Prevalence of Factor V Leiden-G1691A and MTHFR-C677T Thrombosis Gene Modifier in Iron Deficiency Anemia: A Pathophysiological Effect in Indian Isolates

S. K. Pandey¹ · S. Pandey¹ · R. M. Mishra² · M. Indurkar³

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Abstract Normal iron levels are required to prevent thrombocytosis by inhibiting thrombopoiesis. Thrombocytosis is usually associated with a mild iron deficiency and is the result of a lack of inhibition of thrombopoiesis. Study participants were 430 iron deficiency anemia (IDA) patients. Ten (10) mL of venous blood were collected for the subjects. Ferritin analysis was done by ELISA method while Hemogram analysis was done by auto-analyzer. Factor V Leiden, PRTG20210A, and MTHFR C677T genotype analysis was performed by PCR–RFLP method. Among the patients, 9 were heterozygous (G>A) and 2 were homozygous (A>A) carrier of FV Leiden; while 20 were heterozygous (C>T) and 3 were homozygous (T>T) for MTHFR polymorphism. None of the patient was identified with PT mutation. Patients with thrombosis gene marker had lower hemoglobin, mean corpuscular volume, mean corpuscular haemoglobin levels, and mean corpuscular hemoglobin concentration than patients without thrombosis gene marker. Serum ferritin was elevated in subject with the absence of thrombosis gene markers. Our data suggest a high impact of inherited hypercoagulability risk factors in the pathogenesis of IDA and its complications.

Keywords Factor V Leiden · MTHFR · Phenotype · IDA

✉ S. K. Pandey
pandeysanjaybt@rediffmail.com

¹ Faculty of Life Sciences, School of Environmental Biology, Centre for Biotechnology Studies, Awadhesh Pratap Singh University, Rewa, M.P. 486003, India

² Department of Environmental Biology, Awadhesh Pratap Singh University, Rewa, M.P. 486003, India

³ Department of Medicine, SS Medical College, Rewa 486001, India

Introduction

Iron is an important regulator of thrombopoiesis and required to prevent thrombocytosis by inhibiting thrombopoiesis and certain amount need to maintain platelet production. Mild iron deficiency may be associated with thrombocytosis and is the result of a lack of inhibition of thrombopoiesis that is called thrombocytopenia. Iron deficiency anemia (IDA) results in reactive thrombocytosis [1–4]. Higher platelet count increased the risk of thrombosis and decrease in antioxidant defence in iron deficiency anemia may cause increased oxidant stress, that result in a tendency toward platelet aggregation [5]. Therefore abnormality in platelet count and function cause thrombosis and setting in atherosclerosis disease [6]. *Hypercoagulability* may be affected by iron deficiency through altering flow of blood in vessels due to increased viscosity and reduced deformability of microcytic red blood cells [7, 8]. Thrombosis gene markers i.e. factor V (FV) Leiden (G1691A), Methylenetetrahydrofolate reductase (MTHFR) C677T and prothrombin (PRT) G20210A association with genetic modifiers of sickle cell disease have been studied extensively [9–13]. A study also reported MTHFR mutation as a risk factor in the development of avascular necrosis [14]. The pathophysiology of reactive thrombocytosis in iron deficiency anemia remains incompletely understood. Thus our aim was to estimate the frequency of thrombosis gene marker and their effect on pathophysiology of iron deficiency anemia in Indian isolates.

Materials and Methods

Study participants were 430 iron deficiency anemia patient and all were Indians. Ten (10) mL of venous blood were collected after obtaining the signed informed consent form.

Study was approved by the institutional ethic committee. All IDA patients were diagnosed by iron profiling and complete blood count analysis. Ferritin analysis was done by ELISA method while Hemogram analysis was done by auto-analyzer (SYSMEX K-4500, Kobe Japan).

Detection of Polymorphism

Total genomic DNA was isolated from peripheral blood leukocytes by the kit (bioserve) method. Factor V Leiden, PRTG20210A, and MTHFR C677T genotype analysis was performed by PCR–RFLP method according to published literature [9–11]. Student's *t* test was used to compare the means of groups using GraphPad software (version 3.06). *P* value <0.05 was considered statistically significant.

