DETECTION OF HEMOPHILIA A CARRIERS AMONG EGYPTIANS

Thesis

submitted for partial fulfilment of the requirement for

the M.D. Degree in Clinical Pathology

By

Azza Mohamed Sadek El Danasoury

Under the supervision of

Prof. Wagih Nagib Ibrahim

Professor of Clinical Pathology

Ain Shams University

Prof. Ahmed Samy Khalifa

Professor of Pediatrics

Ain Shams University

Prof. Sawsan Abd El-Moety Fayad

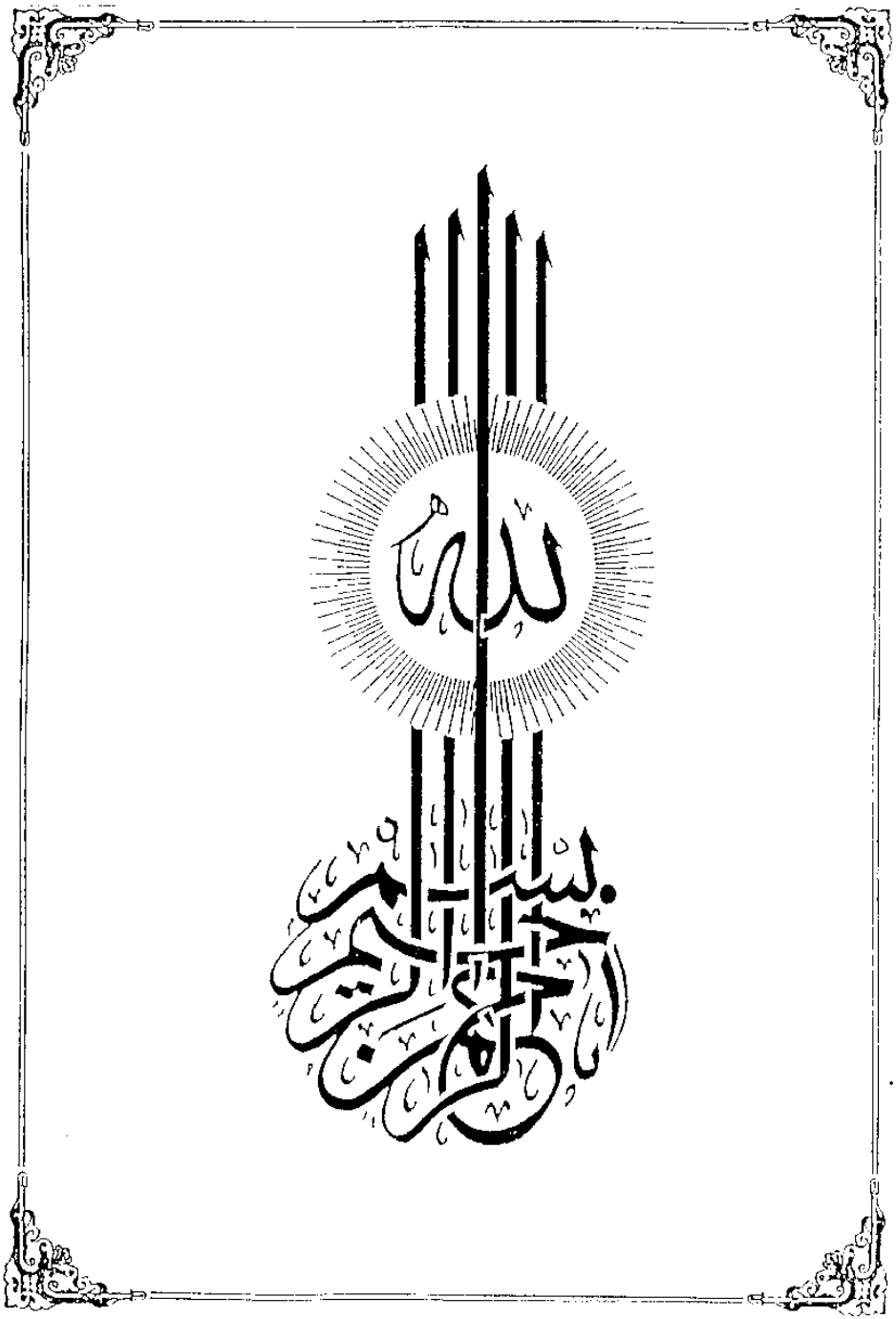
Professor of Clinical Pathology

Ain Shams University

FACULTY OF MEDICINE

AIN SHAMS UNIVERSITY

1991



ACKNOWLEDGEMENT

I wish to express my deep gratitude to Prof. Wagih Nagib Ibrahim, Professor of Clinical Pathology, Ain Shams University for his meticulous supervision, scientific support and judicious guidance despite many other demands on his time.

I am very grateful to Prof. Ahmed Samy Khalifa, Professor of Pediatrics, Ain Shams University for his kind supervision, continuous fatherly encouragement throughout the course of this thesis.

I find no words to express my gratitude to Prof. Sawsan Abd El-Moety Fayad, Professor of Clinical Pathology, Ain Shams University for her constant advise, enthusiastic support and great help without which this work would not have come to light.

I also wish to thank Prof. Sawsan Elwan, Professor of Clinical Pathology, Assiut University. She was very kind to give me the opportunity to work in her department. I will always remember her valuable advices.

I am greatly indebted to Dr. Refaat Gaballah, Assistant Professor of Clinical Pathology, Ain Shams University for his honest assistance and precious guidance during most of the stages of this work.

