

Investigating the prevalence of FXIIIVal34Leu polymorphism in the thalassemic patients of Ilam City

Farajolah Maleki¹, Mitra Azizian¹, Masoumeh Shohani², Mansour Amraei^{*3}

1. Clinical Microbiology Research Center, Paramedical Faculty, Ilam University of Medical Sciences, Ilam, Iran
2. Department of Nursing, Paramedical Faculty, Ilam University of Medical Sciences, Ilam, Iran
3. Department of Physiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

*Corresponding author: Tel: +98 8432235724; fax: +98 8432235724

Address: Department of Physiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran

E-mail: amraei-m@medilam.ac.ir

Received; 2015/03/16 revised; 2015/03/20 accepted 2015/04/7

Abstract

Introduction: The coagulation factor 13 has a fundamental role in homeostasis, protective effects on thrombosis, and some other associated diseases. Due to increasing the chronic coagulability of major thalassemic patients, this study was conducted with aim determining the prevalence of Val34LeuFXIII polymorphism in the thalassemic patients.

Materials and methods: The present case-control study was conducted on 100 patients with major thalassemia with a gender ratio of 50 females and 50 males as case and 100 healthy persons as control with a gender combination of 50 males and 50 females that were selected with the referral order on the different days. DNA extraction and the polymerase chain reaction (PCR) were conducted. The genotypes of this polymorphism were identified by RFLP technique and CfoI restrictive enzyme. The analysis of data was conducted by SPSS 11.5 software, chi-square, and logistic regression statistical tests. $P < 0.05$ was considered as a significant level.

Results: The prevalence of FXIIIVal34Leu polymorphism was achieved 18% that its 83% and 17% were related, respectively, to the heterozygote genotype (Val/Leu) and homozygote genotype (Leu/Leu). 82% of these patients also had the genotype of wild type (Val/Val) among which 66.7% and 33.3 % of mutants were, male and female. 26% of them had Val34Leu polymorphism in the control group that 73% and 27% of them were, respectively, heterozygotic and homozygotic.

Conclusion: So far, the prevalence of FXIII Val34Leu polymorphism has not been reported in patients with Major thalassemia. A significant relationship wasn't seen in this study between the prevalence of FXIIIVal34Leu polymorphism in the thalassemic patients compared with the control group.

Keywords: FXIIIVal34Leu polymorphism, Thalassemic patients, PCR-RFLP

Introduction

The coagulation XIII factor of blood is an inactive enzyme with 731 amino acids that is converted into an active transglutaminase by thrombin and calcium ion. Its duty is binding to the end of alpha

and gamma amino group of fibrin chains. This binding causes to increase the strength of fibrin network. Its plasmatic and intracellular structures are, respectively, tetrameric and dimer. Its

form of the plasma has been formed from two similar pre-enzymatic subunits (FXIIIA) and two carrier / transporter protein subunits (FXIIIB).

The gene of FXIIIA subunit has had 200 kb of length, is located in the area of P24-256 bands (1). The active XIII factor is essential for maintaining homeostasis (2), as well as has a role in the vaginal delivery, wound healing, cellular migration and clot resilience (clot retraction) and its defects is associated with the wound non-healing, risk of miscarriage and haemorrhagic complication after surgery or trauma (3).

There are four types of XIII factor common polymorphism that are due to the amino acidic changes in Val34Leu, Pro564Leu, Val650Ile and are Glu651Gln (3). Tyr204Phe polymorphism recently has been known (4). Among these polymorphisms, G to T common polymorphism leads to Val substitution by Leu at a distance of 4 amino acid of being activated site of thrombin (Arg37-Gly38) in A subunit with position 35 (5, 6).

This polymorphism leads to the excessive interaction with thrombin surface of proximate of the amino acidic changes to being activated site of thrombin and causes to increase in vitro cross bindings and this causes the excessive activation of factor in the body.

In vivo antithrombotic effects may be observed because of being consumed the mutant protein of circulation (5). Val34 wild-type thrombin activates XIII factor, two and a half times slower than Val34Leu polymorphism .

Fibrin faster cross bindings and the structural differences have caused to increase polymorphism activity. Trans glutaminase proprietary activity of FXIIIA factor and its plasmatic rate as a result of this polymorphism do not change (2, 7), only accelerates being activated plasmatic XIII factor Leu allele due to the release of activation peptide through thrombin from plasmatic protein of mutant dramatically and therefore, occurs more quickly (2, 8).

Due to high rate of being activated, XIII factor can cause the ineffective cross-linking and or because of FXIIIA effects on other proteins disturbs their kinetics of the binding reactions (6).

