PLASMA, ANTITHROMBIN-III AND NATURAL ANTIPLASMINS IN MATURITY ONSET DIABETES, COMPLICATED WITH RETINOPATHY IN EGYPTIANS

Thesis

Submitted By

Nabila Barsoum
M.B., B. Ch. & D.M.

In Partial Fulfilment for the Degree of
M.D. General Medicine

Supervisors

Professor Dr. Adel Shaker
Professor Dr. Amira Monir
Professor Dr. Fikry Goubran
Professor Dr. Mohammed Abdel Fattah
Professor Dr. Mohammed Ramadan

Faculty of Medicine
Ain Shams University

1985

ACKNOWLEDGEMENT

This thesis was done in the department of General Medicine, Ain Shams University, Cairo - Egypt.

I would like to express my deepest gratitude and cordial thanks to Prof. Dr. Adel Shaker, Professor of Medicine, Ain Shams University, whose fatherly attitude, moral support and constant encouragement and supervision throughout the work, helped much for its completion.

It is my great pleasure to aknowledge my indebtness to Prof. Dr. Amira Monir, Assistant Professor of Ophthalmology, Ain Shams University, for her very sensitive tender touch to this work.

I am very grateful to Prof. Dr. Mohammed Abdel Fattah, Assistant Professor of General Medicine, Ain Shams University, for his enthusiastic supervision and final touch to this work.

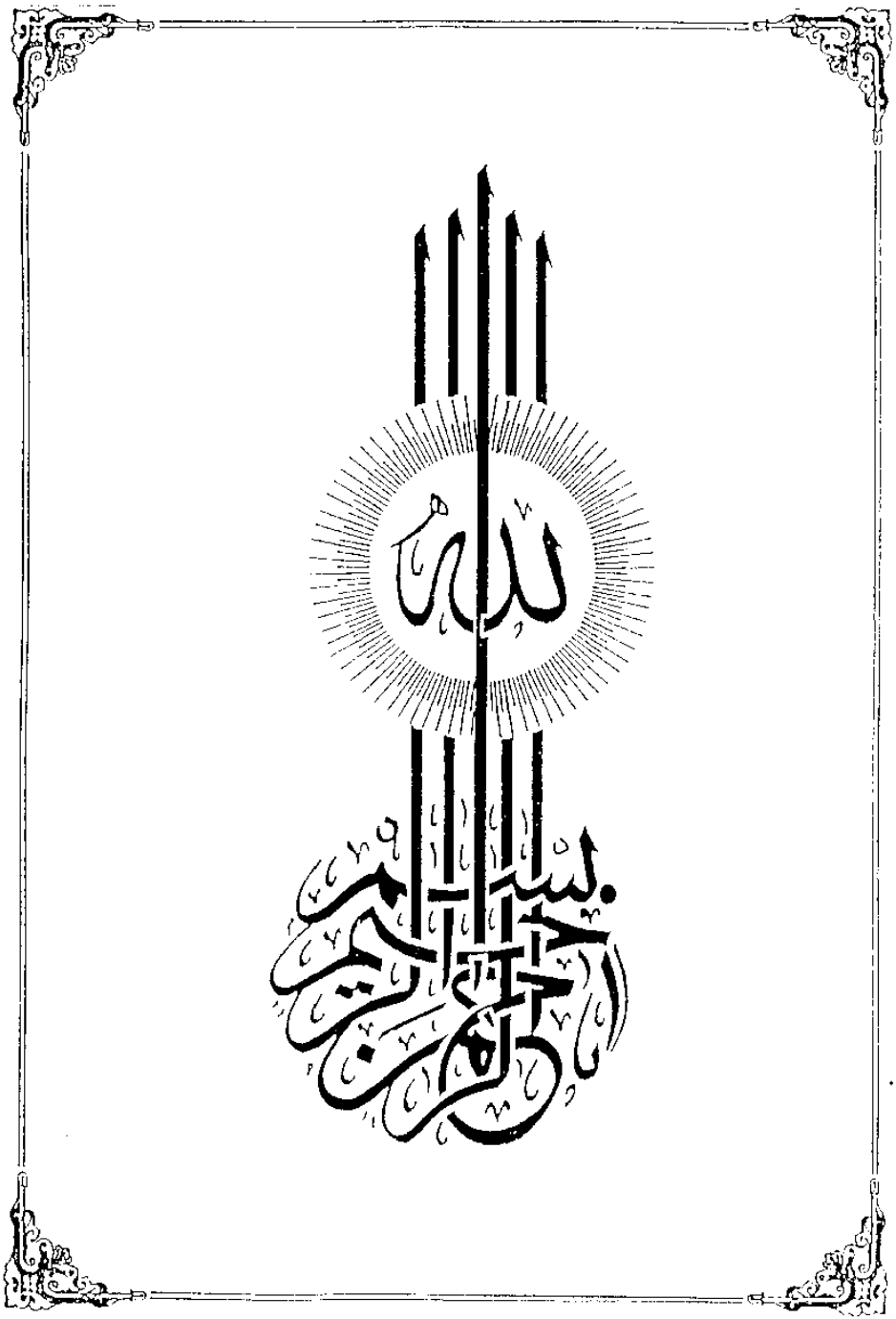
I find no words, by which I can express my extreme thankfulness, appreciation, and profound gratitude to Prof. Dr. Fikry Goubran, Assistant Professor of Haematology, Suez Canal University, for desgning the work, for teaching me how to read in the library, and how to perform the practical part of the work, for reviewing some chapters and lastly for pushing me all the time for hard work.

