Factor V- Leiden Gene Mutation Among Natural Population of Maiduguri, North East Nigeria

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ABSTRACT

Background: The emergence of inexplicable thrombotic events with unrecognised mechanism in the recent times warranted the investigation of otherwise-uncommon risk factors for thromboembolic phenomena. It is a common cause of inherited thrombophilia associated with venous thromboembolism (VTE), recurrent pregnancy loss, infertility, contraceptive or hormone replacement related coagulopathy, and cerebral palsy. This study therefore aimed at exploring the prevalence of factor V–Leiden (FVL) gene mutation among the natural population of Maiduguri.

Methods: This is a cross-sectional study which was carried out between January 2013 and March 2014. Ninety-eight (98) healthy blood donors from ethnic population of Maiduguri, northeast of Nigeria were recruited prospectively & consecutively. They were investigated for factor V–Leiden genotype by- Amplification Created Restriction Enzyme Site (ACRES) polymerase chain reaction. Data was presented as percentage and Newman-Keuls post hoc was used to compare variables.

Result: Factor V-Leiden mutation was not detected in any of the 98 subjects screened; all expressed normal genotype for factor V gene (F5) 1619 G/G. Protein C (PC) and Proteins S (PS) analysis revealed that all the subjects had normal plasma percentage (%) activities for these natural anticoagulants.

Conclusion: FVL mutation is probably a rare genetic trait among ethnic population of Maiduguri northeast of Nigeria.

KEYWORDS: FVL-Mutation, Rarer, Maiduguri Northeast Nigeria.

Introduction

Factor V-Leiden (FVL) mutation is an autosomal dominant trait; the mutant gene is located on chromosome locus F5, 1q 23. [1].

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The disorder is reported as one of the most common cause of prothrombotic genetic abnormality leading to thrombophilia1,2,3. Individuals with activated protein C (APC) resistance have the same point mutation in the gene for factor (F5) with guanine to adenine transition at nucleotide position 1691 in the axon 10 of F54,5. The mutation causes a substitution of glutamine for arginine at position 506 (Arg 506 GLn); this is the major site of factor V (FVa) cleavage by activated protein C (APC)5. Mutant factor V- Leiden FVL (Arg 506–GLn) is therefore resistant to APC leading to hypercoagulable state.

The latter results from the inability of APC to limit activated factor V (FVa) enhancement of
factor X in the conversion of prothrombin to thrombin, consequently the conversion of fibrinogen to fibrin clot continues uninhibited.6,7,8

Factor V Laden mutation is usually diagnosed following screening tests in venous thromboembolism (VTE)6,8, recurrent miscarriage and infertility9,10,11, combined oral contraceptive or hormones replacement therapy related hypercoagulability11,12, acute respiratory distress syndrome13, gestational induced high blood pressure and placental abruption14,15,16; bleeding phenotype in anticoagulant sensitive patients17 and in hemiplegic cerebral palsy18,19,20.

Factor V Laden (Arg 506 GLn) mutation is the most common heritable thrombophilia in the Caucasian population and has been reported to have a prevalence rate between 2%-15%; in Africans, Asians, and Southern Europe, the population rates of between 1%-4% were reported.21,22

However, in a relatively recent report, prevalence of 14.4% among Lebanese patients diagnosed of VTE was documented; this was deduced as one of the highest prevalence rates in the world.23 Prevalence rate of this mutation varies per ethnic and geographic distribution of a population. In our environment, there appear to be a dearth of information on the prevalence of FVL mutant among the natural population of Maiduguri north eastern Nigeria.

Materials and Methods

Study Area

Maiduguri, a cosmopolitan settlement north east of Nigeria is in the Sahel Savannah Zone of Sub-Saharan Africa. It is a Metropolitan area with representation of more than one ethnic group of which the predominant is the Kanuris, others include Bura, Marghi and Shuwa.

Subjects

Ninety-eight (98) healthy blood donors were recruited after informed consent and pre-test counselling were instituted. Financial implication of ACRES PCR assay per subject did not allow the expansion of the sample size beyond 98. Socio-demographic data were obtained with semi structured questionnaire; ethical clearance was obtained from the research and ethics committee of University of Maiduguri Teaching Hospital (UMTH).

Sample Collection

Eight and a half millilitres of blood were collected, 4ml was introduced into EDTA vacutainer and 4.5ml was dispensed into a plastic bottle containing 0.5ml sodium citrate (0.11 molar solution) to give a blood/citrate ratio of 9:1.

Sample Analysis

DNA product was extracted from frozen EDTA blood samples by Sodium Dodecyl Sulphate (SDS) extraction method and subjected to molecular analysis using Amplification Created Restriction Enzyme Site (ACRES) Polymerase Chain Reaction (PCR), using forward primer in FVL gene with low cost deliberate mismatch (-5’GTAAGAGCAGATCCCTGGACAGtC3’) and a reversed primer without a mismatch (-5’TGTATTCACACACTGTTGCTAA3’). The PCR product was subjected to agarose gel electrophoresis. The procedure was carried out at Safety Molecular Pathology Laboratory (www.safety.biomeedical.org) at the University of Nigeria Enugu Campus (UNEC) Enugu State Nigeria.

