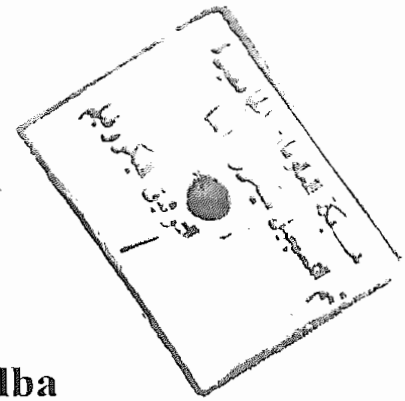
Protein C And Protein S In Sick Neonates

Thesis
Submitted in partial fulfillment of Master Degree
in Pediatrics



By

Sherief Soliman Salem
M.B; B.Ch; (1990)

Under the supervision of

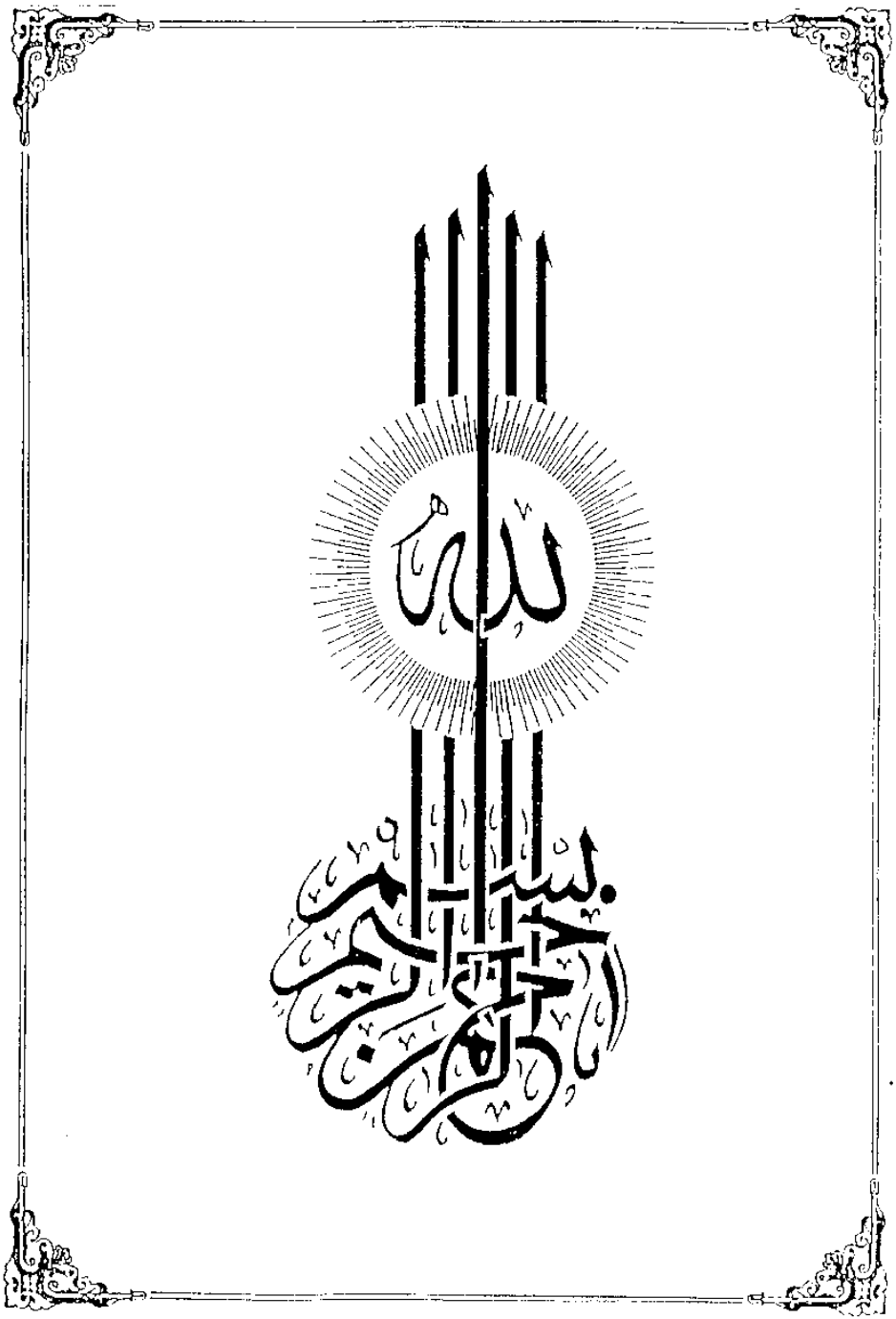
Prof. Dr. Kotb Ahmed Tolba
Professor of Pediatrics
Faculty of Medicine
Ain-Shams University

