ESTIMATION OF SERUM ALPHA 1 - ANTITRYPSIN LEVEL IN PATIENTS PRESENTING WITH INTERSTITIAL PULMONARY FIBROSIS

Thesis

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In

CHEST DISEASES AND TUBERCULOSIS

By

HANI ZAKI MEHANNI

M.B., B.Ch.

Supervised By

Prof. Dr. SAID EL-HILALY

Prof. of Chest Diseases
Ain-Shams University

Prof. Dr. ADEL GOMAA

Prof. of Chest Diseases
Ain-Shams University

Prof. Dr. MONIR BOLLUS

Prof. of Biochemistry,
Ain-Shams University

Faculty of Medicine
Ain-Shams University

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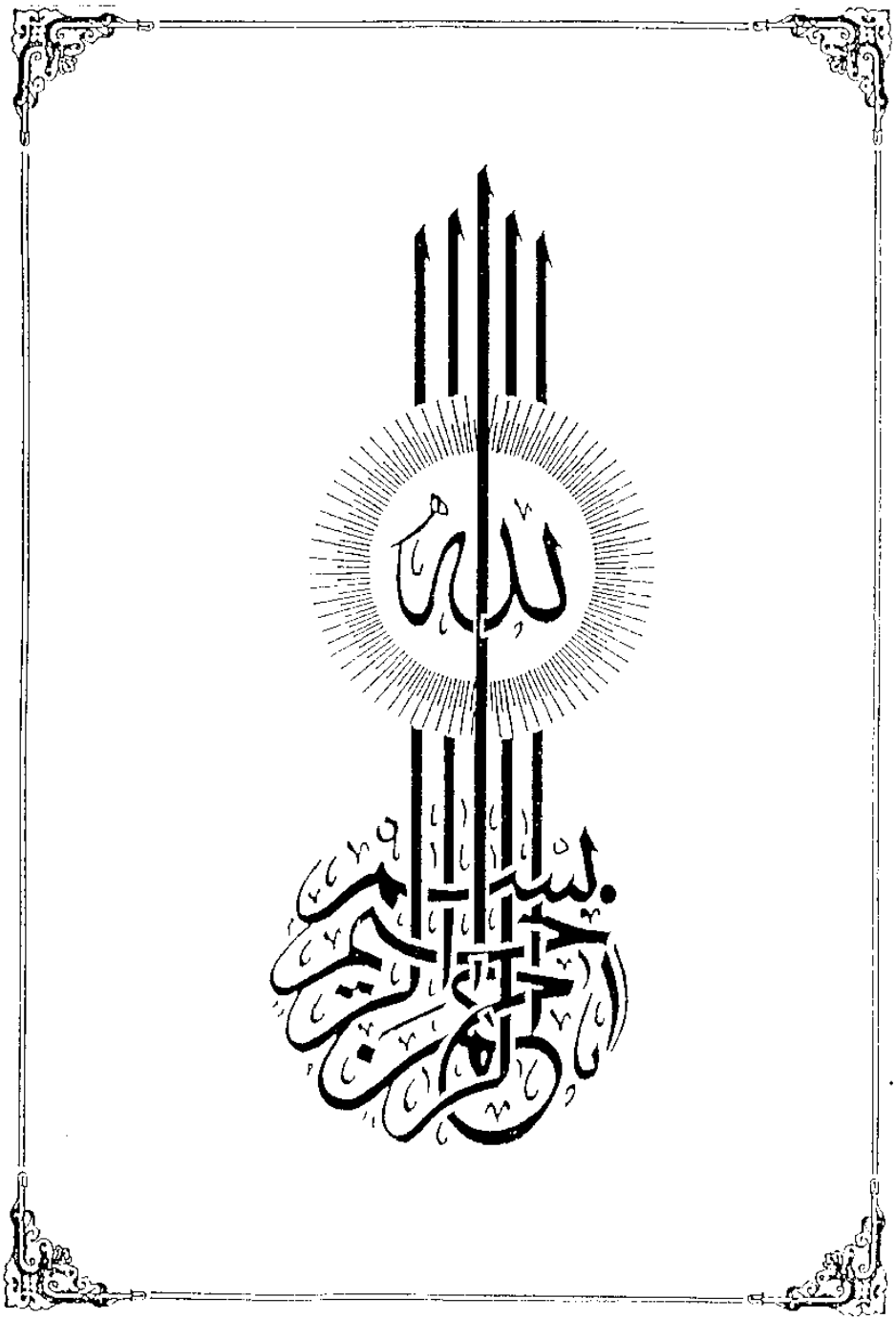
C O N T E N T S



