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Assessment of Coagulation State and its Related Factors in Thalassemia Intermedia Patients Referred to Thalassemia Research Center at Booali Sina Hospital Sari/IR Iran in 2007

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Abstract: A high incidence of thromboembolic events have been reported among thalassemia intermedia patients especially in splenectomized patients. This study has been conducted to evaluate the coagulation state of patients referred to thalassemia research center at Booali Sina Teaching Hospital, Sari, Iran. This descriptive-cross sectional study was performed in 2007. Sixty thalassemia intermedia patients aged older than 10 years were enrolled. After recording demographic and therapeutic data of the participants, the plasma levels of Coagulant factors were measured. Obtained Data were analyzed using SPSS 13 software and t-test and chi-square tests. Of 60 studied patients, 62% were female. The mean age of the patients was 26.6±9.3 years. ASA tablet were used in 53.3%. None of the patients have experienced thromboembolic events. Decrease in protein C has been observed in 60%. Antithrombin III was decreased in 42% and protein S declined in 10% of the patients. Mutation in factor V leiden was seen in 3%. Splenectomy performed in 53.3% and there was no significant relation between low level of protein C and protein S, antithrombin III and presence of factor V leiden with splenectomy ($p>0.05$). Regarding to the high incidence of low levels of protein C, protein S and antithrombin III among thalassemia intermedia patients, there is an increased risk of thromboembolic events in the patients.

Key words: Beta-thalassemia intermedia, protein C, protein S, antithrombin III, factor V leiden

INTRODUCTION

Thalassemia is a quantitative defect in globin chain synthesis. The prevalence of the disease is high in several parts of the world particularly in Mazandaran Province/ Iran (Kosaryan *et al*., 2007). β-thalassaemia homozygous usually manifests like a chronic hemolytic anemia, as in form of β-thalassaemia major, usually patients need frequent blood transfusion for survival, whereas in thalassaemia intermedia, there is no need for frequent blood transfusion (Kosaryan *et al*., 2007; Dennis *et al*., 2005). The disease can involve several organs by different mechanisms such as chronic hypoxemia, increased iron overload and etc., that their complications on heart, liver, skin, pancreas and hypothalamus are known. Such complications should be considered in routine therapeutic protocol of thalassaemia patients and follow up should be made based on the side effects the (Moratelli *et al*., 1998; Ismaeel *et al*., 2006). Although, the clinical evidences appear the frequent occurrence of thromboembolic events in thalassemia intermedia patients, specially hypercoagulable state in very young patients, this complication has not been emphasized or comprehensively reviewed and in some cases the results of studies were controversial (Eldor *et al*., 1999; Naithani *et al*., 2006; Shebl *et al*., 1999; Eldor *et al*., 1993).

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With regard to importance of thrombotic event on survival of the patients and development of organ failure such as cardiovascular, pulmonary and brain complications and existence of a few evidence related to coagulation state among these patients, this study provides to evaluate coagulation state in thalassemia intermedia patients referred to Booali Sina Hospital in 2007.

MATERIALS AND METHODS

It was a descriptive cross- sectional study in 2007. The studied population was thalassemia intermedia patients aged more than 10 years who referred to Booali Sina Hospital in Sari/Iran. Diagnosis of thalassemia intermedia was based on hemoglobin electrophoresis recorded in the patients' chart. These patients need only occasional or no transfusions. According to previous studies the number of 60 cases (4-10) were selected by systematic random sampling. After selecting cases and taken informed consent from all patients, obtained data about background, therapeutic and diagnostic information from the patients' chart were recorded in a questionnaire. Venous blood (5 cc) in citrate was collected. Plasma were separated by centrifugation and frozen at -80°C. Plasma levels of protein C and protein S, antithrombin III and factor V leiden were measured using France Stago Set. After sampling and separation of plasma, all laboratory tests were done based on kit information. The first stage of sample preparation was performed and the test was done after reassurance of calibration and quality control of the Stago Set in appropriate temperature.

All measurements were performed by oneself and at the same laboratory. Obtained data were analyzed using SPSS software 13 (Holford, 2002) and descriptive statistics as Mean, ±SD and statistical tests such as χ^2 and t-test. $p<0.05$ was considered significant.

RESULTS

Among the 60 patients with demographic data recorded in Table 1, two cases (3%) had prolonged PT and 8 cases (13%) had prolonged PTT. In addition, Antithrombin III concentration were decreased in 42% of patients (CI95%=41.88-42.12) as 60% of them were splenectomized. Decreased Antithrombin III concentration was significant in men ($p<0.017$). However, there was no significant relationship between decreased Antithrombin III concentration and splenectomy ($p<0.3$) (Table 2).

Factor V Leiden was positive in the 2 splenectomized patients (3%) (CI 95% = 2.9-3.1). No significant correlation was detected between Factor V Leiden and sex ($p<0.7$) or splenectomy ($p<0.1$) (Table 2).

