ORIGINAL ARTICLE

EVALUATION OF PRO-C GLOBAL FOR IDENTIFICATION OF DEFECTS IN PROTEIN C/S ANTICOAGULANT PATHWAY

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Background: Detection of protein C and S deficiency forms a major investigation in the laboratory evaluation of thrombophilia screening. It has key role in the diagnosis of protein C and S deficiency. The objective of this study is to determine the utility of ProC Global as a screening test for identifying the defects of protein C and S anticoagulant pathways. **Methods:** Two Hundred patients with venous thromboembolism were studied at the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, from October 2004 to March 2006. ProC Global test (Dade Behring Diagnostics) was performed and was followed up by protein C and S assays. ProC Global is an activated partial thromboplastin time based assay in which Protac (snake venom from Aghistroden contortrix) is used for activation of the endogenous protein C of the plasma sample. The protein C activation time in the presence of the activator was set in relation to a parallel determination of PCAT/O with addition of a buffer instead of activator reagent. The ratio PCAT: PCAT/O was transformed in normalized ratio by relating them to a calibrator. Control plasma for normal range and ProC control plasma for pathological range (Dade Behring Diagnostics) were assayed in each run for quality control. **Results:** A total of 200 patients, 132 (66%) males and 68 (34%) females with age ranging from 1 to 68 years were studied. ProC Global was positive in 29/200 (14.5%) patients. ProC Global was found to be 86% sensitive, 94% specific and its overall efficiency turned out to be 94%. **Conclusion:** Pro-C Global can be used effectively as a screening test to detect abnormalities in protein C and S anticoagulant pathways.

Keywords: Protein C deficiency, Protein S deficiency, Thrombophilia

INTRODUCTION

An inherited defect in protein C/S anticoagulant pathway has been demonstrated in 30–75% patients presenting with arterial[1] and venous thrombosis[2]. Thus, detection of protein C/S deficiency forms a major investigation in the laboratory evaluation of thrombophilia screening. Recently a new functional screening test, ProC Global (Dade Behring Diagnostics), has been introduced, which may facilitate the detection of protein C/S deficiency. ProC Global is an activated partial thromboplastin time (APTT)-based assay in which Protac (snake venom from *Agkistrodon contortrix*) is used as activator of the endogenous protein C and intrinsic coagulation cascade of the plasma sample (Figure-1). The objective of this study is to determine the utility of ProC Global as a screening test for identifying the defects of protein C and S anticoagulant pathways.

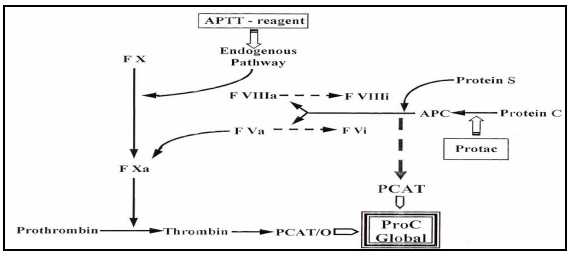

Figure-1: Relationship of Protein C Pathway and various coagulation factors with Pro C Global Assay

PATIENTS AND METHODS

The study was done at the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi on patients with a history of venous thromboembolism. Only samples with a normal prothrombin time (PT) were included in the study. Patients on oral contraceptives or anticoagulant therapy were excluded. Relevant clinical details were recorded and 1.8 ml venous blood in 0.2 ml trisodium citrate (0.11 M) was collected by venepuncture under aseptic measures. Samples were centrifuged at 4000 rpm for 10 minutes to make platelet poor plasma and were kept frozen at -20 °C until testing.

The protein C activation time (PCAT) was measured in presence of the activator reagent and a parallel determination of the PCAT/0 (without the addition of activator reagent) was done. For PCAT, 100 µl of activator reagent and 100 µl of APTT reagent were added to 100 µl of the citrated plasma sample. For PCAT/0, 100 µl of buffer and 100 µl of APTT reagent were added to 100 µl of the citrated plasma sample. Both tubes were incubated for 3 min at 37 °C, and the clotting time was measured after adding 100 µl of $CaCl_2$.

To ensure inter-laboratory comparability of results, which may vary due to different analysers, a normalised ratio (NR) was calculated using the following formula: $NR= (PCAT:PCAT/0)_{sample} \times CF$

where CF is a calibration factor which is calculated from Standard Human Plasma (SHP) for every lot of reagent using the following formula:

$$CF = SV/(PCAT:PCAT/0)_{SHP}$$

where SV is the sensitivity value of SHP.