I am grateful to Prof. Dr. Mohammed Ramadan, Assistant Professor of General Medicine, Ain Shams University, for his stimulating suggestions and for guiding me in choosing my patients.

Two others, I can never forget their help; Prof. Dr. Hussein Shaker, Lecturer in Ophthalmology Department, Ain Shams University, and Dr. El-Awady in the Department of Community and Environmental Medicine, Ain Shams University, for fulfilling the statistical part of this work.

I wish to thank all my colleagues, laboratory assistants, and nursing staff in General Medicine Department, Ain Shams University, for their help and cooperation.

Also, I wish to thank all my patients and volunteers, who shared in this thesis and sacrificed their blood and time for the sake of this work.
Lastly, I wish to thank, with great admiration Mr. Ibrahin Riad, for typing this thesis, with great patience.



CONTENTS

LIST OF ABBREVIATIONS

ACTH	:	Adrenocorticotrphin.
ADP	:	Adenosine diphosphate.
APTT	:	Activated partial thromboplastin time.
DDAVP	:	Synthetic analogue of vasopressin.
DIC	:	Disseminated intravascular coagulopathy.
EACA	:	Epsilon-amino caproic acid.
FDP	:	Fibrin/fibrinogen degradation products.
HMWK	:	High molecular weight kininogen.
IgG, IgM	:	Immunoglobulins.
DLE	:	Disseminated lupus erythematosus.
DM	:	Diabetes mellitus.
ESR	:	Erythrocyte-sedimentation rate.

INTRODUCTION AND AIM OF THE WORK

It is well known that maturity-onset diabetics, may suffer from vaso-occlusive disorders. One of the major unsolved mysteries in diabetes research, is the extreme variability in susceptibility of individuals to the development of large and small vessel disease complications (Wesk, 1978, New York Elsevier). Several investigations suggest that a hypercoagulable state, manifested by elevated coagulation factors : V, VII, VIII and X (Bellet et al., 1961 - Valdorf-Hansen, 1967 - Fuller et al., 1979 - Mayne et al., 1970), and by increased platelet reactivity (Colwell et al., 1976 - Kwaan et al., 1972 - Fuller et al., 1979 - Burrows et al., 1978 - Grigniani et al., 1982), may develop, in some patients, with diabetes. In addition, a short firbinogen survival has been reported in insulin-dependent diabetes, which denotes an increased fibrinogen utilisation (Ferguson et al., 1975).