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INTRODUCTION

INTRODUCTION :

Alpha 1- antitrypsin is a glycoprotein in human serum that inhibits several proteases. The liver is propably the only organ that synthesizes alpha 1- antitrypsin. Its normal concentration is 150-280 mg. per 100 ml. Its level in serum increases considerably under various conditions including pregnancy and inflammation. (Kueppersand Black, 1974).

The reason for the higher serum concentration with acute and chronic infections and pregnancy are unknown. Present evidence suggests that the synthesis of alpha 1 - antitrypsin is controlled by a series of allelic autosomal genes at a single locus. Persons described deficient in this protein have serum alpha 1 - antitrypsin concentrations only 10-15 % of normal and are believed to be homozygous for a recessive deficiency gene. (Litwin, 1969).

Eriksson in 1964, had reported the association of a marked reduction in serum alpha 1 - antitrypsin with 1^{ry} pulmonary emphysema. (Eriksson, 1964).

Evans and associates in 1970, had suspected a relationship between respiratory distress syndrome and low levels of alpha 1-antitrypsin. (Evans, 1970).

Rawlings and co-workers believed that the simultaneous

occurence of both alpha 1 - antitrypsin deficiency and hepato cellular carcinomas was probably greater than a chance event. (Rawlings, 1974).

AIM OF THE WORK

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The aim of this work is to evaluate and match the level of alpha 1 - antitrypsin with the extent of the disease as reflected by the Blood gases (P_aO_2 - P_aCO_2) in arterial blood in cases presenting with interstitial pulmonary fibrosis.

REVIEW OF LITERATURE

PROTEASE INHIBITORS IN HUMAN SERUM :

The capacity of human biological fluids to inhibit the enzymatic activity of proteases has attracted the interest of investigators for sometime.

Camus and Gley in 1897, were the first to recognize this activity in human serum. (Camus and Gley, 1897).

Landsteiner in 1900, in experiments to fractionate antitrytic components of serum had shown that this activity was mainly associated with the globulin fraction. (Landsteiner, 1900), a fact later confirmed by Opie and Barker in 1907, for sera of several other species. (Opie and Barker, 1907). The important conclusion drawn from these experiments was that the antiproteolytic components were not immunoglobulins.

During the last decade considerable progress had been made in the isolation and characterisation of protease inhibitors in human serum.

At present six distinct antiproteases (Table 1) are known with the exception of alpha 1 - antichymotrypsin, which appears to be specific for chymotrypsin most of these inhibitors are capable of inactivating several proteases. (Heimburger, 1971), (Kueppers, 1967).

Table (1)

PROTEASE INHIBITORS IN HUMAN SERUM

Inhibitor	Mean Concentration (± S.D.)	Molecular Weight
Alpha 1 - Antitrypsin	212.0 ± 32.0	45,000 - 54,000
Alpha 1 - Antichymotrypsin	48.7 ± 6.5	69,000
Interα-trypsin Inhibitor	50.0	160,000
Anti thrombin III	29.9 ± 2.9	65,000
CI. Inactivator (*)	23.5 ± 3.0	104,000
Alpha 2 - Macroglobulin	260.0 ± 70.0	720,000

(*) CI - inactivator : Chemotactic factor inactivator.

As shown from Table (1) alpha 1 - antitrypsin and alpha 2 - macroglobulin are present in the highest concentration, both have a wide inhibitory spectrum covering trypsin, Chymotrypsin, Plasmin, Kallikrein, Elastase, Thrombin and a protease from Aspergillus oryzae. (Heimburger, 1971); (Lindvall, 1969).

ISOLATION AND BIOCHEMICAL NATURE OF ALPHA 1 - ANTITRYPSIN :

Alpha 1 - antitrypsin had been isolated by Schultze and associates in 1962 using a series of precipitation steps. (Schultze, 1962). Other isolation procedures using various chromatographic and electrophoretic technique has been described recently. (Kueppers and Black, 1974).

