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Thesis Title: Understanding the structural and mechanistic role of antithrombin in thrombosis

ABSTRACT:

Thrombosis and thromboembolism remain a major cause of mortality and morbidity worldwide. Venous thrombosis is a multi-causal disease caused by the presence of genetic or acquired risk factors. Inherited causes of thrombosis include mutations in the genes that encode Protein C (PC), Protein S (PS), Antithrombin III (AT), Factor V Leiden and Factor II G20210A mutation. The prevalence of inherited risk factors for thrombosis is variable between populations throughout the world. In Indian studies, no risk factor for thrombosis has been identified in half the patients.

AT, a member of the serine protease inhibitor family (SERPIN) is the principal regulator of coagulation cascade and inactivates multiple coagulation proteases like factor Xa, factor XI, and thrombin. The significance of AT in the hemostasis and in sustenance of life is evident from the association of inherited or acquired AT deficiencies in humans with increased risk of thrombosis. The first mutation linked to AT deficiency was characterized in 1983 and the natural variants of AT mostly identified from families with a tendency of thrombosis, are collated together in a mutation database. Although more than 250 mutations in AT gene leading to increased thrombotic risk has been identified throughout the world, not a single genetic variation has been identified and characterized in AT deficient Indian DVT patients. The present study was undertaken to identify the prevalence and molecular basis of AT deficiency in Indian DVT and recurrent pregnancy loss (RPL) patients.

In a time span of more than 3 years (October 2011-December 2014) 2800 Doppler proven thrombotic patients were screened for AT deficiency at Department of Hematology, AIIMS, New Delhi for the presence of different thrombophilic risk markers. After applying the exclusion criteria, 1980 DVT patientswere finally selected for the study of AT deficiency and elucidating its molecular basis of defect. Based on the AT activity and antigen levels of DVT patients, we assessed 62 patients with AT deficiency for its molecular basis of defect. We identified a family

with type I AT deficiency where rs2227589 polymorphism was identified as the underlying cause of type I AT deficiency in symptomatic proband and his younger brother but was absent from the asymptomatic father. This polymorphism was checked in all the 62 AT deficient patients and 62 healthy controls and was found to be significantly associated with reduced plasma AT levels (p value < 0.001). In another family with type II AT deficiency, the proband was found to be carrying dual mutations in AT gene. One of these mutations was a previously reported heparin binding defect, Arg47Cys and the other was a single nucleotide insertion g.13362_13363insA. The novel single nucleotide insertion was observed to affect the global protein conformational change causing*in vivo* polymerization of the variant AT in patient's plasma as confirmed by Western blot and TEM analysis. We also identified and characterized two novel point mutations in AT gene in Indian DVT patients. AT variant Thr280Ala present on s3B was purified using hi-trap heparin affinity chromatography and gel filtration chromatography and showed a tendency of polymerization as assessed by SDS-PAGE, NATIVE PAGE, Western blot, fluroscence spectra, far-UV CD and thermal denaturation studies. *In silico* studies showed disruption of hydrogen bond interactions post mutation and TEM analysis confirmed the polymeric nature of variant AT. The other novel point mutation, Ala427Thr was present on C-terminal region of the AT and caused an increase in the melting temperature of variant AT, decrease in emission intensities of tryptophan and bis-ANS, decrease in percentage ASA and loss of hydrogen bonding interactions post substitution. All these changes indicated polymerization of the variant which were further confirmed by TEManalysis. In addition to identifying novel mutations, we also observed previously reported variants like C-4X, *Dde*I and *Pst*I in Indian DVT population for the first time. In patients where no mutation was identified in the protein coding region, known polymorphisms like g.67G>A and rs3138521 and novel polymorphisms like g.25G>A and g.-1A>T were identified in the 5' UTR of AT gene in Indian DVT patients. We also studied the deficiency of this critical endogenous anticoagulant in RPL population. While we did not find any mutation in the AT gene in RPL patients, we did identify homozygous rs2227589 polymorphism in one patient. Frequency of this allele was observed to be more frequent in Indian RPL patients as compared to the Western population.

Our study indicates that AT as a risk factor for DVT in Indian population is not different fromthat reported in Western population and similar studies should be carried out to understand the prevalence and molecular basis in large Indian population.