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ORIGINAL ARTICLE

Thrombophilic Mutations Among Patients with Sickle Cell Disease

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SUMMARY

Background: **Factor V-Leiden (FVL), Prothrombin (PRT) G20210A, and Methylene Tetrahydro Folate Reductase (MTHFR) C677T and A1298C mutations are major inherited risk factors of thrombotic complications. Our aim in this study was to investigate the prevalence of these mutations among Tunisian sickle cell patients.**
Methods: **Study subjects comprised 64 patients and 100 healthy controls. FVL, PRT G20210A, and MTHFR genotypes were determined using a reverse dot blot based method.**
Results: **In the patient population studied, the prevalence of FV Leiden was not statistically different from controls while a significant prevalence of heterozygous PRT G20210A mutation among patients (10.93%) was found. An increased frequency of the MTHFR 677 C>T genotype was seen among patients as well as controls. The results showed no significant association between the MTHFR A1298C mutation and sickle cell disease (SCD). However, the prevalence of carrier among studied patients was 15.62% compared to 7% among healthy subjects.**
Conclusions: **In conclusion, our data suggest a significant association between PRT G20210A and MTHFR C677T and sickle cell disease among Tunisian patients.**
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KEY WORDS

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INTRODUCTION

Hemoglobin S (Hb S) is the most frequent hemoglobin variant resulting from the substitution of valine for glutamic acid at the sixth amino acid (ß6 Glu→val) of the β-globin chain [1].
This hemoglobin in homozygous state or in combination with one of the structural Hb variants such as Hb D-Punjab, Hb O-Arab, Hb C or β-thalassemia mutation results in sickle cell disease (SCD) that is characterized by chronic hemolytic anemia and tissue injury secondary to vaso-oclusion [2].
SCD, one of the most common inherited diseases in the world, is known as a hypercoagulable state in which various hemostatic systems both in steady state and during vaso-occlusion are perturbed with increased activation of the coagulation system and platelets, thrombin generation, and occurrence of thrombosis [3-5].

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In the present study, we investigated the hypothesis that the presence of the factor V gene G1691A mutation (factor V Leiden), the prothrombin gene G20210A variant, and MTHFR (methylenetetrahydrofolate reductase) C677T and A1298C polymorphisms may be a risk factor for vascular complications in individuals with SCD.

MATERIALS AND METHODS

Study subjects

The study enrolled 64 patients including 35 with sickle cell anemia (SS), 20 with sickle/thalassemia (S/thal), 7 compound heterozygous HbS/Hb C, and 2 compound heterozygous HbS/Hb O-Arab.

The study population consisted of 26 males and 38 females, aged between 3 and 27 years, were recruited from the Children's Hospital pediatric departments for revaluation of chronic anemia.

This population was gender and age matched with a control population of 100 subjects referred to the Laboratory of Molecular Biology in Military Hospital of Tunis with uncomplicated histories.

The present study was approved by the Ethical Committee of the Military Hospital of Tunis, Tunisia, and it conforms to the provisions of the Declaration of Helsinki. The study was conducted after all patients or their parents had given their full informed consent.

Detection of mutations

Total genomic DNA was isolated from peripheral blood leukocytes by DNA extraction kit (QIAmp blood kit, Qiagen GmbH, Hilden Germany) according to the manufacturer's protocol. A multiplex amplification with biotinylated primers was used to amplify the genomic DNA. The polymerase chain reaction (PCR) products were subjected to a reverse hybridization using the GenoType Factor V Leiden, Prothrombin G20210A, and MTHFR test that is based on the DNA.STRIP technology (Hain Lifescience GmbH, Nehren, Germany) and allowing the molecular characterization of the Factor V Leiden, Prothrombin G20210A, and MTHFR C677T and A1298C mutations of the human gene.

Statistical analysis

All analyses related to the case-control study were performed using the Epi Info™ version 7. Differences between cases and controls were evaluated by using the chi-square test for qualitative variables. In addition, the odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. Probability values $p < 0.05$ were considered statistically significant.

RESULTS

FV Leiden, PRT G20210A, and MTHFR C677T, and A1298C Prevalence

The results showed that there were 8 heterozygous patients (G/A) giving a prevalence rate of 12.5% for the FV Leiden G1691A mutation, and none was a homozygous (A/A) carrier. In the control group, 5 were heterozygous and 1 was a homozygous carrier giving a prevalence rate of 6%, this did not reach statistical significance (Table 1).

The PRT G20210A mutation was identified in heterozygous state with a prevalence significantly higher among patients (10.93%) than in controls (3%) ($p = 0.03$; OR = 3.97; 95% CI (0.85 - 24.51).

In contrast, a significant increase in MTHFR C677T carrier rate was seen in SCD patients (49/64; 76.56%) compared to healthy subjects (40/100; 40%).

The prevalence of C/T genotype was higher among patients (75%) than in controls (35%). Whereas the prevalence of T/T genotype was higher among controls (5%) than in patients (1.56%), this did not reach statistical significance (Table 1).

