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Research paper

High prevalence of factor V Leiden and prothrombin G20101A mutations in Kashmiri patients with venous thromboembolism

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ABSTRACT

Aim: The genetic variants of the factor V (G1691A), prothrombin (G20210A) and MTHFR (C677T) genes have been widely implicated as inherited risk factors for developing venous thrombosis. This study was undertaken to reveal the frequency of these mutations in Kashmiri patients with venous thromboembolism.
Methodology: A case-control study was designed with 250 VTE patients and 250 healthy controls. The mutations were analysed using ARMS-PCR and PCR-RFLP approach.
Result: The factor V Leiden *G1691A* mutation was found in 17/250 (6.8%) VTE patients and prothrombin *G20210A* mutation was found in 7/250 (2.8%) VTE patients while no mutation was found in any of the healthy controls. Both the mutations were found to be significantly associated with the increased risk of VTE (**p = 0.0001** and **0.0150** respectively) while no association of VTE risk with *MTHFR C677T* polymorphism was found (p = 0.53).
Conclusion: The increased frequency of factor V Leiden *G1691A* and prothrombin *G20210A* mutation in VTE patients indicates a significant role of these mutations in the development of VTE in our population. We therefore suggest the routine screening of these two mutations as thrombophilic markers in Kashmiri patients with venous thromboembolism.

1. Introduction

Thrombophilia is defined as a tendency of inappropriate formation of blood clot. Venous thromboembolism (VTE) is the most common clinical manifestation of thrombophilia and is a serious public health burden affecting about 2 in 1000 individuals per year and is associated with a mortality rate of 10% (White, 2003). The recurrence risk of VTE is 6% per annum and 25% of patients suffer from post-thrombotic disease within 5 years following a VTE episode (Prandoni et al., 2004). VTE has a multifactorial etiology and is influenced by both the acquired and genetic risk factors. In acquired thrombophilia the hypercoagulation is attributed to specific causes including immobilisation, obesity, trauma, surgery, malignancy, hormone replacement therapy, myeloproliferative disorders, use of contraceptives, etc. Besides the number of genetic risk factors causing inherited thrombophilia like the deficiency of naturally occurring anti-coagulants like antithrombin, protein C and protein S, the two well characterised mutations, factor V *G1691A*, commonly known as factor V Leiden (FVL) and prothrombin *G20210A* and a *C677T* polymorphism in MTHFR gene have been widely implicated as important genetic risk factors in individuals with or without an apparent cause for developing VTE, and with a tendency to recur (Rosendaal and Reitsma, 2009).

FVL mutation, the most important genetic cause for inherited VTE is a G to A substitution at position 1691 of the factor V gene. This mutation results into an altered form of coagulation factor V that is not inactivated by activated protein C, thereby leading to the state of hypercoagulability. Among different world populations there is a varied frequency of FVL mutation. A high frequency of FVL mutation is present in Caucasian and Arab population while it is non-existent in most Asians and African countries (Ridker et al., 1995; Ridker et al., 1997a; Mansourati et al., 2000; Doggen et al., 1998; Tanis et al., 2003; Juul et al., 2002; Zoller et al., 1996; Schroder et al., 1996; Pecheniuk et al.,

Abbreviations: FVL, factor V Leiden; MTHFR, methylene tetrahydrofolate reductase; ARMS, amplification-refractory mutation system; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; VTE, venous thromboembolism; DVT, deep venous thrombosis; PTE, pulmonary thromboembolism; SKIMS, Sher-i-Kashmir Institute of medical sciences; SMHS, Shri Maharaja Hari Singh Hospital; EDTA, ethylenediaminetetraacetic acid; DNA, deoxyribonucleic acid; mRNA, messenger ribonucleic acid

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Table 1
Prevalence of FVL *G1691A* gene mutation in different populations.

Population	Country	*FVL G1691A* controls	*FVL G1691A* VTE	Reference
Caucasian	USA	6	11.6	(Ridker et al., 1995)
	USA	3.7	–	(Ridker et al., 1997a)
	France	5	–	(Mansourati et al., 2000)
	Netherland	2.9	19.53	(Rosendaal and Reitsma, 2009)
	Netherland	5%	6.8%	(Doggen et al., 1998)
	Netherland	5.5%	–	(Tanis et al., 2003)
	Denmark	7.45%	–	(Juul et al., 2002)
	Sweden	14.74	–	(Zoller et al., 1996)
	Germany	7.12	–	(Schroder et al., 1996)
	Australia	3.6%	–	(Pecheniuk et al., 1997)
	Spain	3.33%	–	(Garcia-Gala et al., 1997)
	Spain + Italy	2.94%	–	(Pepe et al., 1997)
	Greek	2.5%	–	(Antoniadi et al., 1999)
	Iran-Kurds	2.97%	–	(Rahimi et al., 2008)
	Iran-Tehran	5.5%	–	(Rahimi et al., 2008)
Arab	Saudi Arabia	2%	–	(Almawi et al., 2005)
	Bahrain	3%	–	(Almawi et al., 2005)
	Tunisia	6.4%	–	(Almawi et al., 2005)
	Lebanon	14.75%	–	(Almawi et al., 2005)
	Tunisia	6.1%	24%	(Bouaziz-Borgi et al., 2006)
	Lebanon	14.63%	52%	(Bouaziz-Borgi et al., 2006)
	Lebanon	16.7%	–	(Irani-Hakime et al., 2000)
	Syria	13.6%	–	(Irani-Hakime et al., 2000)
	Syria	7.7	–	(Dajani et al., 2013)
	Jordan	12.3%	–	(Awidi et al., 1999)
	Jordan-Chechens	18.3%	–	(Eid and Rihani, 2004)
	Jordan-Circassian	7.7%	–	(Dajani et al., 2012)
	Circassian Israel	1.3%	–	(Falik-Zaccai et al., 2003)
Asian	Indian	0%	–	(Gupta et al., 2003)
	Indian	0%	3.8%	(Garewal et al., 1997)
	Indian	0%	2.39%	(Pawar et al., 2001)
	Japan	0%	–	(Takamiya et al., 1995)
	China	0%	0%	(Ho et al., 1999)
	Korean	0%	–	(Kim et al., 1997)
	Kashmir	0%	6.8%	[This study]
Africans	Senegal	0%	–	(Rees et al., 1995)