Dr. Gamal Ahmed Helmy Mottier
Assistant Professor of Pediatrics
Faculty of Medicine
Ain-Shams University

Dr. Hala Mahmoud Hamdy Abaza
Assistant Professor of Clinical Hematology
Faculty of Medicine
Ain-Shams University

Faculty of Medicine
Ain-Shams University
1994

DEDICATED TO
MY PARENTS
MY WIFE
&
MY SON
AHMED



ACKNOWLEDGMENT

I would like to express my deepest gratitude and appreciation to **Professor Dr. Kotb Ahmed Tolba,** Professor of Pediatrics, Faculty of Medicine, Ain-Shams University, for his patience, continuous guide and valuable suggestions. I felt a great honor to work under his kind supervision.

I am also greatly indebted to **Assistant Professor Dr. Gamal Ahmed Helmy Mottier,** Assistant Professor of Pediatrics, Faculty of Medicine, Ain-Shams University, for his faithful help, sincere guidance, and honest assistance that facilitated the completion of this work.

I am again sincerely thankful to **Assistant Professor Dr. Hala Mahmoud Hamdy Abaza**, Assistant Professor of Clinical Hematology, Faculty of Medicine, Ain-Shams University, for her continuous interest, willing cooperation, persistent support and the precious time she has given me throughout the course of this work.

Contents

List Of Abbreviations

APC	Active Protein C
APTT	Activated partial thromboplastin time
AT III	Antithrombin III
BUN	Blood urea nitrogen
C4b-BP	C4b- binding protein
CPAP	Continuous positive airway pressures
CSF	Cerebrospinal fluid
DIC	Disseminated intravascular coagulopathy
EGF	Epidermal growth factor
FIO2	Fragmented inspired oxygen
FFP	Fresh frozen plasma
Gla	Gamma carboxyglutamic acid
HpG	Serum haptoglubin
IgG	Immunoglobulin G
MW	Molecular weight
NEC	Necrotizing enterocolitis
PAI	Plasminogen activator inhibitor
PC	Protein C
PCI	Protein C inhibitor
PEEP	Positive end-expiratory pressure
PG	Phosphatidyl glycerol
PI	Phosphatidyl inositol
PS	Protein S
SHBG	Sex hormone binding globulin
TAT	Thrombin - antithrombin
Th	Thrombin
TM	Thrombomodulin
tPA	Tissue plasminogen activator

List Of Tables And Figures

Page

Introduction
And
Aim Of Work

Introduction

The coagulation system in healthy preterm and full-term neonates is immature and gradually evolves postnatally towards the mature adult system. The plasma concentration of both procoagulants and inhibitors differ in healthy neonates, compared to adults. The difference in the concentration of procoagulatns results in diminished ability to generate thrombin in neonates, compared to adults. Similarly, the concentration of thrombin inhibitors are also altered, resulting in an impaired ability to inhibit thrombin in neonates, compared with adults ***(Andrew et al., 1990).***

Presumably, the plasma concentration of coagulants and inhibitors, although significantly differ from those of adults, are in a physiological balance. So in healthy neonates, thrombin expression is controlled such that neither hemorrhagic nor thrombotic complications occur ***(Andrew et al., 1990).***

In contrast, sick neonates with problems such as respiratory stress syndrome ***(Jay et al., 1992)*** and neonatal sepsis ***(Roman et al., 1992)*** are prone to develop both hemorrhagic and thrombotic complications indicating that the coagulation abnormalities may have implications not only on pathogenesis and complications, but also on the management of such diseases.

