Introduction: Portal vein thrombosis (PVT), is a thromboembolic disorder with a genetic etiology. In developing countries, PVT presents in 40% of portal hypertension cases. Anti-thrombin (AT) deficiency is a major risk factor for venous thromboembolic disorders. The SERPINC1 gene encodes AT-III, a protease inhibitor. A comprehensive literature review revealed that a variant of the SERPINC1 gene (rs2227589, g.5301G>A), is associated with an increased risk of thrombosis in South Asian populations. The objective of this study was to design and implement an assay for determining the presence of the SERPINC1 gene variant in a cohort of PVT patients.

Methods: The study population comprised of 80 PVT diagnosed individuals, who were referred to the Human Genetics Unit, Faculty of Medicine, University of Colombo, for genetic screening. Their blood samples were acquisitioned following ethical clearance. DNA extraction was conducted by a silica membrane based nucleic acid purification technique. A novel tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) assay was designed and optimized to genotype the SERPINC1 gene variant. The optimization included a gradient PCR to detect the optimum melting temperature and also primer concentration series to detect the optimum primer concentrations.The optimized protocol was validated by Sanger sequencing.

Results: The presence of two simultaneous peaks at the desired location in the Sanger chromatogram confirmed the PCR protocol. The allele frequency for the normal variant (G/G) and the heterozygous variant (G/A) was 62.4% and 31.2%, respectively. No homozygotes for the pathogenic variant were identified. The minor allele frequency for the g.5301G>A genotype was 0.01 (p=0.90) according to the Hardy Weinberg equilibrium.

Conclusions: The designed T-ARMS-PCR assay can be implemented to genotype the SERPINC1 gene variant. Strong conclusions regarding the sensitivity and specificity cannot be drawn from a single study using a small sample size. Furthermore, no homozygotes for the pathogenic variant were detected in this study.