Being homozygote causes more activity whereas the heterozygote carriers show an intermediate enzymatic activity (9) so that in Leu34 variant, Lag phase between the formation of fibrin and FXIIIA separation is obviously shorter than Val34 variant (10). So far, the several types of different polymorphism have been reported about XIII factor.

FXIIIVal34Leu polymorphism has the most important role in the activity of XIII factor. Many investigations have been conducted in relationship with this polymorphism and the cardiac, venous, and cerebral thrombotic events that their results are contradictory. The results of some researches show that this polymorphism is considered as a protective factor for the risk of coronary heart, myocardial infarction, early inside hemorrhage, venous and cerebral thromboembolism diseases (7, 11, 12)

The protective effects of this polymorphism on the mentioned factors have not been established in other number of investigations (13,14). The protective effects of this polymorphism on thrombosis have been reported in some studies only in one gender. The proposed mechanisms in often investigations show that FXIIIVal34Leu point mutation probably causes to form weaker fibrin structures and thus, leads resistance against the cerebral and cardiac infarction and reduces the susceptibility to thrombosis (8).

Considering that major thalassemic patients are susceptible to increase of the chronic coagulability with the increased incidence of thromboembolic events (18-15) and furthermore, so far, a study hasn't been conducted to investigate the prevalence of FXIIIVal34Leu polymorphism in major thalassemic patients in Iran and the world thus, this

study has been conducted with aim to determine the prevalence of FXIIIVal34Leu polymorphism and its relationship to the gender in patients with major thalassemia referring to Thalassemia Center of Ilam City.

Materials and methods

The study has been from the case-control type and the studied population was the thalassemic patients referring to Thalassemia Center of Ilam City. The sampling was conducted as census with the referral order on the different days so that based on calculation for each case group, the number of 100 patients with thalassemia, including 50 males and 50 females were investigated .

The number of 100 healthy subjects compatible in terms of age and gender was selected as control. After preparing 10 ml of venous blood sample from participants, DNA extraction was conducted from the bloody samples.

Designing primers was conducted in making-kit ward of Iran (Tehran) Blood Transfusion Organization. DNA after extraction by using Roche kit was investigated by the proliferation method and creating the restrictive area. Sequence of the primers was as follows.

R: GTTGACGCCCCGGGGCACCG

F: ACTTCCAGGACCGCCTTTGGAGGC

Following proliferative instruction was used to duplicate FXIIIVal34Leu for PCR reaction of thermo cycler program.

Initial denaturation for 5 min at 95 °C, 40 cycles of denaturation (Denaturation for 1 min at 95 °C, Annealing for 1 min at 55 °C, Extension for 1 min at 72 °C) and a final extension phase for 5 min at 72 °C were conducted. It should be mentioned because of ensuring FXIIIVal34Leu correct and appropriate proliferation for any sample, electrophoresis was conducted in agarose gel 1.5 % (with voltage of 120 V for 20 min), and if the absence of seeing a band bp 114 for the related sample, PCR

again was conducted. RFLP was conducted for FXIIIVal34Leu. DNA pieces (bands) that had become to the orange color in the presence of existed ethidium bromide in the gel against UV ray were seen. Existence of band bp 114 indicated the accuracy of extraction and DNA adequacy as well as absence of Taq polymerase inhibitor. The electrophoresis of PCRFXIIIVal34Leu products was conducted after RFLP.

While the length of PCR product was 114 bp that after the enzymatic digestion due to lacking the site of break for Cfo1 enzyme in homozygotic individuals, only one band (114 bp), in heterozygotic individuals, both the bands, intact and digested (94 and 114 bp) and in Wild Type individuals, only a 94 bp are observed that indicates the complete digestion. The used oligonucleotide sequences in PCR were as follows.

R: GTTGACGCCCCGGGGCACCG

F: ACTTCCAGGACCGCCTTTGGAGGC

The characterized G nucleotide shows an incongruity in 3' end of the reverse primer (original nucleotide A) that is for establishing a site of break for Cfo1 enzyme in the normal sequence (GCGC) that is lost in the presence of Leucine allele (GCTC).

Analysis of the data was conducted by SPSS 16 software, chi-square, and logistic regression statistical tests. $P < 0.05$ was considered as the significant level.

Results

The results showed that 18% (18 persons) had Val34Leu polymorphism that 83% and 17% (15 and 3 persons) of them, respectively, were the heterozygotic and homozygotic. 26% (26 persons) had Val34Leu polymorphism in the control group that 73% and 27% (19 and 7 persons) of them were, respectively, heterozygotic and homozygotic. Based on the findings, a significant relationship weren't observed between the prevalence

of FXIII A Val34Leu polymorphism in the thalassemic patients ($p= 0.07$) and also

gender ($p= 0.08$) compared with the control group.