Alpha 1 - antitrypsin is a glycoprotein with carbohydrate moiety of 12 % consisting of galactose, mannose, acetyl hexosamine, N- acetyl neuraminic acid and fucose. The amino-acid composition does not show any unusual features. The contents of aspartic acid (9.8 %), glutamic acid (12.9) and Leucine (9.9 %) are relatively high. It contains only 2 moles of cysteine per mole which could give rise to one disulphide bridge, resulting in a loop of polypeptide chain. (Kueppers and Black, 1974).

According to Ozawa and Laskowski's (1966) general model of protease inhibitors, the critical peptide bond is situated in such a loop. (Ozawa, 1966).

The molecular weight has been determined by several authors. The reported values ranged from 45,000 to 54,000 daltons. The true value is probably 50,000. (Rimon and co-workers, 1966).

The sedimentation constant ($S.^{0}_{W,20}$), has been reported as 3.3 to 3.4 S. (Bundy and Mehl, 1959).

A simple but accurate method for the determination of alpha 1 - antitrypsin in the presence of other protease inhibitors is radial immunodiffusion. (Mancini, 1965).

With this method the concentration of an antigen is determined as a function of area of a circular immune precipitate that forms in an antibody containing agarose gel. A constant amount of the sample to be tested is applied to a well in the agarose layer, and appropriate standards are used. The serum concentration of alpha 1 - antitrypsin in healthy persons is 150 to 280 mg. per 100 ml. (Kueppers,1967) ; (Heimburger, 1971). These values best agree with trypsin inhibiting - activity of 0.9 to 1.5 mg. of trypsin inhibited per ml. of serum. (Kueppers,1968).

There is a close correlation between immunologically measured concentration of alpha 1 - antitrypsin and the trypsin inhibitory activity. The reason for this is that approximately 90 % of the total trypsin inhibitory activity is due to alpha 1 - antitrypsin. The remaining 10 % is contributed by other inhibitors mainly alpha 2 - macroglobulin and inter alpha - trypsin inhibitor. (Homer, 1963).

ELASTASE INHIBITORY ACTIVITY (E.I.A.) :

Units of elastase inhibited per ml. of serum.

PHYSIOLOGIC ROLE OF ALPHA 1 - ANTITRYPSIN :

Alpha 1 - antitrypsin is an acute phase reactant protein. Its concentration in human sera increases considerably under various physiologic and pathologic conditions.

Opie in 1905 was the first to draw attention to the occurence of proteases and protease inhibitors in inflammatory exudate. (Opie, 1905).

Kueppers in 1968, had shown that during pregnancy and bacterial infections or after an intravenous injection of typhoid vaccine, the alpha 1 - antitrypsin in serum increases twice the normal. (Kueppers,1968).

Contraceptive drugs in women lead to an elevation of alpha 1 - antitrypsin concentration of approximately 50 percent (Laurell,1968), an effect which was shown later by Lieberman and associates in 1971 to be due to estrogens. (Lieberman, 1971).

Ohlsson in 1971 had shown that alpha 1 - antitrypsin can inhibit proteases from human granulocytes including an elastase and a collagenase. These enzymes are probably major contributors to tissue destruction during an inflammatory process. (Ohlsson, 1971).

In addition sera from patients with alpha 1 - antitrypsin

deficiency also have low level of "Chemotactic factor inactivator". (Ward and Talmo, 1973). This factor was later demonestrated by Kueppers and Black in 1974, not to be identical to alpha 1 - antitrypsin.

More commonly both defects are interrelated in a more indirect way. In the absence of this inactivator more granulocytes may remain at the site of inflammation for a prolonged period which may lead to excess local proteolysis.

Regarding the activity of alpha 1 - antitrypsin against Thrombin, Plasmin and Kallikrein; Kueppers and Black in 1974, suggested that alpha 1 - antitrypsin may be capable of influencing fibrin formation and interfering with kinin system. (Kueppers and Black, 1974).

In addition Heck and Kaplam in 1971, had recently shown that alpha 1 - antitrypsin inhibits plasma Thromboplastin antecedent and accordingly by such an action alpha 1 - antitrypsin could influence clotting. (Heck and Kaplan, 1971).