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EVALUATION OF PROTHROMBIN TIME AND PARTIAL THROMBOPLASTIN TIME AS TESTS FOR MONITORING THE DOSE OF ORAL ANTICOAGULANT THERAPY

THESIS

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BY

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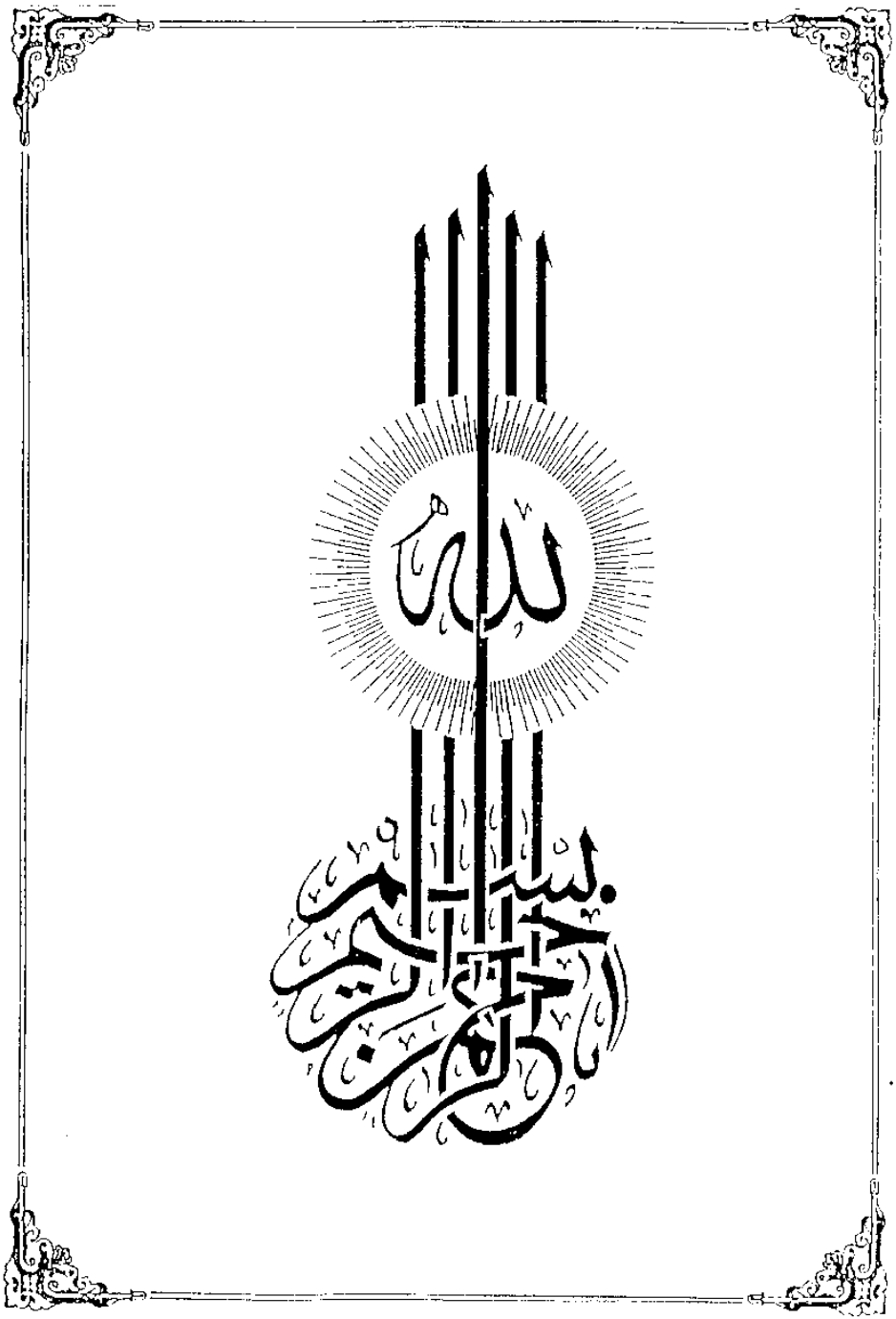

