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Determination of Serum C, S Proteins and Factor V Leiden among Patients with Sickle Cell Disorder at Khuzestan Province, Iran

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Abstract

Background: Sickle cell disease occurs due to a mutation in β chains and the substitution of valine instead of glutamate in the sixth position of the ß-chain that causes polymerization and vascular blockage. The aim of this study was to compare the serum C, S proteins and factor V Leiden between sickle cell patients and the control group.

Materials and Methods: In this case-control study, performed in Khuzestan province, Iran, from 2008 to 2009, C and S proteins as well as factor V Leiden activity were measured in 100 patients with sickle cell disease and compared with 50 patient in the control group that matched in age, gender and race. The type of sickle cell disease, hydroxyurea intake, blood transfusions, the level of HbF, age and gender were also analyzed.

Results: Out of 100 sickle cell patients entering the study 47 patients were males and rests of them were females. The mean age of patients was 20.2± 1.03 years (Range 3 to 58 years). The level of protein C and S were low in 35% and 24% of patients respectively, but in controls both were normal (P<0.0001). Factor V Leiden was increased in 27% of patient and 4% of controls (P=0.001). A significant relationship was detected between protein S levels and patients' age (P=0.02).

Conclusion: Among patients with sickle cell disease, protein C and S levels are reduced and factor V Leiden activity is increased compared to controls, which might cause hyper-coagulation state among these patients.

Keywords: Sickle cell disease, protein C, protein S, Factor V Leiden

Introduction

Sickle cell disease (SCD), prevalent in malaria –prone areas, is one of the oldest diseases and its mutation has happened 50000 years ago [1].The severity of disease depends on the source of hemoglobin S mutation like Senegal, Benin, Bantu or Car mutations [1]. Hemoglobin S is created by a mutation in hemoglobin β chain. Blood vessel stenosis among these patients causes prevalent complications such as pain attacks, acute chest syndrome and splenic sequestration. Recently hypercoagulopathy has been considered in these patients as a cause for thrombosis formation and increased complications, but limited information is available regarding this subject [1].There have been some studies with variant results considering the use of anticoagulant drugs among these patients and the positive effect of Heparin has been reported [2, 3]. Protein C and its cofactor, protein S, are the main anticoagulant proteins that inhibit the coagulation by inhibiting the active V and VIII factors [4]. The probability of protein C deficiency has been reported from 0.2% in general population to 20% in sickle cell patients and considering the protein S these numbers are from 0.7% in general population to 15% among patients with sickle cell anemia [5-9]. Also, factor V Leiden has been considered as another factor which increases the coagulability and thrombosis among these patients [10].

Because of the high incidence of SCD in our area and different kinds of SCD accruing among our patients, we decided to determine the amount of protein C and S as well as factor V Leiden in patients

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with sickle cell disorders in Khuzestan province which has the most numbers of sickle cell patients in Iran.

Materials and Methods

In this case control study Arab ethnicity patients, who were suffering from sickle cell disease in the Khuzestan province, south of Iran, and were referred to the Shafa hospital, entered the study. Those patients, in whom the sickle cell disease was in doubt or did not have positive sickle PorB, or were using Heparin or Warfarin before sampling, were excluded from the study. The controls were selected from cases who had no history of hemoglobinopathy or anticoagulant drugs usage and had Arab ethnicity. Patients and control group were matched through gender and age. After receiving consent from the patient and filling up the questionnaire, 1.8cc blood was taken from each patient and mixed with 0.2cc sodium citrate in a plastic container. The samples were sent to laboratory after coding. Samples were taken from the control group in the same manner. The samples were centrifuged (2500 round per second) for 10-15 minutes and then the surface plasma was collected and maintained in freezer in -20 centigrade. Samples were evaluated for protein C, protein S and factor V Leiden content using special ACL kits and a full automatic coagulometer analyzer (Beckman/Instrumentation Laboratory ACL 8000 - Coagulation Analyzer). The normal range of protein C was considered (40%-92%) for ages 1 to 5, (45%-93%) for ages 6 to 10, (55%-110%) for ages 11 to 16 and (64%-128%) for over 16 years of age; and the normal activity range of protein S was considered; (58%-118%) for ages 1 to 6, (41%-114) for ages 6 to 11, (52%-92%) for ages 11 to 16 and (60%-113) for over 16 years of age. To evaluate the factor V Leiden we used resistance to activated protein C (APCR) method. Patients were divided in four age groups , 1-6 , 6-11, 11-16 and over than 16 years. We also grouped patients considering the amount of F hemoglobin in electrophoresis into three groups: under %10, %10-20, and over 20%. The levels of protein C and S and factor V Leiden were compared between patients and the control group according to the kind of disease, age and gender group, receiving hydroxyurea and transfusion. We estimated the sample size based on the deficiency of protein C and S and high level factor V Leiden to be found in 20% of patients with sickle cell disease. The results were evaluated using SPSS software (version 16.0, SPSS Co, Chicago IL) P values less than 0.05 were considered statistically significant.