Hirsh, J., in 1978, claimed that, increased levels of coagulation factors, did not lead to thrombosis, this is because, they were normally present in excess and only a small amount of any coagulation factor had to be activated, in vivo, thrombus formation.

These observations, lead us to try to throw some light on the natural inhibitors of thrombin and plasmin which may be responsible for the state of hypercoagulability, observed in diabetics. AT-III is known to be the major inhibitor of thrombin (Abildgaard, 1969 - Rosenberg, 1973), and α_2M is one of the most important inhibitors of plasmin in blood.

REVIEW OF LITERATURE

BLOOD COAGULATION

Blood coagulation has long been considered to be an enzymatic process. The concept has received its most complete expression in the cascade (Macfarlane, 1964), or water-fall (Davie & Ratnoff, 1964) sequence for blood coagulation. In this concept, most of the coagulation factors circulate as proenzymes which are converted to enzymes during the clotting process, with some exceptions as fibrinogen, which is converted to insoluble fibrin in the final step. The function of each enzyme formed is to activate the proenzyme which succeeds in the coagulation sequence. The cascade concept was developed for the so-called intrinsic system, but it also readily accomodates the extrinsic system (Straub & Duckert, 1961).

Modern biochemical techniques have approved the validity of the cascade concept with exception of at least three enzymes which involve complex formation instead of enzyme activation; the reaction of tissue factor with f VIIa and calcium (Williams & Norris, 1966 - Nemerson, 1966), the reaction involving f IXa, f VIIIa, phospholipid or platelets and calcium (Østerud & Rapaport, 1970 - Hemker et al., 1970 - Chuang et al., 1972) and the reaction involving f Xa, fVa, phospholipid or platelets and calcium (Barton et al., 1967 - Hemker & Macfarlane, 1967).

So the present concept of coagulation, involves both enzymatic conversion of proenzymes to enzymes, the physical combination of reactants and what is called feed back-effects or autocatalysis. Thus e.g. thrombin activates f V (Hanahan & Day, 1972 - Esmon et al., 1973) and f VIII (Shapiro et al., 1973 - Legaz et al., 1973); factor Xa can also activate f VIII (Vehar & Davie, 1980 Hultin & Jesly, 1981 - Hultin, 1982).

The kallikrein formed by XIIa-catalysed activation of prekallikrein, in turn activates more f XII (Cochrane et al., 1973 - Revak et al., 1974). Factor XIIa can also activate f VII (Radcliffe et al., 1977 - Kisiel et al., 1979) and f IX (Zur & Nemerson, 1980 - Østerud et al., 1977). plasmin can activate f XII (Cochrane et al., 1973 - Revak et al., 1974). Thrombin also activates prothrombin (Stenn & Blount, 1972 - Heldebrant et al., 1973 - Owen et al., 1974). The activation of f X may be autocatalytic under some circumstances (Jesly et al., 1974 & 1975).

Blood coagulation factors;

Fibrinogen "factor I"

Normal plasma coagulation :

1. In premature infant; it is about 1 gm/l.
2. Newborn infant cord blood fibrinogen is 1.81 ± 0.61 gm/l. Synthesis of fetal fibrinogen persists up to 7 - 8 days after birth, adult levels of adult fibrinogen are reached by 2 months after birth. (Foley et al., 1978).
3. Adult level is 2 - 4 gm/l, plasma fibrinogen concentration increases with increasing age and is a positive risk factor for ischaemic heart disease in men (Steinman, 1964 - Meade & North, 1977).
4. In late pregnancy, it is 4 - 6.5 gm/l, and is also increased with oral contraceptives, this partially explains the raised ESR in pregnancy (Designes & Bonnet, 1981).