CONTENTS

ABBREVIATIONS

Ag	Antigen.
α_1- AT	α_1- antitrypsin.
α_2- Ap	α_2- antiplasmin.
α_2- M	α_2- macroglobulin.
$AL(OH)_3$	Aluminium hydroxide.
APC	Activated protein C.
A.P.T.T.	Activated partial thromboplastin time.
Arg	Arginine.
AT III	Antithrombin III.
Ca++	Calcium ion.
$CaCl_2$	Calcium Chloride.
C1-inh.	1st component of complement inhibitor.
CJD	Creutzfeldt jakob disease.
CTAD	Citric acid theophylline adenosine and dipyridamole.
Factor I	Fibrinogen.
Factor II	Prothrombin.
Factor IV	Calcium.
Factor V	Proaccelerin.
Factor VII	Proconvertin stable factor.
Factor VIII	Antihemophilic globulin (A.H.G).
Factor IX	Christmas factor.
Factor X	Stuart-prower factor.
Factor XI	Plasma thromboplastin antecedent (PTA).

Factor XII	Hageman factor.
FDP	Fibrin degradation product.
Gla	Glutamic acid.
HC II	Heparin-cofactor II.
HEPES-buffer	4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid.
HMW-Kininogen	High molecular weight kininogen.
I.C.T.H.	International committee on thrombosis and haemostasis.
Ile	Isoleucine.
I.N.R	International normalised ratio.
I.S.I	International sensitivity index.
MW	Molecular weight.
NaCl	Sodium Chloride.
PC	Protein C.
PF4	Platelet factor 4.
PIVKA	Protein induced by absence of vit.K.
Ps	Protein S.
P.T	Prothrombin time.
P.T.T	Partial thromboplastin time.
WBCT	Whole blood clotting time.
WF	Von willebrand factor.
Zn++	Zinc ion.

INTRODUCTION
&
AIM OF THE WORK

INTRODUCTION

Many disease conditions necessitate administration of oral anticoagulant for long periods,some times life-long.Although the follow-up of these patients by prothrombin time as a test for monitoring the dose of anticoagulant is strict,however there is a significant percentage of patients complicated with haemorrhagic or thrombotic complications due to over or under-dose used.

Despite the extensive research to find out the ideal test in following-up of those patients,it is still unsuccessful.It is well known that oral anticoagulants affect mostly factors of extrinsic pathway of coagulation. However, factor IX is also affected, although late,but on long use of these drugs certainly it will be affected and should be also monitored with other factors.

Aim of the work:

Is to find out if prothrombin time alone is the best test in following-up patients on oral anticoagulant therapy or other test (or tests) can be used.

REVIEW OF LITERATURE

THE BLOOD COAGULATION CASCADE

The view that the coagulation mechanism is composed of a series of reactions which function as a biological amplifier was recognized by Macfarlane (1964), and Davie and Ratnoff (1964) and termed the "cascade" or "waterfall" hypothesis. Currently, twelve plasma coagulation factors have been recognized,including 10 plasma proteins in addition to, Ca++ and tissue factor which are designated as factors IV and III respectively (table 1).

The complexity of the original coagulation sequence has been increased by the recognition of the inter-relation ships between the intrinsic and extrinsic mechanisms and a number of functionally important feed-back reaction. Finally the co-factor roles of such factors as high molecular weight Kininogen, factor V, factor VIII and tissue factor have been partially elucidated (Ogston and Bennett, 1985).

