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Post Splenectomy Protein C Levels in Beta Thalassaemia Major Patients

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Abstract

Objectives: To compare protein C levels with splenectomy status in beta-TM patients.
Methodology: Cross-sectional comparative study conducted in the Department of Pathology, Al-Nafees Medical College & Hospital, Islamabad in collaboration with the Thalassemia center of Pakistan Institute of Medical Sciences, and Islamabad Diagnostic Center, Islamabad. After written informed consent, 60 diagnosed beta-thalassemia major patients were enrolled based upon inclusion and exclusion criteria, there were 30 splenectomized and 30 non-splenectomized patients. Protein C levels were measured by chromogenic assay. The mean values of protein C were compared between splenectomized and non-splenectomized beta-TM patients by student's t-test. Pearson correlation test was done to find the correlation between protein C and serum Ferritin, ALT, and serum albumin. A p-Value < 0.05 was considered statistically significant.
Results: The mean protein C values in non-splenectomized and splenectomized patients were 62.11±9.65% and 41.64±6.37% respectively with a significant difference by independent samples t-test (p=0.000).There were 45% (n=27) male and 55% (n=33) female patients. The mean age for the selected patients was 12.95 ± 5.43 years. The mean protein C activity in all thalassemia patients was 51.87 % ± 13.12. The mean protein C activity in non-splenectomized patients was 62.11 % ± 9.66.
Conclusion: Splenectomy in thalassemia increases the risk of hypercoagulability and thromboembolic complications. Splenectomized beta-TM patients have decreased protein C activity as compared to non–splenectomized beta-TM patients, signifying decreased activity of anticoagulant mechanisms.
Key Words: Beta thalassemia major, Protein C, Anticoagulant, Hypercoagulability, Splenectomized, Non-splenectomized.

Introduction

Beta thalassemia major (β-TM) is a serious disease burden worldwide. Globally about40 000 infants are born with this disease each year, of whom about 25,500 have transfusion-dependent β-thalassemia.[1]In Pakistan the carrier rate for β-thalassemia is 5-7%.[2] About 5000 children are diagnosed every year in Pakistan with an estimated rate of birth of affected infants of 1.3 per 1000 live births.[3] Transfusion-dependent beta-thalassemia leads to many complications one of these being a chronic hypercoagulable state with increased incidence of thromboembolic episodes, which is variably reported as 4% and 9.6 %.[4,5] Therapeutic splenectomy increases the risk of hypercoagulation in these patients due to decreased removal of procoagulant particles from the circulation.[6]

Cure of β-TM is quite expensive and it is available to only a minority of patients, new research is directed towards the prevention of its complications. Decreased protein C activityis a significant indicator of hypercoagulability in thalassemia patients.[5] No research is done to date in Pakistan to assess levels of natural anticoagulants in thalassemia patients or their relation to splenectomy. This study was done to compare protein C levels between splenectomized and non-splenectomized β-TM patients. It will help to demonstrate the relation of hypercoagulability in thalassemic patients, with splenectomy status. So that, these patients can be vigilantly monitored with protein C levels and timely identified and managed with prophylactic anticoagulants if needed. This will help in preventing prothrombotic complications in these patients.

Methodology

The study was conducted in the Department of Pathology, Al-Nafees Medical College & Hospital,

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Islamabad in collaboration with the Thalassemia center of Pakistan Institute of Medical Sciences, and Islamabad Diagnostic Center, Islamabad. A comparative cross-sectional study design was used. A convenient sampling technique was applied for study proceedings. A total of 60 (N) thalassemia patients were registered in the study. There were 30(n) splenectomized (Sp) and 30(n) non-splenectomized (NSp) patients. Both the groups were further divided into 3 subgroups depending upon the age i.e. Group I included patients from 6 - 10 years, Group II included patients from 11-18 years and Group III included patients from 19 to 35 years. Patients who were seropositive for hepatitis B or C or had liver enzymes more than 5 times the normal range, or who had undergone a splenectomy less than 6 months before the time of enrollment in the study were excluded. Patients with prolonged PT or APTT or on vitamin K antagonist anticoagulation therapy were also excluded. All data were collected by using a research proforma.

Protein C was measured by a chromogenic assay using citrated venous blood. To determine the integrity of liver function serum Albumin, Alanine transferase (ALT), was also measured using an automated chemistry analyzer. Serum ferritin levels were measured by the ELISA method.