Plasma fibrinogen is an acute phase reactant and its concentration increases in response to a variety of stimuli (Koj & Macfarlane, 1968), including subcutaneous saline injection (Bocci & Pacini, 1973), or administration of endotoxin (Atencio & Lorand, 1974), ACTH by some mechanisms other than stimulation of the adrenal cortex (Chen & Reve, 1974) - Seligsohn et al., 1973), growth hormone (Jeejeebhoy et al., 1970), serum (Pickart & Pilgeram, 1974), or fibrinogen degradation products (FDP) (Bernhart & Noonan, 1973 - Bocci & Pacini, 1973 - Bell et al., 1983) and is not specific. Human plasma fibrinogen is increased in pregnancy (Desvignes & Bonnet, 1981), postoperatively (Aronsen et al., 1972) and in diabetes (Jones & Peterson, 1979).

Although fibrinogen is synthesized in the liver, hypofibrinogenaemia is rare in patients with liver disease, except in the end stage, and when present it may be more a result of fibrinogenolysis (Finkbiner, 1959), or DIC (Tytgat et al., 1971), than of deficient production.

In congenital afibrinogenaemia, heterozygotes are symptomless while homozygotes suffer from haemarthrosis after injury and postoperative bleeding. Their plasma fibrinogen levels are less than 100 mg/l (Girolami et al., 1975). Congenital dysfibrinogenaemia is also rare. Heterozygotes

are usually asymptomatic, while homozygotes show signs of excessive bleeding after trauma, or an increased tendency to thrombosis. It is inherited as an autosomal dominant trait (Al-Mondhiry et al., 1971 - Jandrot-Peirus, 1981 - Lane, 1980 & 1981 - MacDonagh, 1980). Acquired dysfibrinogenaemia can occur in patients with hepatoma (D'Souza, 1979 - Gralnick et al., 1978).

Production of fibrinogen :

Fibrinogen is synthesized by the hepatocytes in the liver, Kupffer cells appear to be utilized for storage, or removal of degradation product (Barnhart & Noonan, 1973). It is synthesized in response to circulating FDP, fragments D & E removed by the liver (Bell et al., 1983).

Platelets contain fibrinogen which constitutes up to 15 percent of the total platelet protein (Salmon, 1958), it appears to be synthesized in the megakaryocytes (platelet factor 5) (Sandler et al., 1974), but no evidence is obtained (Nachman et al., 1978), it exists both as plasma fibrinogen adsorbed to the platelet surface and as intraplatelet fibrinogen in the α-granules (Nachman et al., 1964 - Karaca, 1971).

Platelet fibrinogen is not completely coagulated by thrombin and its carbohydrate content, sedimentation and intrinsic viscosity are all different from plasma fibrinogen (Karaca, 1970 - Ganguly, 1972). These differences may be due to partial degradation of the platelet fibrinogen, i.e. platelet and plasma fibrinogen are products of the same gene (Doolittle, 1974).

Life span of fibrinogen :

The biological half-life is from 1.5 - 6.3 days (Collén, 1972 - Davies, 1973). The catabolic rate is from 31 to 46 mg/kgm per day (Tytgat et al., 1971 - Regoeczi, 1971). From 10 to 25 precent of the total body fibrinogen exists as extravascularly (Takeda, 1966 - Pilgeram, 1968). Fibrinogen is also present in human lymph (Stutman & Shinowara, 1965).

Catabolism of fibrinogen :

From 2 to 3 percent of normal fibrinogen catabolism can be accounted for by conversion to fibrin (Nossell et al., 1974). Fibrinogen is catabolised rapidly once it leaves the circulation and the fibrin is broken down almost instantaneously (Takeda, 1966 - Atencio, 1965).

Fibrinogen may also be degraded by a pathway involving the plasminogen plasmin system, the so-called fibrinogenolytic pathway (Mosesson, 1973 & 1974). Fibrinogen metabolism may be increased in various disease states (Regoeczi, 1971 - Jones & peterson, 1979), and in pregnancy (Regoeczi & Hobbs, 1969), and it is decreased in hypothyroidism (Hart, 1965).

The fractional catabolic rate tends to remain constant in both health and disease, suggesting that the catabolic mechanisms are not altered by disease (Reeve & Franks, 1974 - Sherman, 1977 - Regoeczi, 1971).
In liver cirrhosis, there is increased fibrinogen metabolism, which can be decreased by heparin therapy, suggesting that it is due to associated disseminated intravascular coagulation "DIC" (Green et al., 1976).

