بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالي:

(وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلاَّ قَلِيلاً)

صدق الله العظيم

الإسراء 85

Dedication

To my mother and father souls

To my wife and kids

Acknowledgement

Who does not thank people does not thank God as the Prophet Mohammed said. Thank God first and foremost. Thanks to my supervisor Dr. Maria. M. Satti who was very patient in supervising & revising this work. A great thanks to the Hemophilia center, Sudan, specially sister Ekhlas who was of great help in sample collection and patients data. Thanks go to the hemophilia A patients who agreed to participate in this work. Also I want to thank Dr. Mansoor M. Mansoor my Co-supervisor who helped greatly in the molecular studies. Also thanks extend to Dr. Fathelrahman Mahadi who helped and supported me to complete the work. Thanks also to the staff of Medical laboratory Sciences, Sudan University whom encouraged me in my work, specially Dr. Mohammed S. Abdelaziz, Dr. Mohammed Masaad, and U. Mudathir Abdelraheem. Also my thanks to Dr. Omer M. Khalil, assistant professor Aljouf University, Dr. Abd Allah Osman Associate professor Umm Al qura University. Also I want to thank the staff of the research lab of Sudan University, and all my colleagues in the Faculty of Medical laboratory Sciences, SUST.

Abbreviations

Abbreviation	Name
APC	Activated Protein C
APTT	Activated Partial Thromboplastin Time
BLAST	Basic length alignment search tool
Bp	Base pair
DDAVP	1-deamino-8-D-arginine vasopressin
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
dTTP	deoxythymidine triphosphate
DNTPs	Deoxynucleotides Tri phosphate
EDTA	ethylene diamine tetra acetic acid
Ex	Exon
ExoSAB	Exo-nuclease shrimp alkaline phosphatase
HADB	Hemophilia A database
HAMSTeRS	Hemophilia A Mutation, Structure, Test and Resource Site
Hb	haemoglobin or haemoglobin concentration
HCT	Haematocrit
Int	Intron
Inv	Inversion
Kb	Kilobase
LD PCR	Long distance polymerase chain reaction
µM	Micro molar
mM	Milli molar
mRNA	Messenger ribonucleic acid
nmol	Nano mole
OD	Optical density
pGEM	Professional Gel Electrophoreses Machine
PT	Prothrombin Time
RBC's	red blood cells

RPM	Round per minute.
Taq	Thermo aqueous
TBE	Trisborate EDTA (buffer)
TBS	Tris-buffered saline
PPP	Platelets poor plasma
PCR	Polymerase chain reaction
VIII	Factor 8
WBC	white blood cell

Abstract

Hemophilia A (factor VIII deficiency) is the most common hereditary disorder of blood coagulation. It is due to the absence or decreased function of coagulation factor VIII, resulting from mutations in the factor VIII gene. The aim of the study was to screen the factor VIII gene mutation among Sudanese patients with hemophilia A.

This analytical cross sectional study conducted in Khartoum teaching hospital in patients with hemophilia A who attended to hemophilia center, 72 patients with hemophilia A were selected, 5 ml of blood samples were taken in K_2 EDTA for DNA extraction for the molecular studies and 5ml tri sodium citrate for APTT, PT, factor VIII assay and factor VIII inhibitor by manual methods.

For the molecular studies a master mix and conventional PCR were used. Twenty primers were used as followed: four for Intron 22 inversion mutations. Four for Intron 1 inversion mutation. The twelve primers were used for screening of six exons sequence by sequencer. For exon screening for mutations 120 PCR products were sequenced and analyzed by BLAST and FASTA in the NCBI web site, in which query and subjects procedure was used. PCR products were tested by agrose gel electrophoreses and gel documentation system.

The result showed All the 72 patients were males factor VIII activity less than 1% were 59 patients (81.9%) and 13 patients (19.1%) between 1 - 5%. VIII inhibitor 62 patients (80.9%) were negative while 10 were positive (13.8%). All patients were normal PT and elevated APTT. Two patients out of 72 (2.7%) were positive Intron 1 inversion mutation. For exons sequencing there were deletions mutations in three out of ten samples (30%), in exons 11, 23 and exon 24. About fifty PCR runs were done for intron 22 inversin using conventional LD-PCR, but no products were found except the ladder bands.

المستخلص

الهيموفيليا (أ) هو مرض نزفي وراثي يرجع إلى غياب أو انخفاض وظيفة عامل تجلط الدم الثامن، والناجم عن طفرات في جين العامل الثامن. هدفت الدراسة إيجاد الطفرات الجينية لعامل التجلط الثامن في المرضي السودانيين المصابين بمرض الهيموفيليا-أ.

هذه دراسة مقطعية تحليلية أجريت في مستشفى الخرطوم التعليمي في المرضى الذين يعانون من الهيموفيليا-أ والذين حضروا إلى مركز الهيموفيليا، تم اختيار 72 مريضاً يعانون من مرض الهيموفيليا-أ ، أخذت 5مل من عينات الدم في EDTA K2 لاستخراج الحمض النووي للدراسات الجزيئية و5مل في مانع التجلط tri-sodium citrate لقياس APTT، PT، العامل الثامن مثبطاته, أجريت التجارب بالطرق اليدوية. جمعت العينات بعد موافقة المريض او ولي أمره.

استخدمت عشرون primer في الدراسة أربعة لدراسة انقلاب انترون 22 و أربعة لدراسة انقلاب انترون 1 واثني عشر لمسح ستة من الاكسونات و استخدم master mix و . conventional PCR . حيث تم مسح ال exons بواسطة جهاز الSequencer حيث مسحت 120 عينة PCR مختلفة وتم تحليل الناتج بواسطة برنامج BLASTو FASTA من موقع NCBI حيث تم مطابقة العينات بوضع query and subjects. استخدم جل الاقاروز لكشف وجود ناتج PCR من عدمه.

أظهرت النتائج ان جميع المرضي من الذكور ونتيجة فحص PT طبيعية و APTT وجدت مرتفعة في كل المرضي. نسبة نشاط العامل الثامن حيث وجد 59مريضاُ (81.9%) اقل من 1% و13 مريض (19.1%) بين 1-5%. ووجد 10 مرضي (13.8%) نتائج فحصهم إيجابية لمثبطات العامل الثامن و الباقي 86.2% سلبية. انترون 1 طفرتان انقلابيتان من 72 عينة (2.7%) . كما أظهرت نتيجة مسح ال exons وجود ثلاثة طفرات جينية من عشرة عينات (30%) تم مسحها لل exons الستة (30%) حيث وجدت الثلاث طفرات deletions في كل من exon11 و 23 و 24.

و أجريت اكثر من خمسين محاولة مختلفة لمسح انترون 22 بواسطةconventional PCR ولكن لم يوجد ناتج غير ladder bands.

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