1997; Garcia-Gala et al., 1997; Pepe et al., 1997; Antoniadi et al., 1999; Rahimi et al., 2008; Almawi et al., 2005; Bouaziz-Borgi et al., 2006; Irani-Hakime et al., 2000; Dajani et al., 2013; Awidi et al., 1999; Eid and Rihani, 2004; Dajani et al., 2012; Falik-Zaccai et al., 2003; Gupta et al., 2003; Garewal et al., 1997; Pawar et al., 2001; Takamiya et al., 1995; Ho et al., 1999; Kim et al., 1997; Rees et al., 1995) Table 1.

The substitution of *A* for *G* at position 20,210 of the prothrombin gene is the second most important genetic risk factor for inherited VTE. This mutation increases the plasma prothrombin levels higher than normal (upto 25% increased levels) without leading to any functional difference in prothrombin molecule (Kraaijenhagen, 2000; Kyrle, 2000). This mutation almost increases the 2-fold risk of deep vein thrombosis (DVT) and pulmonary emboli (PE) (Kim et al., 2003). The prevalence of this mutation widely varies between different countries (Table 2). In Caucasians it has been roughly been estimated to be 3–17% in VTE patients and 1–8% in healthy controls. On the other hand this mutation in rarely found in populations from Asian and African origin (Jadaon, 2011; Franco et al., 1998; Gibson et al., 2005; Zoossmann-Diskin et al., 2008; Rees et al., 1999; Ruiz-Argüelles et al., 2001; Ruiz-Argüelles et al., 2005; Jun et al., 2006; Lu et al., 2002; Sakuma and Shirato, 2003; Miyata et al., 1998; Chang et al., 2008; Kim and Kim, 2007; Shen et al., 2000; Lin et al., 1998; Newton et al., 1999; Kumar et al., 2005; Ghosh et al., 2001; Angeline et al., 2005; Garewal et al., 2003a; Bennett et al., 2001; Hessner et al., 1999; Gunathilake et al., 2015).

MTHFR is an enzyme which has a pivotal role in modulating the plasma homocysteine levels by converting it into methionine. A *C* to *T* polymorphism at position 677 of the MTHFR gene leads to decreased enzyme activity by rendering the enzyme thermolabile and subsequently elevating the plasma homocysteine status (Toyoda et al., 2004). The elevated total homocysteine levels have a toxic effect on vascular endothelium and clotting cascade and it has been associated with venous thrombosis in young adults and recurrent venous thrombosis in general, with about 10% frequency in patients with first episodes of venous thrombosis (Ueland and Refsum, 1989; McCully, 1969; Hankey and Eikelboom, 1999). There have been conflicting opinions about the role of MTHFR C677T polymorphism and risk of VTE among different populations (Arruda et al., 1997; Cattaneo et al., 1997; Ocal et al., 1997; Salden et al., 1997; Tosetto et al., 1997; Brown et al., 1998; Dilley et al., 1998; Grandone et al., 1998; Kluijtmans et al., 1998; Margaglione et al., 1998; Rintelen et al., 1999; Alhenc-Gelas et al., 1999; Cattaneo et al., 1999; Ho, 2000; Franco et al., 1999; Gemmati et al., 1999; Salomon et al., 1999; Lin et al., 2000; Akar et al., 2000; Angchaisuksiri et al., 2000; Couturaud et al., 2000; de Franchis et al., 2000; Fujimura et al., 2000; Gerhardt et al., 2000; Toydemir et al., 2000; Zheng et al., 2000; Guedon et al., 2001; Hanson et al., 2001; Hsu et al., 2001) Table 3.

Kashmir, which is regarded worldwide as paradise on earth is also burdened with high incidence of venous thrombotic events. The Kashmir valley, located in the north-western region of the Indian subcontinent is lying between the Great Himalayas and the Pir Panjal range. It is situated at an altitude of about 1800 to 2400 m above sea level and is among one of the provincial territories of India. The population here has immigrated mainly from Turkey, Iran, Central Asia and Afghanistan, and settled in the valley. The population here is ethnically conserved through generations as people do not prefer to marry outside the state and majority of the marriages are of consanguineous nature keeping it genetically conserved (Department of Tourism, 2018).

Keeping in view the importance of FVL *G1691*, prothrombin *G20210A* and MTHFR *C677T* mutations in developing