Result and Discussion

A total 430 Iron deficiency anemia patients were evaluated (257 male and 173 female with a mean age of 22.3 ± 3.2 and 20.6 ± 2.9 years respectively). Out of 173 female, 80 were in gestation and 93 were in non gestation period. A complete blood count and iron profiles were performed in all subjects. Among the patients, 9 were heterozygous (G>A) and 2 were homozygous (A>A) carrier of FV Leiden; while 20 were heterozygous (C>T) and 3 were homozygous (T>T) for MTHFR polymorphism (Figs. 1a, b, 2a, b). None of the patient was identified with PT mutation. Patients with thrombosis gene marker had lower hemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) levels, and mean

Fig. 1 a Agarose gel picture of FV Leiden gene amplification. b Factor five Leiden mutations (lane 3, 6 heterozygous)

Fig. 2 a Agarose gel picture of MTHFR gene amplification. b Restriction digestion for MTHFR (lane 2, 5, 8 are heterozygous and lane 7 is homozygous)

corpuscular hemoglobin concentration (MCHC) than patients without thrombosis gene marker. Mean differences of hematological variables among patients with thrombosis gene marker and patients without thrombosis gene marker were statistically significant. Patients without thrombosis gene marker had low severity and fewer frequencies of clinical manifestations. Our study reported significant elevation of serum ferritin without FV Leiden and MTHFR genotype. Value of these parameters is statistically significant. TIBC Level was elevated while % transferrin saturation level was decreased in wild type genotype and *P* values were statistically significant. Comparative Iron profile and CBC of mutant and wild genotype of FV Leiden and MTHFR genotype in IDA patients is given in Table 1. It is interesting to note that although with different pathogenic mechanisms, both iron deficiency and overload have been associated with an increased thrombotic risk in experimental and clinical studies. Thrombotic complications reported in iron deficient children and adults in several studies [8, 15]. Many cases showed secondary thrombocytosis. However reactive thrombocytosis cause usually mild iron deficiency [4]. A study also reported one-third reactive thrombocytosis iron deficiency cases of children [16]. Stolz et al. [17] reported severe anemia, along with thrombophilia and hypercholesterolemia, is a risk factor for cerebral venous thrombosis. Another studies suggested that the relationship between reactive thrombocytosis and iron deficiency is much more complex than a mere consequence of a cross reactivity between erythropoietin and thrombopoietin [18, 19]. An Indian study on

Table 1 Comparative iron profile and CBC of mutant and wild genotype of FV Leiden and MTHFR genotype in IDA patients

IDA Patients (N = 430) Mean $\pm$ SD					
Iron/biochemical profile	MTHFR and FV Lieden				
	(+/+ and +/-) genotype N = 34	-/- genotype N = 396	SE	95 % CI	P value
Serum ferritin (μ g/L)	10.5 $\pm$ 1.4	11.6 $\pm$ 2.8	0.486	0.225–1.575	0.024
TIBC (μ g/dL)	491.3 $\pm$ 80.4	434.2 $\pm$ 7.1	12.679	32.179–82.021	<0.001
% Transferrin saturation	12.4 $\pm$ 2.3	14.6 $\pm$ 3.2	0.561	–3.303 to –1.097	<0.001
Platelet (Ths/ μ L)	417.8 $\pm$ 63.5	380.6 $\pm$ 34.3	6.679	24.073–50.327	<0.001
HGB (g/dL)	7.2 $\pm$ 1.4	8.2 $\pm$ 1.3	0.234	–1.45944 to –0.54056	<0.001
MCV (fL)	57.3 $\pm$ 2.4	64.1 $\pm$ 5.4	0.935	–8.637 to –4.963	<0.001
MCH (pg)	24.3 $\pm$ 3.7	27.8 $\pm$ 5.2	0.911	–5.291 to –1.709	<0.001
MCHC (g/dL)	30.4 $\pm$ 1.7	32.3 $\pm$ 2.7	0.471	–2.826 to –0.974	<0.001

SE standard error, CI confidence interval

sickle patient reported low impact of thrombosis gene markers [20]. Our data suggest a high impact of inherited hypercoagulability risk factors in the pathogenesis of iron deficiency anemia and its complications.

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Compliance with Ethical Standards

Conflict of interest None.

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