My sincere thanks are due to Dr. Salwa Abou El-Hana, Lecturer of Clinical Pathology, Ain Shams University for her helpful suggestions while revising fully the manuscript.

I also wish to express my thanks to Dr. Mohamed El-Kabsh, Lecturer of Clinical Pathology, Assiut University. He helped me very much to get access to hemophilic families attending the Clinical Pathology Department, Assiut University Hospital.

I would like to thank the members of the Computer Unit, Faculty of Agriculture, Ain Shams University for helping in statistical analysis of the results.

This work was partially supported by a grant from the Academy of Science and Technology, Cairo, Egypt.

LIST OF ABBREVIATIONS

A:	Adenine
AHF:	Anti-hemophilic factor
AIDS:	Acquired Immunodeficiency Syndrome
APTT:	Activated partial thromboplastin time
arg:	Arginine
bp:	Base pair
C:	Cytosine
Ca^{++}:	Calcium ions
cDNA:	Complementary DNA = copy DNA
CRM^{+}:	Cross reacting material positive
CRM^{-}:	Cross reacting material negative
CP:	Ceruloplasmin
Da:	Dalton
DNA:	Deoxyribonucleic acid
EIA:	Electroimmunoassay
ELISA:	Enzyme linked immunosorbent assay
f:	Relative reproductive fitness
FV:	Factor V
FVIII:	Factor VIII
VIII:Ag:	Factor VIII antigen
VIII:C:	Factor VIII coagulant activity
FVIII R Ag:	Factor VIII related antigen
FVIII/VWF:	Factor VIII/von Willebrand
FIX:	Factor IX
FX:	Factor X

FXII:	Factor XII
FIA:	Fluorescent immunoassay
G:	Guanine
GP:	Glycoprotein
G6PD:	Glucose 6 phosphate dehydrogenase
HIV:	Human immunodeficiency virus
IRMA:	Immunoradiometric assay
IRP:	International reference preparation
IS:	International standard
IU:	International unit
Kb:	Kilobase
KDa:	Kilodalton
K_m:	Concentration of substrate at which the reaction rate is one half of the maximum
Le^a:	Lewis antigen
Mr:	Relative molecular weight
mRNA:	Messenger RNA
RFLP:	Restriction fragment length polymorphism
RIA:	Radioimmunoassay
RNA:	Ribonucleic acid
SD:	Standard deviation
SDS PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
T:	Thymine
t 1/2:	Half life
Vmax:	Maximum velocity
vWF:	von Willebrand factor
ln	natural logarithms

vWF:Ag:	von Willebrand factor antigen
vWD:	von Willebrand's disease
WHO:	World Health Organization

TABLE OF CONTENTS

INTRODUCTION

AND AIM OF THE WORK

INTRODUCTION AND AIM OF THE WORK

Hemophilia A (classic hemophilia) is the commonest of the genetic coagulation defects. It is due to the functional deficiency of factor VIII coagulant activity (VIII:C). The clinical severity of the disease usually correlates with the level of VIII:C, the spectrum of defects ranging from mild to severe (Forbes and Madhok, 1991).

Hemophilia A is inherited as an X-chromosome linked disorder and thus limited almost exclusively to males. All the sons of affected hemophilic males are normal while all their daughters are obligatory carriers of the factor VIII defect. Sons of female carriers have a 50 percent chance of being affected while daughters of carriers have a 50 percent chance of being carriers themselves (Roberts and Jones, 1990).

Despite the great advance in treatment of hemophiliacs, the disease still is a serious one that has profound implications on the individual affected, his family and society in general (Markova et al., 1986).

Detection of carriers is an important aspect of genetic counseling in hemophilia A and has received special attention in the last decade particularly as prenatal

diagnosis of hemophilia is starting to be successfully available, though in few centers (White and Shoemaker, 1989).

Recently, methods that determine the genotype of carriers directly from their DNA have been available (Lillicrap et al., 1987). However, in view of the inherent limitations of these DNA methods, there is still a place for the conventional methods which use the laboratory bioaasay data to detect carriers of hemophilia A (Graham et al., 1985 and White and Shoemaker, 1989).

This work aimed to introduce, in our laboratory, a method for detection of hemophilia A carriers among Egyptians. This method is based on simultaneous evaluation of VIII:C and vWF:Ag measurements in combination with genetic risk estimated from pedigree analysis.

REVIEW OF LITERATURE

SECTION I: FACTOR VIII AND HEMOPHILIA A

MAGNITUDE OF THE PROBLEM OF HEMOPHILIA A

Hemophilia A (classic hemophilia) is a hereditary bleeding disorder that is due to defective and/or deficient FVIII molecule (Roberts and Jones, 1990). It is known to be a worldwide disorder without ethnic or geographic limitations (Graham, 1979). It occurs almost exclusively in males. It has an estimated incidence of 1 in every 10000 male births (Antonarakis et al., 1987).

The inheritance pattern of hemophilia A is shown in table (1).

Table (1): Inheritance Patterns for Hemophilia A

		Hemophilic male	
		X^h	Y
Normal female	X	X X^h (Carrier female)	X Y (Normal male)
	X	X X^h (Carrier female)	X Y (Normal male)
		Normal male	
		X	Y
Carrier female	X^h	X^h X (Carrier female)	X^h Y (Hemophilic male)
	X	X X (Normal female)	X Y (Normal male)

X = normal X chromosome
X^h = abnormal X chromosome with hemophilic gene.