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ORIGINAL ARTICLE

Prevalence of activated protein C resistance (Factor V Leiden) in Lagos, Nigeria

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Abstract

Objectives: Hereditary resistance to activated Protein C (Factor V Leiden) is the commonest genetic defect known to confer a predisposition to thrombosis. This study aims to determine the prevalence of activated protein C resistance (APCr) in Lagos, and to determine if any association exists between APCr and ABO, Rhesus blood types, and hemoglobin phenotypes.

Materials and Methods: A functional APCr test was conducted on healthy adult volunteers to get a Factor-V-related activated protein C ratio (APC-V ratio). APCr due to Factor V mutation was indicated when the APC-V ratio is below a cut-off value that was determined by calibration. Subjects' hemoglobin, red cell ABO, and Rhesus phenotypes were determined by standard methods.

Results: Six (2%) of 297 participants with normal baseline coagulation screening tests had functional resistance to activated protein C (APC-V ratio < 2). None of the six subjects with APCr had history of venous thromboembolism. One of the six subjects was a female but the male sex did not demonstrate a risk of inheritance of APCr (P = 0.39). Four (67%) of the six subjects with APCr were non-O blood group. Whereas only two (0.9%) of 226 non-A subjects (blood groups 0 and B) had APCr, 4 (6%) of 71 subjects with A gene (blood groups A and AB) had APCr. The inheritance of A gene appears to constitute a risk to inheritance of APCr (P = 0.03). No association was demonstrable between APCr and hemoglobin phenotypes.

Conclusion: Only 2% of the studied population had resistance to APC. The inheritance of blood group A may be a predisposition to APCr.

Key words: Activated protein C resistance; Factor V leiden; lagos; Nigeria; venous thromboembolism

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Introduction

Venous thromboembolism (VTE) is a common disorder.[1] Inherited thrombophilia that is a genetically determined predisposition to thrombosis describes a life-long increased risk of venous thromboembolism. Specific hereditary thrombophilias can now be identified in 30% to 50% of patients with a first episode of venous thromboembolism, with even higher percentages found in subjects with recurrent thrombosis.[1] A clinical suspicion of an underlying inherited thrombophilia should be considered for patients with an unprovoked idiopathic VTE onset at a younger age (before the age of 50 years), recurrent thrombotic events, and a family history of VTE, VTE at unusual anatomic sites (cerebral, mesenteric, portal, or hepatic veins, or the vena cava), or multiple adverse pregnancy outcomes.[2]

The laboratory approach to thrombophilia testing changed in 1993, when Dahlback and colleagues[3] described a new and very common familial thrombophilia, *hereditary resistance to activated Protein* C (APC resistance). Several

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laboratories subsequently reported the specific genetic defect responsible for the APC resistance – a single well-conserved G to A missense mutation at nucleotide 1691 of the factor V gene.[4,5] The resulting amino acid substitution, namely, arginine (R) to glutamine (Q) at amino acid 506, occurs precisely at 1 of the 3 sites where APC normally cleaves and inactivates procoagulant Factor Va.[5] Because of this single amino acid substitution, activated FVL is partially resistant to the anticoagulant action of APC and is inactivated at an approximately 10-fold slower rate than normal, resulting in increased thrombin generation and a prothrombotic state.[4]

The functional APC resistance assay, measures the ratio of APTT clotting times in the presence and absence of a standard amount of exogenous APC. The APC-resistant phenotype is characterized by a minimal prolongation of the APTT in response to APC and a correspondingly low ratio. The functional assay has a very high sensitivity and specificity for FVL.[6,7] However, it will not identify the rare patient with APC resistance not due to factor V abnormalities.[8] Direct DNA-based methods to detect this mutation are increasingly available in clinical diagnostic laboratories as the definitive test for this disorder. Many laboratories now use a combination of plasma and DNA (PCR) testing to assess patient status.

A search through the literature did not reveal any study on the prevalence of activated Protein C resistance in the African population. Therefore, this study sought to determine the prevalence of the reported most common hereditary thrombophilia (activated Protein C resistance) in the Nigerian population and identify any association between activated Protein C resistance and hemoglobin phenotype, ABO and rhesus blood group phenotypes as inheritance of non O blood group has been reported more frequently in individuals with inherited activated protein C resistance.[9]

Materials and Methods

Subjects recruited into the study were apparently healthy adult volunteer blood donors between the ages of 18 and 65 years after obtaining approval from the institution review board (IRB). Women on oral contraceptive, and participants with a prolong baseline PT, APTT and low platelet counts were excluded from data analysis.