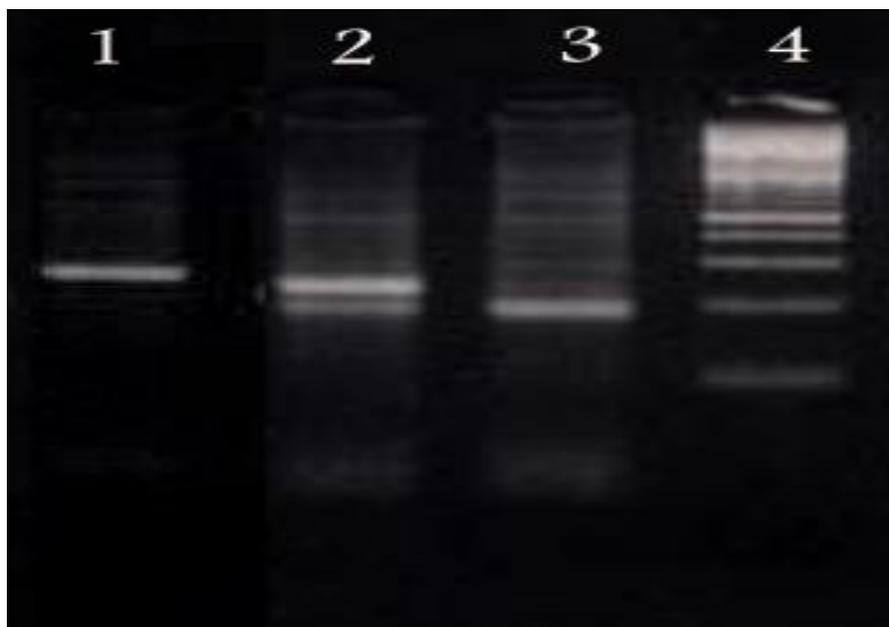


Figure 1. The obtained result after the digestion by CfoI enzyme. 1, Homozygote; 2, Heterozygote; 3, Wild type; 4, Size marker (50 bp).

Discussion

No significant statistical differences existed in present study between the prevalence of FXIII A Val34Leu polymorphism ($p= 0.07$) and gender ($p= 0.08$) in both case and control groups. According the conducted search, so far, the prevalence of FXIII A Val34Leu polymorphism has not been reported in major thalassemic patients but its rate of prevalence has been investigated in other countries in healthy individuals and some certain diseases and in the different races. Its prevalence has been reported in Korea country like Japan at zero limit and in another investigation, 2.5% (19). The prevalence of Leu34 mutant allele among the healthy individuals varies from 3%-44%.

Furthermore, a significant relationship exists between the prevalence of allele and the different races. Negros and Caucasians similarly shows a high prevalence of allele so that its prevalence is, respectively, 44.3% and 28.9% in Caucasians and

Negros, while this amount is obviously lower in Asians. Japanese individuals have a lower rate than Turkish people. Its prevalence also has been reported in Caucasians, 25-30%. The prevalence of Val34Leu polymorphism with Val34 and Leu34 allele frequencies was obtained, respectively, 73% and 27% in an investigation on Caucasian-descent Australian 150 (1, 2, 20).

A significant difference wasn't observed in the present study between the prevalence of this polymorphism and gender in the thalassemic and control individuals but, the protective effect of this polymorphism was observed in an investigation only in males (21).

Obviously, differing group of the present studied group justifies presence of the difference in the results. Investigating this polymorphism can be a basis for the future researches in field of its prevalence in the different races and related diseases. Although thrombosis is common in the

thalassemic patients and these individuals are prone to thrombosis, but in this study, the diagnostic facilities of individuals number with thrombosis didn't existed in the thalassemic patients. Thus, conducting such a study is suggested.

Furthermore, due to the high prevalence of this polymorphism in this study than Asian countries, conducting study with a greater volume of sample and investigation on specific thrombotic groups with the acquired risk factors such as taking oral contraceptive pellets (OCP), surgery

history, and the long-term immobility can help more recognition of its effects on patients.

Conclusion

A significant relationship wasn't seen in this study between the prevalence of FXIII Val34Leu polymorphism in the thalassemic patients than the control group. Conducting studies with larger sample size with the associated genetic risk factors is recommended.