Also, 10% (CI 95% = 2.9-3.1) of the patients had low plasma levels of protein S of which only 16.6% were splenectomized. There was no significant relationship between low plasma levels of protein S and sex as well splenectomy ($p<0.058$) (Table 3). Decreased level of protein C was detected in 60% (CI 95% = 59.88-60.12) and 55.5% of these patients were splenectomized. Although no correlation was observed between

Table 1: Distribution of the thalassemia intermedia patients according to demographic characteristics

Variables	No.	%	Mean±SD
Sex			
Male	23	38	
Female	37	62	
Date of diagnosis thalassemia			
≤3 year	25	42	10.81±10
4- 8year	24	40	
≥9 year	11	18	
Patient's age			
10-20 year	19	32	26.6±9.3
20-30 year	23	38	
≥30 year	18	30	
Age of start transfusions			9.2±8.4
Number of transfusions (month)			2.2±1.4
ASA Consumption	53.3	32	
Duration of ASA (year)			5.2±2.9
ASA (mg day^{-1}) dose		100	
Hydroxyurea consumption	54	90	
Duration of Hydroxyurea (year)			5.8±2.7
Dose of Hydroxyurea (mg/kg/day)			13.4±3.9
Splenectomy	32	53.3	
Splenectomy age (year)			18±7.5
Average three last Hb (g dL^{-1})			9.75±1
Presence of cardiomyopathy	7	11.7	
Average three last feritin (ng mL^{-1})			
<1000	50	84	730±490
1000-2000	10	16	
Average three last platlete			
150-450×000	33	54	450±180
>450×1000	27	46	

Table 2: Distribution of the Thalassemia intermedia patients according to Antithrombin III and V leiden factor concentrations by differentiation of sex as well splenectomy

Variables	↓Antithrombin III concentration (%)	p-value	V leiden factor (%)	p-value
Sex				
Male	61	0.016	0	0.18
female	30		5	
Splenectomy				
Yes	47	0.25	6	0.096
No	36		0	

Table 3: Distribution of the thalassemia intermedia patients according to plasma levels of protein C and protein S by differentiation of sex as well splenectomy

Variables	↓protein S concentration (%)	p-value	↓ protein C concentration (%)	p-value
Sex				
Male	9	0.67	83	0.069
Female	11		46	
Splenectomy				
Yes	3	0.05	63	0.59
No	18		57	

splenectomy ($p<0.6$) and decreased level of protein C. Decreased level of protein C was more common among males ($p<0.005$).

DISCUSSION

This study demonstrates that an optimum 60.12% of the patients had decreased level of protein C, whereas, Naithani *et al*. (2006) reported low protein C in 26.2% that was lower than our results, that can be due to more severe vitamin K deficiency, hepatic dysfunction and hemosiderosis among our patients (Naithani *et al*., 2006; Shebl *et al*., 1999).

In present study concentration of Antithrombin III was decreased in 42% of patients. This finding was 46.8% in Naithani *et al*. (2006) that was similar to our study. In another study, decreased Antithrombin III concentration was shown in the majority 42.12% of the patients (Shebl *et al*., 1999).

Protein S was decreased in 10.07% of our patients, which was similar to finding has reported in France (Eldor *et al*., 1999).

In this study, mutation of factor V leiden was seen in 3.1% of the patients. Similarly, the incidence of congenital thrombotic mutation such as factor V leiden was 4% in Eldor study (Eldor *et al*., 1999). Giordano *et al*. (1997) have been reported that mutation of factor V leiden is responsible of more than 50% of known thrombotic events. This obvious different can be related to genetic and regional predisposition. Cappellini *et al*. (2005) have found that the prevalent of thromboembolic events was more in patients who have undergone splenectomy. But in this study no significant correlation was detected between splenectomy and thrombolytic system dysfunction.

It seems that patients' age, usage of asprin and hydroxyurea and the time elapsed after spelenectomy may have roles in thromboembolic events. Our results were confirmed by Shebl *et al*. (1999) in Egypt.

Reduction of protein C, protein S and antithrombin III levels in males were more significant than females in this study that was not considered in pervious study. With regad to this result, thrombolytic events has to be noticed more cautiously in male patients and treatment with aspirin be started for them at lower ages. Consequently, anticoagulant therapy associated with aspirin is recommended in patients with positive clinical findings (Eldor *et al*., 1999).

Coagulation and platelet activation factors are obviously increased in these patients even in lack of thromboembolic events. Low levels of protein C, protein S and antithrombin III in the risk of thromboembolism events is high among thalassemia intermedia patients due to low levels of protein C, protein S and antithrombin III. Of course, most of these thrombosis have no clinical significance and only found in brain and pulmonary authopsies. These thrombosis may have a role in Pulmonary hypertension, decrease lung capacity, hypoxemia and disseminated defects related to right side heart failure. Several etiologic factors may play a role in the pathogenesis of the hyper-coagulable state such as specific changes in the lipid membrane composition of the abnormal RBCs and the hemosiderosis may contribute to the activation of the coagulation process (Eldor *et al*., 1999; Shirahata *et al*., 1992; Cappellini *et al*., 2000).

Considering to the results of this study in thalassemia intermedia patients with evidence of cerebrovascular involvement including sever headache, dizziness and/or in the patients with clinical signs or symptoms of thrombosis as cludication, Pulmonary hypertension, decrease lung capacity, hypoxemia and specially in older male patients with thalassemia, periodical assessments including coagulation tests as well as treatment with anticoagulant with or without aspirin is recommended.

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