MOLECULAR STUDY OF FACTOR VIII GENE IN EGYPTIAN PATIENTS WITH HEMOPHILIA A

Thesis

Submitted for partial fulfillment of MD degree in clinical pathology

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2009

دراسة جزيئية لجين العامل الثامن في المرضى المصريين المصابين بمرض هيموفيليا أ

مقدم من

الطبيبة حنان محمد علي أحمد

ماجستير الباثولوجيا الاكلينيكية

توطئة للحصول على درجة الدكتوراة في الباثولوجيا الاكلينيكية

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ABSTRACT

Hemophilia A is an X-linked hereditary bleeding disorder caused by deficient or defective coagulation factor VIII. Multiple molecular defects may affect factor VIII gene such as point mutations, premature stop codons, deletions, insertions and inversions. The most common sequence alterations leading to a severe disease condition are the partial gene inversion with a breakpoint in intron 22 of the factor VIII gene, responsible for about 40-50% of the severe hemophilia A cases. The aim of this work was genotyping of *int22h*-related rearrangements of factor VIII gene by Inverse shifting-PCR (IS-PCR) in Egyptian patients with hemophilia A in order to facilitate carrier detection and prenatal diagnosis.

Our study was conducted on 30 Hemophilia A patients following up regularly at the Hematology Clinic, New Children Hospital, Cairo University, and revealed that among severe cases, 6/13 patients (46.1%) had intron 22 inversion, 3/13 patients (23.1%) had intron 22 deletion and 4/13 patients (30.8%) carried the wild type (normal) allele. Only one out of 10 patients (10%) with moderate disease was positive for intron 22 inversion, whereas the rest of moderate cases carried the wild type (normal) allele. All mild cases were negative for *int22h*-related rearrangements and carried the wild type (normal) allele. We concluded that the genotyping of *int22h*-related rearrangements of factor VIII gene by Inverse shifting-PCR (IS-PCR) can be used in molecular diagnosis of severe and moderately severe hemophilia A and it is able for rapid discrimination between inversions (type 1and 2) and deletions (type 1and 2) and duplication of intron 22. We suggest that the spectrum of intron 22

inversion/deletion in the Egyptian hemophilic patients is similar to that reported in other populations.

Keywords:
Hemophilia A - FVIII gene - Intron 22 inversion - Inverse shifting-PCR.

CONCLUSION

Inverse shifting-PCR (IS-PCR) diagnostic and complementary tests of intron 22 of Factor VIII gene have proven to be rapid, robust and reliable technique and represent the method of choice at first line mutation screening of severe and moderately-severe HA cases.

The genotyping of *int22h*-related rearrangements of factor VIII gene by Inverse shifting-PCR enables rapid discrimination between inversions (type I and II) and deletions (type I and II) and duplication of intron 22. This work suggests that the spectrum of intron 22 inversion/deletion in the Egyptian hemophilic patients is more or less similar to that reported in other populations.

IS-PCR is cheap and suitable for carrier detection, preimplantation and prenatal diagnosis in developing countries with limited health resourses.

RECOMMENDATIONS

1. All patients with hemophilia A should be first screened for intron 22 inversion, as intron 22 inversion occurs in 40 – 50% of severe hemophilia A patients.
2. Genotyping of intron 22-related rearrangements by Inverse-shifting PCR could be used for carrier detection and prenatal diagnosis.
3. Further molecular studies are needed for intron 22 inversion negative patients with hemophilia A, in order to study the spectrum of different factor VIII gene mutations in Egyptian population.
4. In Egypt, molecular studies of low cost such as IS-PCR should be widespread, to facilitate genetic diagnosis and more understanding of the molecular background of hemophilia A.
5. Special health programs should be directed to severe hemophilia A patients, in order to facilitate replacement therapy to prevent complications of the disease.
6. Studies in gene therapy in developing countries with limited resources should take place, as gene therapy is the treatment of choice for hemophilia A patients in the near future.

ACKNOWLEDGEMENT

My grateful acknowledge for ***Prof. Dr. Hanaa Hamed Arnaout*** *Professor of Clinical Pathology, Faculty of Medicine, Cairo University for her kind supervision, precious guidance, helpful instruction, and powerful support.*

I wish to express my warm and sincere thanks to ***Prof. Dr. Hanan Nour Raslan****, Assistant Professor of Clinical Pathology, Faculty of Medicine, Cairo University for valuable help, sincere concern and support.*

I would like to express my great thanks and deepest appreciation to ***Prof. Dr. Heba Mohammed Hassan Abou Elew*** *Assistant Professor of Clinical Pathology, Faculty of Medicine, Cairo University for her great support, continuous guidance and valuable advices.*

My appreciation is expressed to ***Prof. Dr. Magy Smir Abdelwahab****, Assistant Professor of Pediatrics, Faculty of Medicine, Cairo University, for her encouragement and valuable suggestions.*

Finally, I want to thank the members of clinical pathology department Faculty of Medicine, Cairo University for their continuous help, moral support all over the work, and also all those who gave me a hand for this work to see light.

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List of abbreviations

AMD: :Amplification mismatch detection
aPTT :activated partial thromboplastin time
APC :Activated protein C
AS-APEX :Allele-specific arrayed primer extension
APCCs :Activated prothrombin complex concentrates
BiP :immunoglobulin-binding protein
BT :Bleeding time
BU :Bethesda Unit
CG :Cytosine guanine
CJD :Creutzfeldt-Jacob disease
CMC :Chemical mismatch cleavage
CRM: :Cross reacting material
CSGE :Conformation Sensitive Gel Electrophoresis
CVS :Chorionic villi sampling
DDAVP :1,8-desamino-d-arginine vasopressin
ddNTP :dideoxy nucleotide triphosphate
Del22 :Intron 22 deletion
DGGE :Denaturating gradient gel electrophoresis
DHPLC :Denaturing high performance liquid chromatography
dNTP :deoxy nucleotides triphosphate
Dup22 :Intron duplication
ED :Extragenic downstream.
ELISA: :Enzyme linked immunosorbent assay
FVIII :Factor VIII
FIX :Factor IX
FVIII:C :level of FVIII activity
HA :Hemohpilia A
HAMSTeRS:Haemophilia A Mutation, Structure, Test and Resource Site
HRM :High resolution melting analysis
ID :intragenic downstream
Int: :Intron
int22h-1 :Sequence within intron 22 (within FVIII gene)
int22h-2 :Homologous sequence (copy) outside FVIII gene
int22h-3 :Homologous sequence (copy) outside FVIII gene
Inv22 :Intron 22 inversion
Inv1 :Intron 1 inversion
IU :Intragenic upstream
IVS :Intervening sequence
I-PCR :Inverse polymerase chain reaction
IS-PCR :Inverse shifting-polymerase chain reaction
Kb: :Kilo base

LD-PCR: :Long distance polymerase chain reaction
LINE :Long interspersed nuclear elements
PCCs :Prothrombin complex concentrates
PCR: :Polymerase chain reaction
PGD :Preimplantation genetic diagnosis
PT :Prothrombin time.
RFLP :Restriction fragments length polymorphism
RT-PCR :Revrse trascriptase- Polymerase chain reaction
S-PCR :Subcycling-PCR
SSCP :Single standard conformation polymorphism
SSRs :Simple Sequence Repeats
STRs :Short Tandem Repeats
VNTRs :Variable number tandem repeats sequence
vWD :von Willebrand disease
vWF :von Willebrand Factor
Xase :Tenase
XCE :X controlling element
XCI :X-chromosome inactivation

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