References

1. Kopyta IA, Emich WE, Balcerzyk A, Niemiec P, Zak I, Pilarska E, et al. Polymorphisms genes encoding coagulation factors II, V, VII, and XIII in relation pediatric ischemic stroke: family-based and case-control study. *Neurologist*. 2012; 18(5):282-6.
2. Corral J, Iniesta JA, Gonzales R, Villalon M, Rivera J, Vicente V. Factor XIII Val34Leu polymorphism in primary intracerebralhaemorrhage. *Hematology*. 2000; 1(4):269-73.
3. Mikkola H, Syrjala M, Rasi V, Vahtera E, Hamalainen PL, et al. Deficiency in the A- subunit of coagulation factor XIII: two novel point mutations demonstrate different effects on transcript levels. *Blood*. 1994; 84(2):517-25.
4. Balogh I, Szoke G, Karpati L, Wartiovaara U, Katone E, Kiomaromi I, et al. Val34Leu polymorphism of factor XIII : biochemistry and epidemiology in familial thrombophilia. *Blood* 2000; 96(7):2479-86.
5. Lange M, Andrew T, Snieder H, Ge D, Futers T, Standeven K, et al. Joint Linkage and Association of Six Single-Nucleotide Polymorphisms in the Factor XIII-A Subunit Gene Point to V34L As the Main Functional Locus. *ArteriosclerThrombVasc Biol*. 2006; 26(8):1914-19 .
6. Guangyun W, Zhikang Z, Xiucai J, Qingshan N, Zhongli M. Factor XIII-A Val34Leu polymorphism might be associated with myocardial infarction risk: an updated meta-analysis. *Int J ClinExp Med*. 2014; 7(12):5547-52.
7. Francisco M, Rocío GC, Kaeng WL, Javier C, Vanessa R, Francisca L, et al. A pharmacogenetic effect of factor XIII Val34 Leu polymorphism on fibrinolytic therapy for acute myocardial infarction. *J Am Coll Cardiol*. 2005; 45(1):25-9.
8. Catto AJ, Kohler HP, Coore J, Mansfield M, Stickland M, Grant P, et al. Association of common polymorphism in the factor XIII gene with venous thrombosis. *Blood*. 1999; 93(3):906-8.
9. Elbaz A, Poirier O, Canaple S, Chedru F, Cambien F, Amarenco P, et al. Theassociation between the Val34Leu polymorphism in the factor XIII gene and brain infarction. *Blood*. 2000; 95(2):586-591.
10. Bereczky Z, Katona E, Muszbek L. Fibrin stabilization (factor XIII),fibrin structure and thrombosis. *Pathophysiol Haemost Thromb*. 2003/2004;33(5-6):430-7.
11. Wells PS, Anderson JL, Scarvelis DK, Doucette S, Gagnon F. Factor XIII Val34Leu variant is protective against venous thromboembolism: A huge

- review and meta analysis. *Am J Epidemiol* 2006; 164(2):101-9.
12. Marin F, Gonzalez-Conejero R, Lee KW, Corral J, Roldan V, Lopez F, et al. A pharmacogenetic of factor XIII Val34Leu polymorphism on fibrinolytic therapy for acute myocardial infarction. *J Am Coll Cardiol*. 2005; 45(1):25-9.
 13. Eldor A, Durst R, Hy-Am E, Goldfarb A, Gillis S, Rachmilewitz EA, et al. A chronic hypercoagulable state in patients with beta thalassemia major is already present in childhood. *Br J haematol*. 1999; 107(4):739-46.
 14. Rahimi Z, Ghaderi M, Nagel RL, Muniz A. Prevalence of thrombotic risk factors among beta thalassemia patients from western Iran. *J thromb Thrombolysis*. 2008; 26(3):229-33.
 15. Sipahi T, Kara A, Kuybulu A, Egin Y, Akar N. Congenital thrombotic risk factors in beta thalassemia. *Clin Appl Thromb Hemost*. 2009; 15(5):581-4 .
 16. Shemirani AH, Haramura G, Bagoly Z, Muszbek L. The combined effect of fibrin formation and factor XIII A subunit Val34Leu polymorphism on the activation of factor XIII in whole plasma. *Biochim Biophys Acta*. 2006; 1764(8):1420-3.
 17. Panigrahi I, Agarwal S. Thromboembolic complications in beta thalassemia: beyond the horizon. *Thromb Res*. 2007; 120(6):783-9.
 18. Eldor A, Rachmilewitz EA. The hypercoagulable state in thalassemia. *Blood*. 2002; 99(1):36-43.
 19. Attie-Castro FA, Zago MA, Lavinha J, Elion J, Rodriguez DL, Guerreiro JF. et al. Ethnic heterogeneity of the factor XIII Val34Leu polymorphism. *ThrombHaemost*. 2000; 84(4): 601-3.
 20. Emtiazi Giti, Karimr Mohsen. [Fundamental of molecular and cell and gene engine]. 4th ed, Mani publication, 2001. [in Persian]
 21. Dentali F, Romualdi E, Ageno W, Cappellini MD, Mannucci PM. Thalassemia trait and arterial thromboembolic events: a systematic review and a meta-analysis of the literature. *J Thromb Hemost* 2011; 9(5):917-21.