For quality control, Control Plasma N for normal range and ProC Control Plasma (Dade Behring Diagnostics) for the pathological range, were assayed with each batch.

Quantitative determination of protein C and S was done on plasma samples using Protein C and Protein S kits.

RESULTS

A total of 200 patients, 132 males (66%) and 68 females (34%) with age ranging from one to 68 years were studied. ProC Global was positive in 29/200 (14.5%) patients. Protein C and S assays done on the patients showed that 17/200 patients were positive for Protein C, while protein S deficiency was found in 6/200 patients.

For statistical analysis, the test results were divided into four groups:

- True Positive (TP): Samples positive with ProC Global and having Protein C/S deficiency on quantitative assay
- True Negative (TN): Samples negative with ProC Global and not having Protein C/S deficiency on quantitative assay
- False Positive (FP): Samples positive with ProC Global and not having Protein C/S deficiency on quantitative assay
- False Negative (FN): Samples negative with ProC Global and having Protein C/S deficiency on quantitative assay

The predictive values of Pro-C Global assay were determined as follows:

- Sensitivity(%)= [TP/(TP+FN)]×100
- Specificity(%)= [TN/(TN+FP)]×100
- Efficiency(%)= [(TP+TN)/(TP+TN+FP+FN)]×100

Table-1: Clinical Utility of ProC Global Assay

	Calculation	Result
True Positive (TP)	n	20
True Negative (TN)	n	168
False Positive (FP)	n	9
False Negative (FN)	n	3
Sensitivity	[TP/(TP+FN)]×100	86
Specificity	[TN/(TN+FP)]×100	94
Efficiency	[(TP+TN)/(TP+TN+FP+FN)]×100	94
Positive Predictive Value	[TP/(TP+FP)]×100	69
Negative Predictive Value	[TN/(TN+FN)]×100	98

ProC Global was found to be 86% sensitive, 94% specific and its overall efficiency turned out to be 94%. The positive and negative predictive values of the test were 69% and 98% respectively. The NR values of ProC Global test in positive cases turned out to be significantly lower than the normal group with a cut off value of 0.8.

Of the 29 positive ProC Global assays, 12 patients were suffering from deep vein thrombosis, while eight had history of cerebrovascular accident. Three females had history of recurrent abortion, two had pulmonary embolism, while there was one patient each of retinal vasculitis, inferior vena caval thrombosis and myocardial infarction, respectively.

DISCUSSION

In the circulation, the predisposition to thrombosis is evaded by the delicate equilibrium between pro-coagulant and anticoagulant factors. Protein C and protein S are among the major natural anticoagulants of the body.[3] Disturbances in the protein C/S anticoagulant pathway, either hereditary or acquired, result in increasing the thrombotic tendency of the body. The common hereditary defects in this pathway include protein C deficiency, protein S deficiency, activated protein C resistance and prothrombin gene mutation.[4] Several acquired factors like oral contraceptives, pregnancy and antiphospholipid antibodies can also interfere with the protein C/S anticoagulant pathway thus producing a thrombophilic state.[5]

Laboratory evaluation of the Protein C/S anticoagulant pathway includes measurement of Proein C and Protein S antigen levels/activity and DNA-analysis to identify the Factor V Leiden and prothrombin gene mutations. These tests are laborious and expensive[6], whereas most hospitals and laboratories in Pakistan lack the facilities and technical skills to perform them all. A sensitive screening assay that would allow the monitoring of the proper interplay of all factors interacting in the protein C/S pathway could therefore be very beneficial as part of routine thrombophilia screening. ProC Global is an easy to perform, cost effective screening test which can be carried out at most health care centres. It is based on the measurement of APTT and thus does not require any additional technical skill or instillation of expensive instruments in the laboratory. Our results show that, ProC Global is highly sensitive in determining the defects of Protein C/S deficiency. The positive cases may be further investigated with quantitative assays for Protein and Protein S. This approach provides a practical and applicable solution for the lack of thrombophilia screening facilities in most of the under resource laboratories of our country. Another useful feature of ProC Global Test is that, as per manufacturer's claims, a modified ProC Global assay may also be

used for screening of Factor V Leiden mutations, though, this option was not explored in this study.

CONCLUSION

ProC Global is a sensitive, cost-effective and easy to perform assay based on the measurement of APTT which can be done manually as well as on an automated analyser. Because of these advantages, it is highly recommended as a regular part of thrombophilia screening in our country.

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