Results

In the case group forty seven patients (47%) were male and 53 patients (53%) were female with male to female ratio of 0.88 and in the control group 23 participants (46%) were male and 27(54%) were female with a male to female ratio 0.85. The mean age in the case group was 20.2 ±1.03 (range 3 to 68 years) and in control group it was 20.8±1.08 (range 3 to 52 years). Among 100 patients we had 62 SS patients (62%), 25 SB0 patients (25%) and 13 SB+ patients (13%) and there were no SC cases. Fifty seven percent of patient did not use hydroxyurea; %11 of them had used it for less than 6 months and 32% of them for more than 6 months. Twenty one percent of patients did not give a history of transfusion, 73% had irregular and %6 had regular transfusions. Six percent of patients were in 1-5 age group, 12% in 6-10, 18% in 11-16 and 64% in over 16 years group. In 32% of patients the amount of F Hemoglobin was less than 10% , in 39% it was 10%-20% and in 29% was more than 20%.Serum level of protein C was lower than normal in 35 patients (35%) while in all controls it was normal (p <0.0001). Serum level of protein S was lower than normal in 24 patients (%24) but all controls were in normal range (p=0.0001). Factor V Leiden was more than normal in 27 patients (%27) and in controls

Table 1: Frequency of C/S proteins and factor V Leiden in all kinds of sickle cell.

Range (mean)	SS	SB^0	SB^+	p
Protein C deficiency %	73(11.7-162)	62.5(9.5-96.3)	77.8(41.2-166)	0.136
Protein S deficiency %	80.8(10.1-167)	83.1(25.2-167)	85.35(27.4-172)	0.9
Factor v Leiden increase	2.29(1.4-3.3)	2.32(1.6-3.1)	2.33(1.6-3.1)	0.911

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Table 2: Frequency of C/S proteins and factor V Leiden in sickle cell patients with transfusion.

Amount	Non transfusion	Irregular transfusion	Irregular transfusion	p
Protein C deficiency%	75.3 (11.7-166)	71.2 (9.5-162)	53.5 (39.5-67.3)	0.191
Protein S deficiency%	80.9 (28-150)	84.3 (10.7-172)	57.4(16.4-95.2)	0.197
Factor V Leiden increase	2.44(1.4-3.3)	2.28(1.5-3.1)	2.08(1.6-2.5)	0.096

Table 3: Frequency of C/S proteins and factor V Leiden in sickle cell patients in different age groups.

Amount	Under 5 years	6-10	11-16	Over 16 Years	p
Protein C deficiency%	69.75(9.5-93.8)	60.8(45.9-81.6)	62.2(11.7-104)	75.55(14.1-166)	0.112
Protein S deficiency%	109(82-144)	81.5(26.5-145)	62.6(10.7-150)	85(16.4-172)	0.02
Factor V Leiden increase	2.48(1.9-3.1)	2.35(1.7-2.7)	2.27(1.5-3.3)	2.280(1.4-3.1)	0.679

2 participants (%4) showed more than normal levels (Table 1) (p<0.0001). There was a low level of either protein C or S in 59% of patients and in %12 of patients showed low levels of both proteins. Also in %5 of patients the deficiency of both C and S anticoagulation proteins were concomitant with increased factor V Leiden. The mean level of protein C was higher among SB+ patients and lower in SB0 patients compared to average but the difference was not statistically significant. Although the mean level of protein S in SB+ patients was higher and in SS patients was lower than the average, but again the difference was not statistically significant. There were also no statistically significant difference between the level of factor V Leiden in different kinds of sickle cell diseases. There was a negative relationship between protein C level and the transfusion requirements but it was not statistically significant (p=0.191). Also the level of protein S in cases with regular transfusions was lower than the others but again it was not statistically significant (p=0.197). There was not a statistically significant not relationship between transfusion requirements and factor V Leiden (p=0.096) (Table3). We also did not observe any statistically significant relation between the levels of protein C, S and factor V Leiden with the amount of F hemoglobin. There was no statistically significant relation between the level of protein C and age (p=0.112).The level of protein S was low in 11 to 16 years old age group and high in under 5 years old age group (p=0.02). There was no statistically significant relation between the level of factor V Leiden and age groups (p=0.679).

Discussion

In our study the level of protein C in %35 of patients with sickle cell disease was low compared to controls (p<0.0001), that was similar to results of studies by Bayazit et al. and Westerman et al. [6, 11], but in a study by Francis the level of protein C has been reported to be low in only %20 of patients [9]. Also in line with other studies, the protein S level was low in our patients was significantly lower than the control group [4,6].

The high level of factor V Leiden in our patients might be due to measurement method of V Leiden activity which is influenced by many factors. In a study by Khan et al. factor V Leiden had no effect on sickle cell complications which shows the need for performing more studies in this field [12]. In our study the average level of protein C and S in SB+ sickle cell patients was more than others but this difference was not statically significant. Also, the factor V Leiden level did not show a difference in different kinds of SCD. We also did not find a statistically significant relationship between the average levels of C and S proteins as well as factor V Leiden with hydroxyurea usage. A similar study in turkey on C an S proteins has indicated that both proteins are increased after using hydroxyurea only the C protein increase was significant [8]. Although the average protein C and S levels in patients who had regular transfusions were low and their average factor V Leiden was high but the difference was not statistically significant. There was also no statistically significant relationship between the averages levels of C S proteins as well as factor V Leiden and the amount of hemoglobin F. The

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average of protein C had no statistically significant relation with increasing age . The mean of S protein in 11-16 years old group was less than the other groups and the difference was statistically significant (p=0.02). There was no statistically significant relation between the factor V Leiden and age. In our study %12 of patients showed both a lower level of both protein C and S that mioght increase the risk of thrombosis in these cases.

Conclusion

Among patients with sickle cell disease, protein C and S levels are reduced and factor V Leiden activity is increased compared to controls, which might cause hyper-coagulation state among these patients.

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