Summer Research 2011

Seasonal Variation of Thrombotic and Thrombolytic Factors in Humans by Age and Gender

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Background Information

It is well known there is a delicate balance in the clotting and anti-clotting factors which control blood coagulation. If this balance is disturbed slightly, it is possible for a person to clot to death or hemorrhage. Recent research indicates these clotting factors vary by season but not much is known about why or how this happens. This change was first observed in a hibernating species of turtle. Researchers wanted to know why the animal didn't clot to death while it laid in the mud for long periods of time. It was discovered the turtle had an increase in the concentration of Heparin, a natural blood thinner, during the winter months. However, the environmental trigger was never found in a laboratory setting despite testing conditions from light exposure to solar radiation. People have since suggested causes for the variation, such as change in temperature, but recent studies have refuted the previously plausible explanations leaving many mechanisms to be explained. New techniques for examining clotting proteins and information from other species gives us an opportunity to investigate the changes in these hemostatic proteins in humans. This study seeks to investigate seasonal changes in five related clotting and anticlotting factors, and concurrently assess clotting factors by age and gender.

There has been a fair amount of recent research into the seasonal variation of a few clotting factors but none of these projects have put together all the necessary components to draw conclusions about what kind of role this variation plays in the haemostatic system in the human body. One study's findings showed a variation in the concentration of fibrinogen by approximately 10mg/dl per decade (Abbate). However, this study didn't analyze the effects of seasonal variation or variation of other clotting factors which may or may not rise along with the concentration of fibrinogen. Other studies have shown temperature has no effect on the variations of studied factors (Stout and van der Bom). The factors investigated in another study

showed similar variation in some different clotting factors which could suggest a similar regulatory mechanism (Undar). Another study suggested that rather than a seasonal rise or fall in a particular factor, elderly people lose their ability to balance both sides of the haemostatic system (Sagripan).

Objective

In this project, the Thrombotic factors Fibrinogen and Tissue Plasminogen Activator and the Thrombolytic factors Tissue Plasminogen Activator Inhibitor, Heparin and Thrombin will be quantified to observe variation in the concentration between the winter and summer months (December/January and June/July) from people of different gender and decade of life from 20 to 70 years old from a serum supply company (BiocheMed) to ensure disease free samples.

This project aims to go where the previous studies in seasonal variation of Thrombotic and Thrombolytic factors have failed to go by looking into clotting and anti-clotting factors directly rather than at clotting times and anecdotal evidence of stroke or embolism. These proteins may be working simultaneously and antagonistically with thrombotic factors taking precedence in the winter months. It will also be useful to determine if there is also an age-related change in the proteins, since anecdotal evidence suggests that stroke and clotting disease are increasing with age. We would like to observe how age effects the concentration of the factors, and, by including gender, exclude natural variations between males and females. The studies cited in the background information were typically limited to only one of the variables which we will be monitoring and often one protein or clotting times.

Purpose

This project could serve many purposes. The most important of these could be in the field of surgery. For some people, surgery is not always a life or death situation and their doctors must chose whether or not it is appropriate for a patient to undergo an elective surgery. This research could provide insight into the how seasonal changes lead to an increase in DVT or other clotting based complications. Outside of surgery there is still practical application, with many people now taking blood thinners and other medications which alter the levels and the delicate balance of clotting and anti-clotting factors. Any variation of clotting factors discovered could provide a basis for monitoring and adjusting medication throughout the course of the year to maintain a healthy balance is the haemostatic system.

Techniques

We will be measuring Fibrinogen, Thrombin, Heparin, Tissue Plasminogen Activator (TPA) and Tissue Plasminogen Activator Inhibitor (TPA-I) in human sera samples as collected and described above. Serum samples will go through a testing of serial dilutions to determine and optimize the dilution factor needed to measure each sample. Sera will be added to an Eliza plate, where protein will adhere and then the appropriate detection antibody is added. This step is followed by a chomagen, which is converted to a colored product by an enzyme attached to the detection antibody. Therefore, the amount of color detected by a wavelength reader will be quantitative for the amount of specific protein present in the serum. Heparin, while a naturally occurring anti-clotting factor, is not a protein but rather a glycosaminoglycan. Specific detection antibodies exist for domains within heparin or for heparin co-factor II protein. One of these methods will be used to detect heparin levels in the sera. Heparin co-factor II may be used to

coordinate with the genetic work being done in Keith Garrison's lab. While we are not working on the same questions our results are integrated in the larger picture of how the mechanism for alteration in clotting factors change.

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