A Software Package for Social Sciences, version 20.0 was used for the statistical analysis of data. Quantitative variables i.e. age, and protein C levels, were given as mean ± SD. Qualitative variables i.e. gender were given as frequencies and percentages. Independent samples t-test was applied for comparison of means of protein C levels in splenectomized and non-splenectomized groups. Pearson correlation test was done to find the correlation between protein C and serum Ferritin, ALT, and serum albumin. A p-Value < 0.05 was considered statistically significant.

Results

A total of 60 thalassemic patients were included in the study, with 30 having undergone therapeutic splenectomy at least 6 months ago. There were 45% (n=27) male and 55% (n=33) female patients. The mean age for the selected patients was 12.95 ± 5.43 years. Among the non-splenectomized patients, the mean age was 12.56±5.06 years, among the splenectomized patients, the mean age was 13.35±5.84 years. Independent samples t-test showed an insignificant variation in age among the two groups implying a similar age distribution in both groups (Table I)

The mean protein C activity in all thalassemia patients was 51.87 % ± 13.12. The mean protein C activity in non-splenectomized patients was 62.11 % ± 9.66. In the splenectomized group, the mean protein C activity level was considerably low at 41.64% ±6.36. Independent samples t-test was applied to compare the means of protein C activity values. A highly significant p-value was obtained on comparison i.e. p= 0.000.(Table II).

To determine the effect of iron load mediated liver dysfunction on protein C, a correlation was done between protein C levels and serum ferritin, serum ALT, and serum albumin. Pearson's correlation analysis was used which showed no significant correlation between

Table I: Independent samples t-test for comparison of mean ages (years) in splenectomized and non-splenectomized patients (n = 60)

Group	Splenectomy status		t-test value	p-value
	NspMean ±SD (n=30)	SpMean ±SD(n=30)		
Group I	8.00±1.65(n=12)	7.13±1.36 (n=11)	1.36	0.188
Group II	13.16±2.40 (n=12)	14.92±2.75 (n=13)	1.69	0.104
Group III	20.50±1.87 (n=6)	21.33±2.94 (n=6)	0.58	0.571
Cumulative Mean	12.57±5.06	13.35±5.85	0.55	0.581

Table II: Independent samples t-test for comparison of mean protein C activity % between splenectomized and non-splenectomized patients.

Groups (N=60)	Mean protein C activity in Non-splenectomized patients (n=30)	Mean protein C activity in Splenectomized patients (n=30)	Value of t-test	p-Value
Group I	65.56±13.57(n=12)	46.16±5.20 (n=11)	4.442	0.000
Group II	60.42±5.18 (n=12)	39.44±6.14 (n=13)	9.191	0.000
Group III	58.57±5.11 (n=6)	38.13±4.24 (n=6)	7.527	0.000
Cumulative Mean	62.11± 9.65 (n=30)	41.64±6.37 (n=30)	9.689	0.000

protein C levels and serum ferritin, serum ALT, and serum albumin. This is shown in Figure I. Moreover, serum albumin which represents the synthetic function of the liver was within the normal range. Thus, liver functional impairment due to iron overload was not the cause of low protein C levels in thalassemic patients.

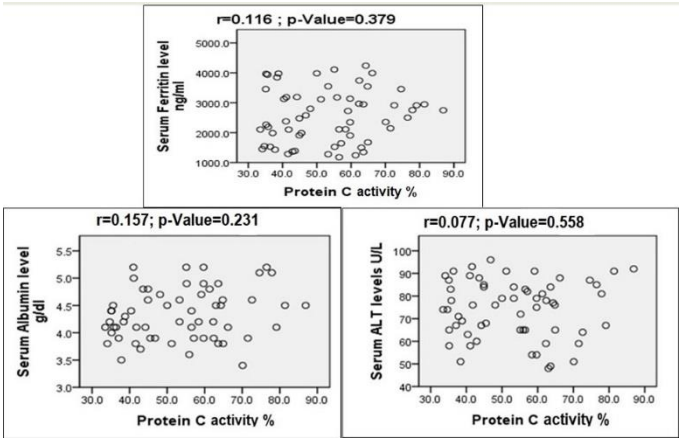

Figure 1. Pearson Correlation analysis of Serum Ferritin, Albumin, and ALT with protein C activity levels showing no correlation for each parameter.