The coagulation factors are classified and discussed in the following groups (Zaverio and Ruggeri, 1985) :-

A. Vitamin -K- dependent factors.

B. Factors V and VIII.

C. Contact activation factors.

D. Fibrinogen and factor XIII.

Table (1) : International nomenclature for blood coagulation factors (Williams, 1984)

Factor	Synonyms
I	Fibrinogen
II	Prothrombin, Prethrombin
III	Tissue factor, Tissue Thromboplastin.
IV	Calcium
V	Proaccelerin, labile factor, Ac globulin
VI	not assigned
VII	Proconvertin, stable factor, autoprothrombin I
VIII	Antihemophilic globulin (AHG), antihemophilic factor (AHF), Platelet co-factor I
IX	Plasma thromboplastin Component (PTC), christmas factor, antihemophilic factor B, autoprothrombin II - platelet co-factor II
X	Stuart- Prower factor, Stuart factor, autoprothrombin III
XI	Plasma thromboplastin antecedent (PTA), antihemophilic factor C
XII	Hageman factor
XIII	Fibrin stabilizing factor, fibrinase, Laki-Lorand factor

A. Vitamin -K- dependent coagulation factors

The necessity of vitamin K,for normal haemostasis in chicks was recognized by Dam in 1935. 30 years later it was shown that vitamin K (vitamin K1= 2 methyl 3 phytyl - 1,4 naphthoquinone) was necessary for the carboxylation of the specific glutamic acid residues of coagulation factors II, VII, IX and X to give gamma-carboxy glutamic acid which enables calcium binding to take place. Additionally, vitamin K is necessary for the gamma carboxylation of protein C which inhibits the active forms of factor V and VIII (Lammle et al., 1985).

1) Prothrombin (factor II):

Prothrombin is a single-chain glycoprotein of an approximate 72000 MW consisting of about 600 amino-acids.Its concentra-tion in plasma is 100 Ug/ml (Suttie and Jackson,1977).Prothrombin consists of a carboxy terminal half,the thrombin forming part of the molecule and an amino-terminal half,Prothrombin fragment 1-2 that is liberated during activation by factor Xa (Stenn and Blout,1972).Prothrombin fragment 1-2 is composed of the amino-terminal prothrombin fragment 1 (Owen et al.,1974) and the carboxy terminal prothrombin fragment 2 (Jackson,1978).At the amino-terminal end of prothrombin fragment 1 is the glutamic acid domain, containing 10-glutamic acid residues (Magnusson et al.,1975) [Fig.1]