Platelet poor plasma was separated after centrifugation at 3000g and immediately assayed for functional protein C (PC) and free protein S (PS). Chromogenic Protein C kit ref. OUVVI5z and Protein S Kit ref. OWRH were used. Results were expressed as % activity of standard Human Plasma ref. ORKL17 (Kraus, 1986). These reagent kits were acquired from Siemens Health Care Diagnostic Products GmbH 35041 Marburg Germany. Estimations were carried out with automated coagulometer Sysmex CA 560.
S/N F1016 (Sysmex Corporation Kobe Japan). Data was presented as percentage/frequency and Newman Keul post hoc was used to compare variables where appropriate.

Results
Out of the 98 subjects enrolled for the study, Kanuris were 69, Bura 20, Marghi 13, Shuwa 6; the predominant ethnic group in the population is the Kanuris. The population constituting 32(32.7%) females and 66(67.3%) males (Fig 1). Their ages ranged from 18-69 year with the highest proportion between the ages 18-30 years, means age was 36.16 ± 1.4 years. Factor V-Leiden mutation was detected in none of the 98 subjects screened; all expressed normal genotype for factor V gene (F5) 1619 G/G (Table I) Protein C (PC) and Proteins S (PS) analysis revealed that all the subjects had normal plasma percentage (%) activities for these natural anticoagulants (Table II). There were no statistical differences (P>0.05) between the mean values of males as compared to the females (Table 2).

![Fig. 1: Age (%) and Sex Cross Distribution of all Subjects.](image)

<table>
<thead>
<tr>
<th>FVL GENOTYPE</th>
<th>MALE n (%)</th>
<th>FEMALE n (%)</th>
<th>TOTAL n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1691 G/G</td>
<td>66(67.3)</td>
<td>32(32.7)</td>
<td>98(100)</td>
</tr>
<tr>
<td>1691 A/G</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>1691 A/A</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>66(67.3)</td>
<td>32(32.7)</td>
<td>98(100)</td>
</tr>
</tbody>
</table>

KEY:
1619 G/G:Normal genotype for FV gene
1619 A/G:Heterozygous genotype for FVL mutation
1619 A/A:Homozygous genotype for FVL mutation
FVL:Factor five (v)-Leiden mutation
Discussion

Resistance to activated protein C anticoagulant properties is caused by a mutation in the factor V gene referred to as factor V-Leiden (FVL) mutation—named after the Dutch City where it was first identified in 1994. In the general population the prevalence varies between 0%-15%. It was reported that the mutant allele incidence is low in African, Asian and South European populations, with prevalence rates between 1-4%. However relatively higher rates (2-15%) were reported for European and American Caucasians. Reports from, Lebanon lately, unexpectedly revealed one of the highest rates (14.5%) in the eastern Mediterranean and in the world.

Our study revealed that FVL mutant allele was present in none of the ninety-eight ethnic subjects of Maiduguri investigated, as all expressed normal genotype (1691 G/G) for factor V-gene and this was in consonance with reports that FVL mutation incidence is low among the black African population.

Factor V Laden mutation was adduced as the most prevalent risk factor for heritable thrombophilia. Heterozygotes (1619 A/G) was reported to have 3-5 times increased risk of thrombotic events. Homozygotes (1619 A/A) are much less common but associated with higher thrombotic risk which was about 80 times increased. Literature had associated this mutation with hypercoagulability hitherto described as idiopathic or with unrecognised mechanism. Studies recently have clearly demonstrated positive correlation between recurrent pregnancy losses and FVL mutation; suggesting that women with second-trimester miscarriage with or without complications such as placental abruption, pre-eclampsia and slow foetal growth should be screened for inheritable thrombophilia including FVL (Arg 506 Gln) mutation. Evolution of this mutation and its association with other complications such as hemiplegic cerebral palsy, acute respiratory distress syndrome, contraceptive and hormone replacement pro-coagulable states are well documented.

The apparently prevailing normal factor V-Leiden genotype among our study subjects in Maiduguri may be a possible reflection of a low incidence of this genetic risk factor for thromboembolic phenomena in our environment. Clinical study is however needed to illuminate its relationship with thrombotic events in Maiduguri. The absence of FVL mutation among natural population in our environment could be a positive score card.

Table 2: Mean± (SD) of Protein C(PC) and Protein S(PS) % Activity of all Subjects per gender distribution/statistical comparison.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>males n=66</th>
<th>Females n=32</th>
<th>F-Statistics</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC% Activity</td>
<td>98.20±0.24</td>
<td>96.3±0.42</td>
<td>0.70</td>
<td>0.53</td>
</tr>
<tr>
<td>Ref Int. (70-140%)</td>
<td></td>
<td></td>
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<tr>
<td>PS % Activity</td>
<td>94.4±0.82</td>
<td>95.3±0.65</td>
<td>0.67</td>
<td>0.49</td>
</tr>
<tr>
<td>Ref Int. (70-130%)</td>
<td></td>
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</tbody>
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PC and PS activity was expressed as % activity of STD Human Plasma Ref. ORKL17. % activity <70% is regarded as diminished plasma activity level. P value of <0.05 is considered significant.
References


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