors and prothrombotic haemostatic alterations are not uncommon in TM patients. Data from seven Italian centres on 720 thalassemia patients revealed 1.1% had thrombosis, however same group previously reported, 3.95% of Thalassemia major had thrombosis.[8,20] In another study the prevalence of thrombosis was 3.3% in thalassemia major.[21] Variable number of thalassemia patients have been claimed to pass away due to this complication. Borgna-Pignatti et al., described 4.1% of deaths in thalassemia individuals; while one earlier study identified 2.5% thalassemia patients expired due to thrombotic manifestation.[20,22]

Low levels of coagulation inhibitors have been observed from various racial backgrounds.[23] When compared with earlier reports, our results are in concurrence with studies from Japan and Italy.[17,24] Similar results had been reported from Egypt and Kuwait.[7,16]

One regional study from India, established predominant antithrombin deficiency in 46.8% of subjects. The protein C and protein S deficiency were 26.2% and 28.6% respectively.[6] It seems that Protein C and protein S deficiency are more pronounced in our thalassemia population as compared with Indian thalassemia population. Moreover, a study from Oakland also found deficient protein C and protein S as a major finding.[9] In accordance to our findings, prior Israeli study also reported noticeably low values of protein S and protein C levels.[11]

The Turkey and Israeli thalassemia patients were also found to have considerably low protein C and protein S whereas no antithrombin deficiency was detected in the Israeli patients.[10,12] Sipahi et al., reported protein S, protein C and antithrombin deficiency in 38.2%, 29.4% and 2.9% respectively in Turkish thalassemia patients. [10] Their study revealed 17.6% patients had combined protein C and S deficiency, while our study has shown distinctly high insufficient levels of combined protein S and C, accountable in 37.5% (Table II). As protein C deficiency correlated positively with protein S insufficiency (p=0.035), probably it reflects the mutual function of both inhibitors in anticoagulation process.

The status of protein C and protein S investigated in another Iranian thalassemia study showed predominance of protein S deficiency in 53% while protein C deficiency was encountered in 43% and 30% were antithrombin deficient.[25] In contradiction to Iranian thalassemia patients, our patients' revealed high prevalence of protein C deficiency while deficiency of protein S and antithrombin III were less prevalent. In parallel to our finding, one study from Japan detected stronger protein C deficiency than protein S.[24]

Studies have revealed that natural anticoagulants in thalassemia major are diminished regardless of age, indicating more consumption.[7,12,23] Deformed red cells present from the early months of existence, probably lead to acquired deficiency at an early age. [23] More or less similar mean protein C levels in adults (0.51±0.11 u/ml) compared to children's (0.46±0.09 u/ml) have been reported from Israel. Likewise, we also did not find statistically deficient protein C, protein S and antithrombin levels related to age: the mean protein C in age group >10 years is 57.89±22.44 while 59.0±23.54 in <10 years of age (p<0.05). In disparity to our results one study from Iran established high frequency of natural anticoagulants deficiency in patients >10 years of age.[25] This difference could be attributed to relatively narrow range of age group in their series (7-20 years) compared to wider age in our series.

In present study, no significant correlation was established between protein C, protein S and antithrombin with serum transaminases and bilirubin, which favours that hepatic impairment, is not the only cause of haemostatic alterations consistent with a prior study.[24] Tripatara also reported that liver damage was not the sole cause of anticoagulants reduction and levels of various coagulation proteins synthesized in liver were not statistically different between patients and control groups.[18]

Furthermore, we considered the consequence of anaemia and established that levels of antithrombin are significantly lower in under transfused patients with haemoglobin <8gm%. The presence of hypercoagulable state in the absence of overt thrombosis in various studies including ours is suggestive of subclinical process of thrombosis in these patients.[13] The same relationship reported earlier, showed pronounced natural anticoagulants deficiency with less pretransfusion haemoglobin.25 But as reported previously,[7] no significant association was detected with protein C deficiency in infrequently transfused patients (<12 transfusions/year), probably accredited to mean haemoglobin of 8.30±1.62 in our series, while all our patients were transfused regularly. As most of the red cells in adequately transfused thalassemia patients are allogeneic in origin so less protein C is likely to be adsorbed because of healthy nature of RBCs.

However beside naturally anticoagulant certain other thrombotic markers are also needs to be evaluated includes factor V leidin mutation and prothrombin polymorphism to evaluate the further thrombotic risk, that's the limitation of this study. Another limitation is the lack of repeat testing, as we detected deficiency on one occasion, which needs to be later reconfirmed on separate occasion.

CONCLUSION

In conclusion, the current study documented that proteins C and protein S are noticeably diminished in thalassemia patients regardless of age, gender, haematological and biochemical status. These outcomes raise the concern whether all the thalassemia individuals need screening for the thrombotic risk and will it be advantageous to suggest prophylactic anticoagulation especially in high risk groups (pregnancy, bed bound, post-operative, post splenectomy, cardiac or pulmonary complications and sepsis). Another question to be answered is to identify risk factors for thrombosis, both acquired and genetics, to have risk stratification. Large prospective controlled trials will be required for specific recommendations regarding screening and for the indications of prophylactic anticoagulation therapy; to whom and at which threshold of deficiency.

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CONFLICT OF INTEREST

None to be declared.

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