Protein C is one of the important coagulation inhibitors, which is a central protein in blood coagulation process. Activated Protein C exhibits its anticoagulant activity through the proteolytic inactivation of two blood coagulation cofactors Va and VIII a. This reaction requires

phospholipids originating from platelets or endothelial cells and a cofactor protein, "Protein S" which enhances the binding of activated Protein C to phospholipid. So, high incidence of thromboembolic complications is seen in congenital or acquired Protein C and Protein S deficiency *(Walker, 1986).*

Aim of Work:

The aim of this work is to study Protein C and Protein S concentrations in neonates with respiratory distress syndrome (RDS) and those with neonatal sepsis, and to find out the changes in Protein C system (Protein C and Protein S) with those neonates, which may have implication on the management of such diseases and their complications.

Review Of Literature

Protein C

Structure:-

Protein C circulates in the plasma at concentration of 4 - 5 ug / ml, as inactive zymogen of two chains proteins held together by a single disulfide bond ***(Esmon, 1984).*** Protein C was formed and secreted as a single chain protein of about 65000 MW. Protein C must undergo a post-secretion processing event leading to the generation of its two-chains plasma form (***Fair and Marlr, 1986***). The heavy and light chains comprising 260 and 155 amino acids respectively, studies employing cDNA sequences have shown that there is a close homology between human and bovine Protein C (***Foster and Davie, 1984***).

The anticoagulants Protein C and Protein S, and the vitamin K-dependent procoagulant factors II, VII, IX and X, have similarities in their protein structure. And with the exception of Protein S, all of them circulate as zymogens that must be cleaved to be activated to their functional forms of serine proteases (***Furie and Furie, 1988***).

The nucleotide sequence of Protein C gene is composed of nine exons. These DNA elements code for a leader sequence, a domain containing 9 gamma-carboxyglutamic acids (Gla-domain), two epidermal growth factor-like domains, an activation peptide and a catalytic domain which is highly homologous in amino acid sequence to other serine proteases ***(Plutzky et al., 1986).*** The Gla region residues are within the first 35 light chain residues (***Owen, 1987***). Protein C possesses two epidermal growth factor (EGF) domains, these are situated in the proximity of Gla region of the molecule (***Stenfelo and Ohlin, 1988***).

The properties of Protein C are summarized in table (1).

Table (1): Properties of Protein C.

I.	**Structure:** a. Two chains glycoprotein joined by sulfahydryl bonds. b. Molecular weight of 62,000 Daltons. c. Vitamin K-dependent, contains 10 Gla residues.
II.	**Activation:** a. Peptide cleaved from N-terminal end of heavy chain. b. Slow activation by thrombin in-vitro (inhibited by calcium ions). c. Rapid activation by the thrombin-thrombomodulin complex (requires calcium ions).
III.	**Function (s):** a. Proteolytically degrades factor Va. b. Inactivates factor VIIIa. c. Facilitates thrombolysis by neutralizing an inactivator of plasminogen activator.
IV.	**Normal concentration :-** 4.8 ± 1.0 ug/ml of plasma. Activity concentration 100 ± 30% of pooled normal plasma.

Quoted from Clouse and Comp,(1986).

The components of the Protein C system are summarized in table (2).

Table (2): Components of Protein C.

Component	Molecular Weight (Daltons)	Biological function(s)
Protein C	62000	-Neutralization of factor Va and factor VIIIa -Enhancement of fibrinolysis.
Thrombomodulin	78000	-Potentiates the activation of Protein C by thrombin.
Protein S	69000	-Enhances inactivation of factor Va and factor VIIIa by Active Protein C.
Activated Protein C inhibitor	57000	-Inhibits activated Protein C.
C4b- binding protein	550000	-Binds Protein S in an inactive form.

Quoted from Foster and Dave, (1984).

The Biosynthesis Of Protein C:-

Protein C is synthesized in the liver as a single protein. Its synthesis requires several post-translation modifications including carboxylation of glutamic acid residues and hydroxylation, and glycosylation of aspartic acid ***(Walker, 1990).*** Meanwhile, ***Triplett (1985),*** stated that carboxylation of glutamic acid at gamma position is very essential in order to be able to bind calcium and hence, attachment to phospholipid surface. This process of carboxylation is carried out in the