No significant association between the MTHFR A1298C mutation and SCD was found. There were 2 homozygous (CC) and 8 heterozygous (AC) MTHFR A1298C individuals, while 7 control subjects were heterozygous.

DISCUSSION

Several genetic factors contribute to the phenotypic diversity of SCD, and genetic polymorphisms associated with thrombophilia were implicated as genetic modifiers of SCD [6].

Insofar as FV Leiden, PRT G20210A, and MTHFR C677T were implicated in the pathogenesis of thrombotic events, we aimed to assess their prevalence among SCD patients.

In the study population, the prevalence of FV Leiden was not different from controls. Our results are in agreement with the Wright et al. study conducted among SCD patients from Jamaica, where no FV Leiden mutation was found [7]. In another study from Brazil, Andrade et al. studied the prevalence of the FV Leiden mutation, MTHFR C677T polymorphism, and prothrombin gene variant in SCD patients [8]. No significant difference was reported between SCD patients and the control population for the prevalence of the studied thrombophilic mutations. However, among nine SCD patients with vascular complications of stroke or deep vein thrombosis they found only one patient to be a carrier for FV Leiden.

In contrast, in spite of the moderate prevalence of FV Leiden mutation (2.97 - 5.5%) among normal population of Iran [9,10], a high prevalence of FV Leiden mutation (14.3%) among Iranian sickle cell anemia (SCA) patients has been found to show association between

Table 1. FV Leiden, PRT G20210A, and MTHFR C677T, and A1298C Prevalence.

Mutation	Genotype	Patients	Controls	X^2	OR	95% CI	p	R
Factor V	1691GG	56	94	2.11	0.44	0.12 - 1.56	0.14	NS
	1691AG	8	5	3.00	2.71	0.73 - 11.01	0.08	NS
	1691AA	0	1	0.05	0.00	0.00 - 60.93	0.82	NS
Factor II	20210GG	57	97	4.29	0 .25	0.04 - 1.16	0.03	A-
	20210AG	7	3	4.29	3.97	0.85 - 24.51	0.03	A+
	20210AA	0	0	0.51	0.00	0.00 - 4.24	0.47	NS
MTHFR C677T	677CC	15	60	21.02	0.20	0.09 - 0.43	< 0.0001	A-
	677CT	48	35	24.97	5.57	2.63 - 12.00	< 0.0001	A+
	677TT	1	5	0.51	0.30	0.0063 - 2.8	0.47	NS
MTHFR A1298C	1298AA	54	93	3.12	0.40	0.12 - 1.26	0.077	NS
	1298AC	8	7	1.42	1.89	0.56 - 6.48	0.23	NS
	1298CC	2	0	1.10	ND	0.29 - (-1.00)	0.29	NS

R - result, NS - no significant, A- - negative association, A+ - positive association.

this mutation and SCA (OR = 6.5 CI [1.19 - 35.33] p = 0.03) [11].

Our study showed a significant prevalence of heterozygous PRT G20210A mutation among patients (10.93%). No homozygous prothrombin G20210A mutation among patients and controls was found, and Moreira Neto et al. reported no prothrombin G20210A mutation in SCD patients from Brazil [12].

On the other hand, MTHFR C677T appears to play a role in SCD, justified by increased prevalence of C/T genotype among SCD patients. In a study from Brazil it was suggested that MTHFR C677T might be considered a risk factor for vascular complications in SCD [12].

According to the literature, there have been three studies of inherited risk factors of venous thromboembolism in SCD patients from southern Mediterranean countries which reported a high prevalence of thrombophilic mutations in SCD patients and their association with thromboembolism in these patients [13-15]. Among Lebanese sickle/β0-thalassemia patients, a high prevalence of thrombophilic mutations of FV Leiden (42%), homozygous and heterozygous MTHFR C677T (59%), and prothrombin G20210A (8%) has been reported [13]. In this report, sickle-β-thalassemia patients were 5.24 and 4.39 times more likely to have FV Leiden mutation as compared to the normal controls and thalassemia intermedia patients, respectively ($p < 0.05$).

Also, the presence of extensive large vessel thrombosis in a sickle/β0-thalassemia patient from Lebanon homozygous for FVL and heterozygous for MTHFR has been reported [14].

Further, in a sickle cell anemia patient from Israel, Koren et al. [15] reported the recurrence of cerebrovascular incidents and deep venous thrombosis. Activated protein C resistance due to heterozygous FV Leiden and C677T MTHFR have been diagnosed and suspected to be the risk factors that contribute to the development of the deep vein thrombosis in this SCA patient.

In contrast, in the study of Fawaz et al. [16], the prevalence of FV Leiden, prothrombin, and MTHFR C677T mutations in SCD patients and controls from eastern Saudi Arabia were similar revealing thus no association between the mutations and SCD.