Structure of fibrinogen :

Human fibrinogen is a glycoprotein with a molecular weight of about 340,000 daltons (Laskowski, 1957 - Blombäck, 1958). The molecule consists of a dimer with A, B, γ -polypeptide chains connected by disulphide bridges and the two halves in turn joined by disulphide bridges $(A\alpha, B\beta, \gamma)_2$ (McKee, 1970 - Blomback & Johnson, 1972).

Fibrinogen isolated from plasma has the electrophoretic mobility of a gammaglobulin. It is moderately stable on storage, not adsorbed on gels or removed by filtration and it is heterogenous as regards solubility, molecular weight, electrophoretic mobility and chromatographic behaviour (Gaffney, 1971 - Mosesson, 1972 - Lipiniska, 1974).

When attacked by thrombin, $A\alpha$, designates the chain yielding all types of fibrinopeptide A, $B\beta$, designates the chain yielding fibrinopeptide β, and γ is used for the chain which is not attacked by thrombin (Lorand, 1953 - Blomback & Blomback, 1966).

The molecular weights of the Aα, Bβ and γ-chains are 63,500, 56,000 and 47,000d. respectively (McKee et al., 1966).

Fibrinogen and fibrin have six free-amino end groups per mole. In human fibrinogen, there are two residues each of alanine (Aα chain), pyroglutamic acid (Bβ chain) and tyrosine (γ-chain) (Blomback, 1958 - Blomback & Blomback, 1966). Aspartic acid is present in small amounts as an N-terminal aminoacid (von Korff, 1963).

Human fibrin contains 4 glycine end groups (α & β chains) and two tyrosine end groups (γ-chain) per mole (Blomback & Blomback, 1966). Fibrinogen and fibrin both contain carbohydrate in amounts accounting for 3 to 5 percent of the molecule (Henschen & Ediman, 1972), hexoamines and hexoses and is bound by covalent linkages to the Bβ and γ-chains (Gati and Straub, 1978 - Henschen et al., 1977 - Gaffney, 1972).
Fibrin monomer from asialofibrinogen polymerises more rapidly than normal (Martiney, 1977 - Collen, 1979). Asialofibrinogen supports platelet aggregation and adhesion (Collen, 1979).

Fibrinopeptides :

Pepetides amounting to about 3 percent of the weight of fibrinogen, are released when fibrinogen is converted to fibrin by thrombin (Lorand, 1953 - Bailey, 1951).

Thrombin releases fibrinopepetide A from fibrinogen at a more rapid rate than fibrinopeptide B (Blomback, 1972). Thrombin attacks only four of about 300 potentially susceptible arginyl and lysyl peptide bonds in fibrinogen (Mihalyi & Godfrey, 1963). Thrombin also attacks additional bonds at a slow rate in native fibrinogen or at a more rapid rate in fibrinogen derivatives (Blomback, 1972 - Henschen, 1972).

An enzyme in the venom of Bothrops "reptilase" (Holleman et al., 1976) and one in the venom of the Malayan pit viper "Ancrod" (EWart, 1970), hydrolise predominantly fibrinopeptide A from intact fibrinogen. Both reptilase and ancrod can release fibrinopeptide B from N-terminal fragment of fibrinogen (Pizzo, 1972) and ancrod also further digests both the α-

and the γ -chains of fibrinogen (Pizzo, 1972 - Edgar & Prentice, 1973). In contrast, the venom of the Southern Copperhead snake (Ancistrodon contortrix) contains an enzyme which releases fibrinopeptide B at a much faster rate than fibrinopeptide A (Herzig, 1970). Visible clotting occurs only after appreciable amounts of finronopeptide A,have been removed (Herzig, 1970).

