**ABSTRACT**

**Background:** Beta-thalassemia is a common genetically inherited blood disorder affecting 4.83% of the world's population. It is caused by defective beta-globin synthesis and has diverse clinical phenotypes ranging from severe to mild. In beta-thalassemia intermedia (β-TI), the moderate form of the disease, anemia caused by ineffective erythropoiesis is primarily ameliorated by an increase in gastrointestinal iron absorption. Since the body has no innate machinery to eliminate excess iron, these patients suffer from severe iron overload. This condition results in the formation of hydroxyl radicals which leads to the oxidation of various proteins such as fibrinogen, the precursor of the molecular mesh of blood clots. Several studies have identified fibrinogen as a major target for oxidation. *In vitro* studies have shown that its oxidation causes the development of aberrant clots. Although, high levels of iron and hypercoagulability are major causes of morbidity and mortality in β-TI, the link between these two symptoms is not fully established. To date, iron-catalyzed oxidation of fibrinogen has not been investigated as a potential contributor to the hypercoagulable state in β-TI. Thus, we hypothesize that ***excess iron and subsequent hydroxyl radical production alter the structure and function of fibrinogen ultimately contributing to a state of hypercoagulability in β-TI.***

**Specific Aim 1: Investigate the biochemical effects of excess iron and hydroxyl radicals on the structure and function of fibrinogen.** Amino acid residues in fibrinogen are known targets for oxidation; however, studies identifying specific sites of oxidation and their effect on function are limited. To gain an overall understanding of how fibrinogen is oxidized, purified fibrinogen will be incubated with varying concentrations of iron and sources of hydroxyl radicals. Its structure and function will be analyzed by general hemostatic assays, microscopy, and mass spectrometry techniques. Structural analysis will also be done on fibrinogen oxidized by ferric iron in the absence of a redox agent. The latter may contribute to recent observations that fibrinogen converted into fibrin-like deposits by ferric iron are resistant to proteolysis.

**Specific Aim 2: Analyze the role of oxidized fibrinogen in β-TI *ex vivo*.** B-TI patients experience hypercoagulable states, which are further pronounced in splenectomised patients. Despite the persistence of hypercoagulability and the spleen status, the occurrence of a major thrombotic event in these patients varies greatly. Moreover, *in vitro* studies on the effects of amino acid oxidation on the function of fibrinogen in coagulation show inconsistencies. A main observation is that clots may have a pro-thrombotic or anti-thrombotic phenotype depending on the modification induced. Thus, samples from splenectomised and non-splenectomised β-TI patients as well as those with and without a history of venous thrombosis will be analyzed. Special attention will be placed on identifying specific sites of oxidation on fibrinogen that favors a pro-thrombotic state versus an anti-thrombotic state.

**Specific aim 3: Investigate the structure and function of fibrinogen in iron overloaded mice:** Iron toxicity and the degree of hypercoagulability in β-TI patients vary significantly. To determine the upper limit iron concentration needed to trigger a hypercoagulable state *in vivo*, C57/Bl6 mice will be iron loaded and compared to β-TI mice. An iron chelator and reactive oxygen specie scavenger, will be used to determine if the effects seen are iron or hydroxyl radical dependent and if they can be reversed. Furthermore, the integrity of clots formed in the presence of lysed red blood cells will also be analysed as the presence of pro-coagulant exposing red blood cells is a known contributor to the hypercoagulable state seen in β-TI patients

**Significance:** Iron overload and the risk of thrombosis continue to be major concerns for β-TI patients. Identifying additional mechanisms by which iron overload can result in hypercoagulability can provide an avenue for the discovery of new drug targets and improved patient management. Moreover, this project will contribute to the ongoing debate on the role of iron metabolism disorders as potential risk factors in cardiovascular disease.