Nine volumes (4.5 mL) of venous blood was collected by venepuncture into vacuum tubes containing 0.5 mL (1 volume) of 0.129 M trisodium citrate after the relevant information was recorded in the questionnaire including the age, sex, past history of venous thrombosis, and family history of venous thrombosis. Venous blood was also collected into EDTA vacuum tubes and plain tubes for the platelet count, hemoglobin phenotype, ABO, and rhesus blood group phenotypes.

In all subjects, prothrombin time (PT) and activated partial thromboplastin time (APTT) were determined using commercial reagents. A full blood count was carried out on the EDTA anticoagulated sample using the sysmex KX 21 N Hematology analyzer. Cellulose acetate hemoglobin electrophoresis at alkaline pH was used to determine hemoglobin phenotypes while ABO and Rhesus blood types were determined using the tube method.

A functional activated Protein C resistance test was used to determine a Factor V related APC ratio (APC-V ratio) that determines the presence of activated Protein C resistance (APCr). A modified (second generation) APTT-based test method was used. A commercial assay kit; Coatest APC Resistance V 823120 manufactured by Chromogenix Instrumentation Laboratory Company, Lexington MA USA and marketed by Diapharma Group Inc. was used to determine the APC-V ratio and hence the presence of activated Protein C resistance. Sample/control plasmas were prediluted in Factor V deficient plasma and incubated with the APTT reagent for a standard period of time (5 min). Coagulation was triggered by the addition of $CaCl_2$ in the absence and presence of exogenous APC and the time for clot formation were recorded. The Factor-V-related APC ratio for the samples and the control were calculated thus:

$$\text{APC-V ratio} = \frac{\text{Clot time with APC/CaCl}_2}{\text{Clot time with CaCl}_2}$$

APC resistance due to a Factor V mutation was indicated when the APC-V ratio is below the cut-off value determined by calibration.

Determining the cut-off value for the APC-V ratio

As recommended by the kit manufacturer, a cut-off value for APC resistance was established by determining the median APC-V ratios for at least 30 plasma samples from healthy individuals in the age range 20–65 years. In this study, the APC-V ratios of 125 subjects whose age ranged between 20 and 59 years were used and the median APC-V ratio was 2.5 with a mean of 2.6 ± 0.41. The Factor-V-related APC resistance cut-off value recommended by the kit manufacturer is a product of the median APC-V ratio and 0.8, when the median APC-V ratio is below 2.8. The cut-off value established therefore was 0.8 times 2.5 equal 2.0. Participants with an APC-V ratio less than 2 were regarded as having activated Protein C resistance.

Statistical analysis

Data were analyzed using statistical soft ware packages: SPSS for windows (version 11.5; SPSS Inc, Chicago, IL), Microsoft excel, and File maker pro. Descriptive statistics,

χ^2 test and student t test were used as appropriate. The critical level of significance was set at $P < 0.05$.

Results

A total of 310 participants consisting of 94 females and 216 males were enrolled for the study. Data from 297 participants were analyzed after excluding 13 subjects with prolonged baseline PT and APTT. The mean age of the 297 subjects was 31.26 ± 8.9 years with a range of 18 to 59 years. The females were younger, aged 28 ± 7.8 years than the males aged 32.6 ± 9.0 years ($P = 0.000$).

Prevalence of APCr

Six (2%) of the 297 participants with normal baseline clotting screening tests had an APC-V ratio below the established cut-off value of 2 with a mean APC ratio of 1.55 ± 0.38. Those without the resistance ($n = 291$) had a mean of 2.55 ± 0.39. Although only one of the six subjects with APC-V ratio below the cut-off value (APCr) is a female, the male sex is not more predisposed to the presence of APCr ($P = 0.39$). None of the six subjects had a past history of venous thrombosis or a family history of venous thrombosis [Table 1].

APCr and ABO blood group phenotypes

One hundred and sixty one (54.2%) of the participants were blood group O. Four (67%) of the six subjects with APCr were non-O blood group (three blood group A and one blood group AB) [Table 2]. Of the 161 subjects with blood group O, only 2 (1.24%) had APCr. None of those with blood group B (0%) had APCr whereas 1 (9.1%) and 3 (5%) of those with blood group AB and A, respectively, had APCr. Thus, if the study population is grouped into A (A and AB) and non-A (O and B) blood groups Table 3, inheritance of the A gene appears to significantly predispose an individual to having APC resistance. $\chi^2 = 6.16$, $P = 0.03$

Ninety seven percent (289) of the 297 subjects were Rhesus D positive. All the six (100%) participants who had APCr were rhesus D positive but the rhesus D antigen was not associated with a predisposition to the presence of APCr ($\chi^2 = 0.466$, $P = 0.5$).