Discussion

Advancements in therapeutics and improved medical care, have increased the survival and life expectancy of thalassemic patients.[7,8] However, the disease course is not free of complications. As such, the number of complications in multiple transfused thalassemia patients is increasingly observed. Abnormalities in the coagulation system are one of the complications in these patients.[6] Derangements in the measured levels of clotting factors and their inhibitors as well as their activity levels are now more frequently noted in thalassemic patients.[9]

It has been postulated that hypercoagulability is due to the accumulation of procoagulant particles released from the damaged RBCs and the endothelial cells.[5,10,11] To demonstrate this subclinical hypercoagulable state in thalassemic patients, various researchers have employed different coagulation parameters. These include protein C, antithrombin, protein S, D-Dimer, fibrinopeptide A and thrombin-antithrombin complex.[5,9]

Splenectomy is one of the common therapeutic strategies to increase patient survival and hemoglobin levels. However, this intervention has its repercussions. With splenectomy comes increased levels of procoagulant microparticles released from the damaged RBCs and the endothelium.[12]It has been observed that post-splenectomized thalassemic patients present with hypercoagulable complications with increasing frequency. [13]

In the current study, it was observed that the protein C activity levels were considerably less than normal. The mean activity level of protein C in all the enrolled thalassemic patients in the study was 51.87% ± 13.12. Sultan, S reported a similar mean protein C activity level of 58.25%±22.5 in thalassemic patients which is in agreement with the current study.[14] Another study reported low levels of Protein C and Protein S in thalassemia major children as compared to normal controls.[10] Similarly, low mean levels of protein C in thalassemic patients vs normal subjects (82.50% vs 104%) and (64.81 ± 17.0vs 102.67 ± 19.21) were reported by Abd El Mabood et al in 2018 and Singh in 2021 respectively.[15,16] Another study in 2020 demonstrated lower mean protein C (71.31%) and protein S (34.3%) levels in thalassemia major patients in comparison to normal controls.[17] These results establish the fact that a hypercoagulable state is present in thalassemic patients as depicted by lower protein C activity levels in these patients.

Some researchers propose the levels of natural anticoagulants are low due to liver dysfunction brought about by serum ferritin overload.[18] However, in the current study the protein C levels were independent of serum ferritin, and ALT levels. Moreover, serum albumin a measure of hepatic synthetic function was normal in all study subjects. This has also been emphasized by Singh S. who states that the deranged hemostatic parameters in thalassemic patients are not due to raised ferritin levels and liver dysfunction. [16]

In the current study, the comparison of mean protein C levels by independent samples t-test resulted in a highly significant difference among the splenectomized and non-splenectomized groups with a (p=0.000). This indicates that the hypercoagulable state in thalassemic patients is more pronounced in splenectomized patients as compared to non-splenectomized ones. Sparse data is however available related to determining the relation of splenectomy status with the hypercoagulable state as determined by protein C levels. In a study by Abd El Mabood, the mean protein C activity level of 75% (32 - 131) in splenectomized and 97.5% (36 - 175) in the non-

splenectomized group was obtained with a significant p-Value (p=0.011) on comparing the two groups. He also observed that the hypercoagulable state was more evident in older patients as compared to younger subjects.[15]

Mostafa M. reported a mean protein C activity level of 58.94 ± 6.52 in splenectomized and 64.27 ±7.03 in non-splenectomized thalassemia groups.(19)Also another study stated similar findings in addition to elevated endothelial protein C receptor levels in splenectomized thalassemic patients.[20]These studies support our findings that protein C values are lower in splenectomized thalassemia patients. However, some researchers have revealed conflicting conclusions as regards the effect of splenectomy on protein C and have failed to find any such significant difference with splenectomy status.[21]

To demonstrate a higher level of hypercoagulability in splenectomized thalassemic patients vs. non-splenectomized patients, some authors have employed other parameters for measuring hypercoagulability including measurements of erythrocyte microparticles and EPCRs.[22–24]They found a significant difference between the two groups and a hypercoagulable state more pronounced in splenectomized patients.

Conclusion

Splenectomy in thalasemia increases the risk of hypercoagulability and thromboembolic complications. Splenectomized beta-TM patients have decreased protein C activity as compared to non–splenectomized beta-TM patients, signifying decreased activity of anticoagulant mechanisms.

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