For the A1298C mutation, no studies about the effect of this polymorphism in the MTHFR gene on the risk of vascular complications in SCD have been reported. Our study showed no significant association between the MTHFR A1298C mutation and SCD. However, the prevalence of a carrier among studied patients was 15.62% compared to 7% from healthy subjects.

CONCLUSION

Our data suggest a significant association between PRT G20210A and MTHFR C677T and sickle cell disease among Tunisian patients. A low impact of Factor V Leiden and MTHFR A1298C in the pathogenesis of SCD and/or its complications was found.

Association between inherited risk factors of thrombosis with sickle cell disease should be further studied in an attempt to clarify the relation between clinical phenotype and these genetic markers.

Ethical:

The present study was approved by the Ethical Committee of the Military Hospital of Tunis, Tunisia and it conforms to the provisions of the Declaration of Helsinki. The study was conducted after all patients or their par-

ents had given their full informed consent.

Declaration of Interest:
There is no conflict of interest.

References:

1. Pauling L, Itano HA, Singer SJ, Wells IG. Sickle cell anemia, a molecular disease. Science. 1949;110:543-8. (PMID: 15395398).

2. Rahimi Z, Parsian A. Sickle Cell Disease and Venous Thromboembolism. Mediterranean Journal of Hematology and Infectious Diseases. 2011;3:e2011024. http://mjhid.org/index.php/mjhid/article/view/2011.024

3. Ataga KI, Cappellini MD, Rachmilewitz EA. Beta-thalassaemia and sickle cell anaemia as paradigms of hypercoagulability. Br J Hematol 2007;139:3-13 (PMID: 17854302).

4. Francis RB. Large-vessel occlusion in sickle cell disease: pathogenesis, clinical consequences, and therapeutic implications. Med Hypotheses 1991;35:88-95 (PMID: 1890982).

5. Nsiri B, Gritli N, Bayoudh F, Messaoud T, Fattoum S, Machghoul S. Abnormalities of coagulation and fibrinolysis in homozygous sickle cell disease. Hematol Cell Ther 1996;38:279-84 (PMID: 8974793).

6. Zimmerman SA, Ware RE. Inherited DNA mutations contributing to thrombotic complications in patients with sickle cell disease. Am J Hematol 1998;59:267-72 (PMID: 9840906).

7. Wright JG, Cooper P, Malia RG, Kulozik AE, Vetter B, Thomas P, Preston FE, Serjent GR. Activated protein C resistance in homozygous sickle cell disease. Br J Hematol 1997;96:854-6 (PMID: 9074431).

8. Andrade FL, Annichino-Bizzacchi JM, Saad STO, Costa FF, Arruda VR. Prothrombin mutant, factor V Leiden, and thermolabile variant of methylenetetrahydrofolate reductase among patients with sickle cell disease in Brazil. Am J Hemato1998;59:46-50 (PMID: 9723576).

9. Rahimi Z, Vaisi-Raygani A, Mozafari H, Kharrazi H, Rezaei M, Nagel RL. Prevalence of factor V Leiden (G1691A) and prothrombin (G20210A) among Kurdish population from Western Iran. J Thromb Thrombolysis. 2008;25:280-3 (PMID: 17700999).

10. Zeinali S, Duca F, Zarbakhsh B, Tagliabue L, Mannucci PM. Thrombophilic mutations in Iran. Thromb Haemost 2000;83:351-2 (PMID: 10739401).

11. Rahimi Z, Vaisi-Raygani A, Nagel RL, Muniz A. Thrombophilic mutations among Southern Iranian patients with sickle cell disease: high prevalence of factor V Leiden. J Thromb Thrombolysis 2008;25:288-92 (PMID: 17619828).

12. Moreira Neto F, Lourenço DM, Noguti MAE, et al. The clinical impact of MTHFR polymorphism on the vascular complications of sickle cell disease. Brazilian J Med Biol Res 2006;39:1291-5 (PMID: 16906320).

13. Isma'eel H, Arnaout MS, Shamseddeen AW, et al. Screening for inherited thrombophilia might be warranted among Eastern Mediterranean sickle-β0-thalassemia patients. J Thromb Thrombolys 2006;22:121-3 (PMID: 17008978).

14. Otrock ZK, Mahfouz RA, Taher AT. Should we screen Eastern Mediterranean sickle beta-thalassemia patients for inherited thrombophilia? J Thromb Haemost 2005;3:599-600 (PMID: 1574 8263).

15. Koren A, Zalman L, Levin C, Abu Hana M, Mader R, Shalev S. Venous thromboembolism, factor V Leiden, and methylenetetrahydrofolate reductase in a sickle cell anemia patient. Pediatr Hematol Oncol 1999;16:469-72 (PMID: 10505325).

16. Fawaz NA, Bashawery L, Al-Sheikh I, Qatari A, Al-Othman SS, Almawi WY. Factor V-Leiden, prothrombin G20210A, and MTHFR C677T mutations among patients with sickle cell disease in Eastern Saudi Arabia. Am J Hematol 2004; 76:307-9 (PMID: 15224376).