APCr and hemoglobin phenotypes

Two hundred and thirty one (77.7%) of the participants had haemoglobin phenotype A with a mean APC-V ratio of 2.53 ± 0.42. Thirteen participant with genotype AC had a lower mean of 2.30 ± 0.28 compared with those of phenotype A ($P = 0.048$). Fifty three subjects with Hb genotype AS have a mean APC-V ratio of 2.55 ± 0.38 that is not different from that of Hb phenotype A ($P = 0.643$) but is significantly different from those with genotype AC ($P = 0.041$). All the six (100%) subjects with APCr had Hb phenotype A. Although subjects with AC genotype tend to have low APC-V ratio when compared with Hb A phenotype and AS genotypes, the AC subjects do not appear to have an increased predisposition to APCr $\chi^2 = 1.75$, $P = 0.4$.

APCr and blood counts

The mean concentration of hemoglobin in subjects with activated Protein C resistance (13.67 ± 0.55 g/dL) is statistically significantly higher than that of subjects without Activated Protein C resistance (13.09 ± 1.23 g/dL) $P = 0.017$.

There is no statistical difference between the mean packed cell volume, white cell count and platelets count in subjects with activated Protein C resistance and subjects without APCr.

Discussion

Resistance to APC is the commonest genetic defect known to confer a predisposition to thrombosis, occurring at least

Table 1: Detailed characteristics of the six subjects with APC resistance

Participant number	Age (years)	Sex	Blood group	Hb phenotype	History of VTE*	Family history of VTE	APC-V ratio
46	47	F	O+	AA	None	None	1.22
49	28	M	A+	AA	None	None	1.11
56	24	M	O+	AA	None	None	1.88
121	28	M	A+	AA	None	None	1.92
196	35	M	AB+	AA	None	None	1.30
202	34	M	A+	AA	None	None	1.88

*VTE – Venous thromboembolism

Table 2: Distribution of APCr according to ABO blood group

Blood group	APC-V ratio < 2 (APCr)	APC-V ratio ≥ 2	Total	Group prevalence of APCr (%)	Overall prevalence of APCr (%)
A	3	57	60	5	50
B	0	65	65	0	0
O	2	159	161	1.24	33.3
AB	1	10	11	9.1	16.7
Total	6	291	297	2	100

Table 3. Association between ABO blood group and APCr

Blood group	APC-V ratio < 2 (%)	APC-V ratio ≥ 2	Total
Non A (O, B)	2 (33)	224	226
A (A, AB,)	4 (67)	67	71
Total	6	291	297

ten times more frequently than other hereditary defects.[10]

Heterozygosity for Factor V Leiden occurs in 3–15% of the normal white population of KInorthern European or Scandinavian ancestry, approximately 12% to 20% of incident VTE patients[11] and approximately 40% to 50% of those with recurrent or familial VTE.[12] The FVL mutation is reported to be much less common in populations of non-European ancestry, with a carrier frequency of 1.2% in African Americans, 2.2% in Hispanic Americans, 1.2% in Native Americans, and 0.45% in Asian Americans.[13,14] Homozygotes account for about 1% of the white population with the FVL mutation, but are disproportionately overrepresented clinically because of their higher thrombotic risk.

The prevalence varies considerably in different populations. As the highest heterozygosity rate is found in Europe, the mutation is said to be extremely rare in Asian, African, and indigenous Australian populations.[14] In a review of the distribution of the FVL mutation in Turkey, the frequency of the mutation from the different part of Turkey ranges from 3.5–15% with a summed up frequency of 7.9%.[15]

Heterozygous carriers of FVL have been shown to have an overall 3- to 7fold increased risk of venous thrombosis, while Homozygotes have a 50- to 100-fold increased risk.

The thrombotic risk in persons with this mutation is amplified by other acquired risk factors, such as oral contraceptive, pregnancy,[16] and orthopaedic surgical settings that predispose patients to thrombosis. The use of oral contraceptives substantially increases the risk of VTE in women with FVL. Factor V Leiden is found in 20% to 30% of women with a history of venous thrombosis during oral contraceptive use.[16]

In this study, a prevalence of 2% of activated Protein C resistance was found. This figure contrast the report of Rendrik F Franco *et al.*,[17] who found an absence of the FVL mutation in African blacks and Asians but a 2.6% heterozygotes state in whites, and 0.6% in Ameridians and found no homozygotes state of the FVL mutation in any of the population groups studied. This also contrast Rees *et al.*[14] and Hooper *et al.*[18] who reported the mutation to be absent in Africans (Negroid). A possible explanation for this discrepant result is that these studies were conducted in Africans in diaspora and the genes if not very common as we have presently documented may not be found in the Negroid populations as reported by these authors. These authors used a combination of APCr test with direct DNA (PCR/RFLP)-based methods that are extremely accurate and precise as opposed to only a APCr test done in this study. However, a modification to the APCr test, using a predilution of test plasma in FV-deficient plasma as was done in our study is said to improve the specificity and sensitivity of the test to FVL by up to 100% in some studies.[19,20]