The thrombin-formin half of prothrombin, the immediate pre-

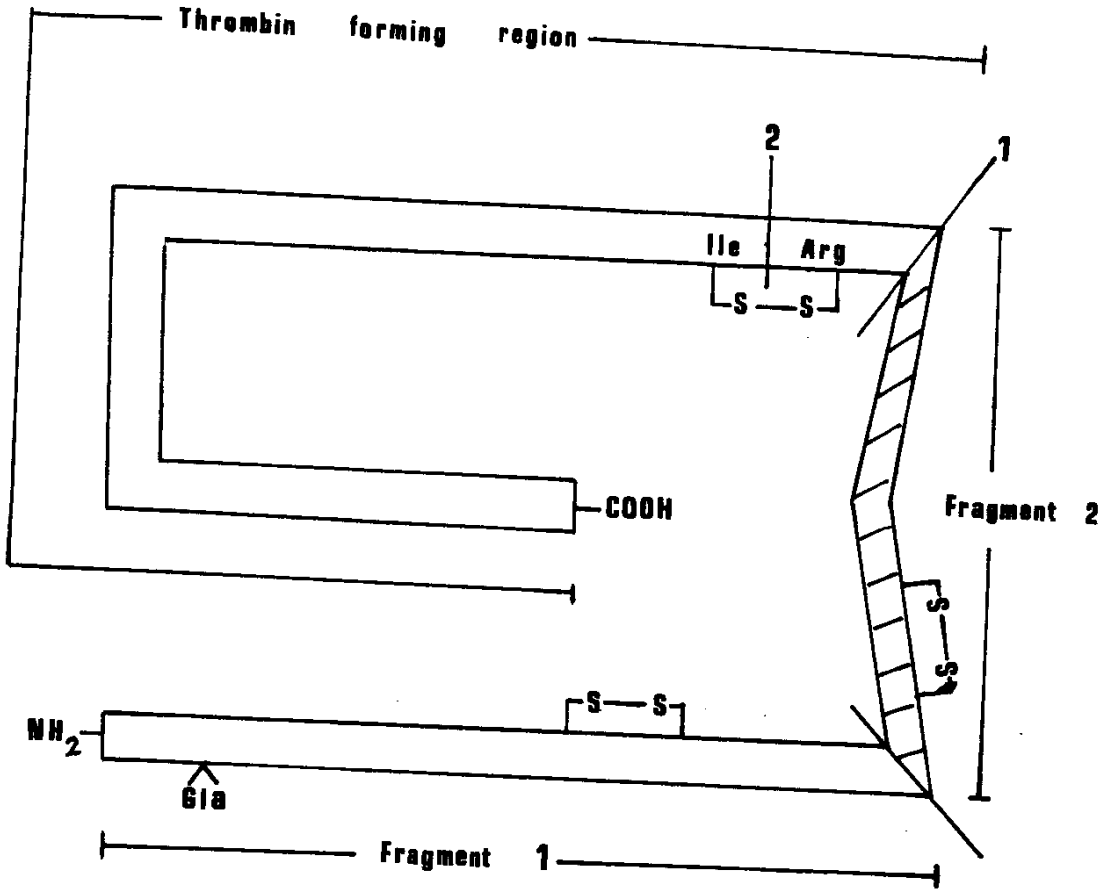

Fig. (1): Schematic Structure of prothrombin.
1 and 2, sites of first and second activation cleavage by factor Xa

Ile = isoleucine

Arg = Arginine

Gla = glutamic acid

(Jackson, 1981)

cursor of active thrombin,is called Prethrombin 2. A cleavage of an internal Arginine-isoleucine bond, (cleavage site 2 in Fig.1) in prethrombin 2 results in the generation of active thrombin, which is composed of small amino-terminal A chain linked by a disulphide bridge to large B chain (Magnusson et al.,1975).

Physiologically, Prothrombin fragment 1, via its glutamic acid residues, is important for the Ca++ mediated binding of prothrombin molecule to phospholipid or platelet membrane surfaces (Esnouf and Jobin, 1965). This binding of prothrombin enhances the factor Xa-induced activation rate about 50 times (Miletich et al., 1978).

Prothrombin fragment 2 seems to express two different functions during prothrombin activation. First, it is involved in the binding of the non enzymatic co-factor, factor Va, which enhances factors Xa induced thrombin formation, even in the absence of phospholipids, about 350-fold (Miletich et al.,1978).

A second role for prothrombin fragment 2 lies in the ability to mediate a non-covalent association of the whole prothrombin fragment 1-2 to the thrombin-forming half of prothrombin (prethrombin 2) after the factor Xa induced cleavage of prothrombin (cleavage 1 in Fig.1) (Esmon and Jackson, 1974). So the prothrombin fragment 1-2 has important function during the prothrombin activation and can not be considered a large "wasted" activation peptide (Jackson, 1981).

2) Proconvertin (factor VII):

Human factor VII is a single chain glycoprotein with MW of about 50.000 daltons it has alanine as the free amino-end group and contains nine-glutamic acid residues per molecule (Broze and Majerus, 1980). Concerning the metabolism, it has a much shorter in-vivo half-life (6-8 hours) (Hasselback and Hjort, 1960) than factors IX, X and II (one to three days) (Biggs and Denson, 1963).

Factor VII is inactive on its physiological protein substrates (i.e factor X and IX), in absence of tissue factor (Williams and Norris, 1966). On the other hand, human factor VII is activated by factor Xa (Bajaj et al., 1981) in the presence of Ca++ and phospholipid to produce two chain molecule with enhanced coagulant activity. Moreover factor IXa, XIIa and kallikrein activate human factor VII (Seligsohn et al., 1979) perhaps indirectly.

3) Christmas factor (factor IX):

Factor IX is a single-chain glycoprotein of MW about 57000 daltons (Discipio et al., 1977). Tyrosine is the free amino end group.

Factor IX activated by factor XIa by cleaving an internal peptide bond forming a two-chain enzymatically inactive intermediate with free amino end group tyrosine and alanine. A second peptide bond is hydrolized by factor XIa with consequent release of carbohydrate rich activation peptide of MW 11.000 daltons