Fibrin polymer formation after release of fibrinopeptide A by thrombin is followed by enhanced release of fibrinopeptide B and further polymerisation (Blomback, 1978). Release of a single fibrinopepetide A may be sufficient to lead to dimer formation (Smith, 1980).
Polymerisation is believed to occur by both end-to-end and side-to-side mechanisms (Blomback, 1981). The initial polymerisation site may be in the carboxy-terminal ends of the γ -chains (Doolittle, 1972). Binding domains (Blomback, 1981) are present in the N-terminal disulphide knot, representing the amino-terminal portion of the molecule (Blomback & Blomback, 1967) and in fragment "D" a portion of the fibrinogen molecule derived from the carboxy-terminal portion of the α, β and γ -chains (Budzynski, 1974). There is evidence for polymerization sites on both α- (Schen, 1977) and γ -chains (Olexa & Budzynski, 1981).
Grossly deficient polymerization is characteristic of fibrinogen Detroit, one of the inherited fibrinogen abnormalities (Mammen, 1968).

Fibrin stabilisation :

The clot formed from purified fibrinogen and thrombin is mechanically weak and is soluble in dilute acid or in concentrated urea solution, in contrast to that formed in plasma (Laki & Lorand, 1948). The difference is due to the presence in plasma of an additional coagulation factor XIII, which catalyses the formation of peptide bonds between the γ -carboxy groups of glutamine and the ϵ -amino groups of lysine in adjacent fibrin molecules (Pisano, 1968 - Lorand, 1968). Cross-linking occurs between γ -chains and between α -chains (Doolittle & Cottrell, 1979 - Frelto, 1978).

Tertiary structure :

By use of electron microscopy, nodular structures, have been demonstrated (Blakey, 1977 - Mosesson, 1979). Physiochemical data and immunological evidences substantiates the complexity of the teritiary structure of fibrinogen (Plow & Edginton, 1975 & 1979 - Cierniewsk, 1977).

Prothrombin "Factor II" and Thrombin

Prothrombin :

Normal plasma level is about 100 µg/ml (60 -155 µg/ml).

The biologic half-life is 2.1 days (Bell et al., 1978).

Prothrombin has been demonstrated in lymph (Stutman & Shinowara, 1965) and in malignant pleural effusion (Bergsagl, 1965). About 30 to 40 percent of the total body prothrombin is present in the extravascular compartment (Shapiro & Martinez, 1969), and is not readily available to replenish reduced plasma levels of prothrombin (Langdell, 1963). Prothrombin turnover was normal in patients with haemophilia (Shapiro & Martinez, 1969). Heparin therapy did not alter the turnover of prothrombin in normal subjects and this suggested that continuous intravascular coagulation did not play a significant role in the catabolism of prothrombin (Hasselback & Hjont, 1960). Fever and hyperthyroidism probably increase the catabolism of prothrombin while hypothyroidism decreases it (Loeliger & Hemker, 1964).

Normal plasma level of prothrombin in the neonate is from 20 - 26 % of adult level reaching adult levels by 60-120 days. In the preterm infants, plasma f II levels are about 30 percent of normal full-term infant (Malia et al., 1980). Pathclogical decrease in f II level is found in vit. K deficiency of different causes, it is a true hypoprothrombinaemia with disproportion of f IIAg and f IIC (Suttie, 1980 - Whitlon, 1978). In liver disease, there is an abnormal depression of both active and inert prothrombin (Blanchard et al., 1981). A disproportion of f IIAg and IIC indicates vit. K deficiency, which should respond, at least partially, to vit. K supplements (Suttie, 1980). With vit. K antagonists, such as warfarin active prothrombin levels fall, with increase in the dysgemma-carboxy form which is inactive (Suttie, 1980 - Whitlon, 1978 - Bell, 1978 - Brozovic, 1976).

Hereditary prothrombinaemia

Both true prothrombinaemia and dysprothrombinaemia are inherited as autosomal recessive characteristic with mild bleeding symptoms or asymptomatic and they do not respond to vit. K treatment (Shapiro, 1969 - Kattlove, 1970 - Josso, 1971 - Weinger, 1980).