Approximately 90% to 95% of those with functional APC resistance measured by the clotting time test as was done in this study, have the identical Factor V R506Q (Leiden) missense mutation. In rare cases, genetic abnormalities other than the Factor V R506Q mutation produce the APC resistance phenotype or modulate its expression in Factor V R506Q heterozygotes. Although 95% of cases of APC resistance reflect the presence of the factor V Leiden mutation, 5% of individuals have repeatedly abnormal APC resistance tests in the absence of the factor V Leiden allele. Depending on the screening assay used, some cases may represent acquired APC resistance caused by high factor VIII levels, pregnancy, or a lupus anticoagulant effect.

Vurkun M *et al.*[21] in their study to determine the prevalence of APC resistance and FVL in a healthy population in Edirne province in Turkey found 22/476 subjects (4.7%) with functional APC resistance and 20 of these 22 subjects (90.9%) had FVL by DNA test. The two subjects who have no FVL mutation had high levels of FVIII to explain an acquired APC resistance. In this study, however, FVL mutation and FVIII levels were not determined for the six participants with functional activated Protein C resistance for lack of facilities.

ABO blood group and more recently high Von Willebrand factor (VWf) and factor VIII (FVIII) levels have been associated with thrombotic disease.[22]

Information on blood group genotypes/phenotypes may play a role in the management of thrombophilia patients especially when they are carriers of the FVL mutation. Morelli *et al.*[9] in the large population based case control study of venous thrombosis (the LEIDEN THROMBOPHILIA STUDY), found among the 471 patient and 471 controls studied, 92 (19.5%) carried the FVL mutation in the patient group compared with 14 (3%) in the control group, yielding an overall risk (OR) for venous thrombosis of 7.9%.[6] In carriers of the Factor V Leiden, non-OO genotype were present in 68/92 (78%) patient versus 5/14 (36%) of controls. The thrombosis risk of the combination of non-OO blood group genotypes and FVL compared with subjects OO genotypes without FVL was 23 fold increased, which was higher than expected for the effects of non OO genotype (OR 1.7) and FVL (0R 4.6) when considered separately, adjustment for age and sex did not influence these risk estimates.

The mechanism by which non-O blood group contributes to the thrombotic risk in carriers of FVL mutation is mainly explained by its effect on FVIII levels. High FVIII levels are associated with decrease responsiveness to activated protein C (APC) in the absence of FVL.[23,24] In carriers of FVL mutation, this additional effect on the APC sensitivity might result in an exponential increase in thrombotic risk.

In the six subjects with APCr in this study, there is an excess of the non O blood group with four (67%) of the subjects having the non O blood group. This does not contrast previous studies. Morelli *et al.*[9] reported 78% non-O blood group for carriers of the FVL with venous thrombosis and 36% for carriers without venous thrombosis (control group). Gonzales *et al.*[25] found 96% non-O blood group in 28 subjects with FVL and venous thrombosis. Non-O blood groups are associated with a higher risk of venous thrombosis than the O blood group, an effect that is mainly explained by Factor VIII levels. Higher levels of Factor VIII have been documented in the non-O blood group persons in whom the risk of thrombosis is reported to be higher.[26,27]

In this study, all the subjects with APCr are rhesus D positive, the rhesus D antigen does not appear to confer an association with APCr.

Although all the six subjects with APCr have hemoglobin phenotype A, the chi-square analysis did not show that Hb phenotype A inheritance could be associated with APCr ($\chi^2 = 1.75, P = 0.4$). There was a significant difference between the mean APC-V ratios between subjects with hemoglobin genotype AC and AA ($P = 0.048$) and between hemoglobin genotype AC and AS ($P = 0.041$) with the mean APC-V ratio for subjects with hemoglobin genotype AC being lower than the mean for phenotype A and genotype AS. It thus appears that the presence of a C gene may be associated with a reduced APC-V ratio. The implication of this finding is yet unclear.

None of the other studied hematological parameters (Packed cell volume, White cell count and Platelet count) were associated with the presence of APCr.

Conclusions

Some Nigerians do have activated Protein C resistance that is usually an inherited predisposition to thrombosis.

Two percent prevalence by a functional APC resistance test in this study suggests the presence of APCr that may be inherited Factor V Leiden in our population.

In addition, these data suggest that the inheritance of non-O blood group may be a predisposition to the presence of APC resistance. However, these data relevant in the clinical management of patients at risk for venous thrombosis and those with recurrent thrombotic disorders need to be confirmed in a larger study.

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