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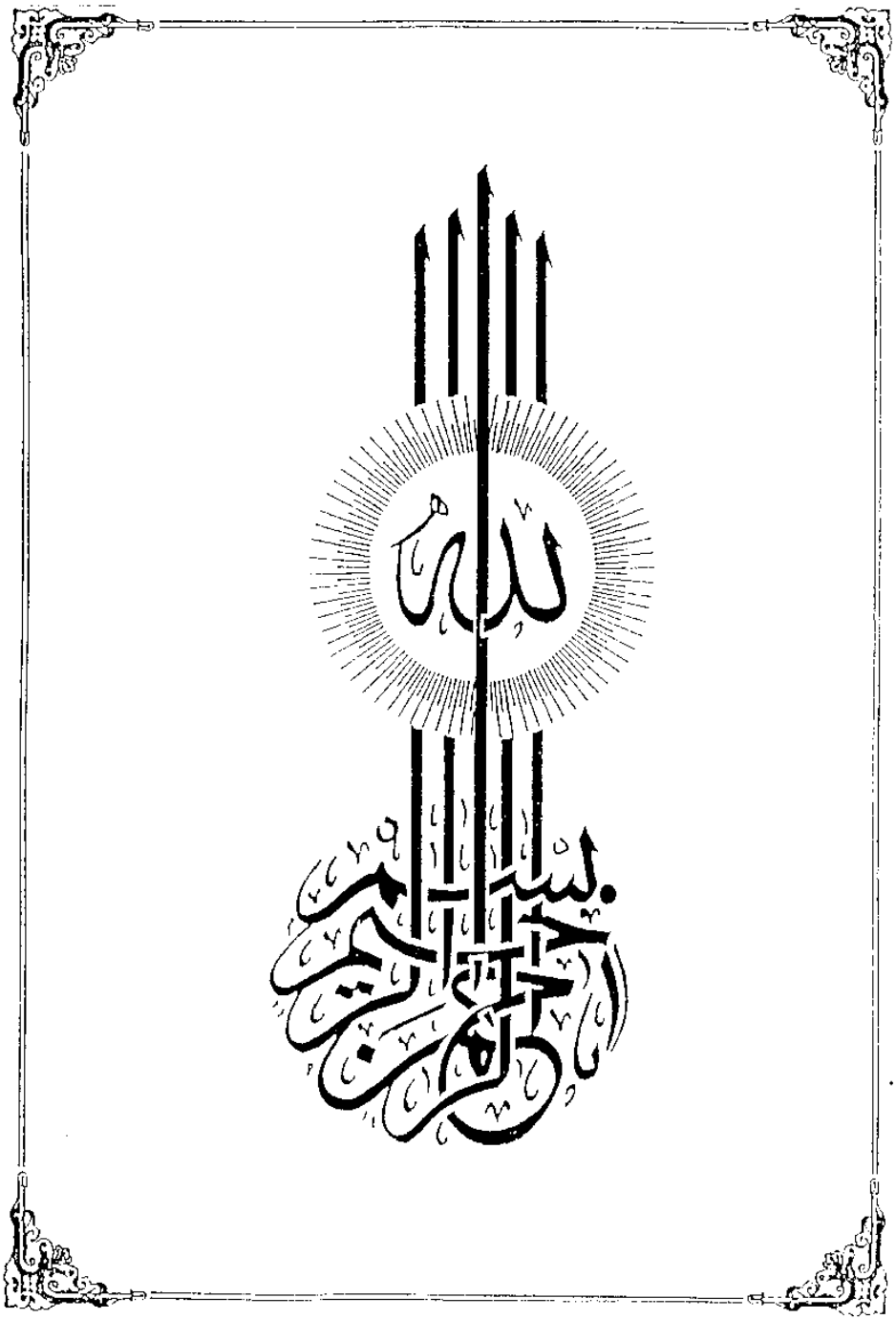

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List of abbreviations:

A	Adenine
Ala	Alanine
Ag-Ab	Antigen-antibody
α_1-AT	Alpha$_1$-antitrypsin
α_1-Pi	Alpha$_1$-protease inhibitor
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
Apo	Apolipoprotein
Asn	Asparagine
AST	Aspartate aminotransferase
Bisacrylamide	N'N' methylene bisacrylamide
C	Cytosine
C_3 & C_5	Complement component factors
CAH	Chronic active hepatitis
%C	Total % of bisacrylamide in total gel composition
DNA	Deoxyribonucleotide
D.W.	Distilled water
G	Guanine
Gal	Galactose
GGT	Gamma glutamyl transferase
Glu	Glutamine
Hb	Hemoglobin
IEF	Isoelectric focusing
Ig	Immunoglobulin
ILD	Interstitial lung disease
LDL	Low density lipoprotein
Lys	Lysine
M	Molar
Man	Mannose
Met	Methionine
µl	Microliter
mRNA	Messenger RNA
ÑAC Glu	N-acetyl glucosamine
nm	nanometer
PAF	Platelet activating factor
APG	Polyacrylamide gel

PAS	Periodic acid schieff
PAS-DR	Periodic acid schieff after diastase reaction
pH	Inverted logarithm of hydrogen ion concentration
pI	Isoelectric point
RDS	Respiratory distress syndrome
RER	Rough endoplasmic reticulum
RID	Radial immunodiffusion
Ser	Serine
Sia	Sialic acid
T	Thiamine
%T	Total % concentration of acrylamide and bisacrylamide
TEMED	NNN'N'-tetramethyl ethelene diamine
VLDL	Very low density lipoprotein

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Introduction:

Alpha$_1$-antitrypsin, a glycoprotein synthesized mainly by the hepatocytes and to a lesser extent by mononuclear phagocytes, is a major inhibitor of several proteolytic enzymes. It is found in a number of body fluids such as tears, lymph and saliva and also in platelets, neutrophils and on the surface of pulmonary alveolar macrophages ***(Graver et al., 1986)***.

Normal persons maintain serum levels of alpha$_1$-antitrypsin within narrow range which increases in case of inflammation, neoplastic disease, pregnancy or estrogen therapy ***(Dalo et al., 1981)***.

Alpha$_1$-antitrypsin deficiency is associated with cases of severe emphysema and liver cirrhosis. However, patients with normal levels of alpha$_1$-antitrypsin may still have increased predisposition to develop certain diseases. These patients may be carriers of the deficiency alleles. That is why, phenotyping of this protein is associated to catalog and elucidate more fully the relationship of the protease inhibitor phenotypes to disease or to a predisposition to disease. Phenotyping is best done using isoelectric focusing which is a well defined easily reproducible

technique. It gives good resolution and separation giving well defined bands ***(Shurkal et al., 1984; Jeppsson and Einarsson, 1992)***.

Aim of the Work:

The aim of the present work was:

1. To study the phenotypic patterns of α_1-antitrypsin in the sera of healthy controls and of patients with hepatoma, liver cirrhosis and chronic active hepatitis by isoelectric focusing in relation to their α_1-AT serum levels.

2. To detect whether there is a relation between certain phenotypes and the occurrence of such liver diseases.

REVIEW OF LITERATURE

ALPHA$_1$-ANTITRYPSIN

The name α_1-antitrypsin (α_1-AT) was suggested by ***Schultze et al. (1962)*** for the protein they had described as α_1-3,5-glycoprotein seven years earlier ***(Schultze et al., 1955)***. During that time, other groups had associated most of the serum trypsin inhibitory activity with the α_1-globulin fraction ***(Moll et al., 1958 and Bundy and Mehl, 1959)***. The protein is known to inhibit a number of other proteolytic enzymes and is therefore more generally known as alpha$_1$ protease inhibitor (α_1Pi). However, the name α_1AT has been maintained for historic reasons to respect the investigators who made the discovery of this inhibitor ***(Carrell & Owen, 1979 and Cox et al., 1980)***.

A. Chemistry of alpha$_1$-antitrypsin:

1. Structure:

α_1-AT is a glycoprotein of molecular weight 52,000 daltons. Its molecule is relatively small and polar allowing ready movement into the tissue fluids. It has a single polypeptide chain of 394 residues with three carbohydrate side chains which are N-linked to asparagine residues (Asn) at position 46,83 and 247 (fig. 1). The

Fig. (1): Structure of AAT molecule. The reactive centre Met-Ser 358-359 is boxed. The attachment points of the three oligosaccharide chains at Asn 46, Asn 83 and Asn 247 are encircled, ▾ shows the mutation sites of the S (264 Glu→Val) and Z (345 Glu→Lys) variants ***(Carrell et al., 1982)***

side chains are composed of N-acetylglucosamine (NAcGLc), mannose (Man), galactose (Gal) and sialic acid (Sia) arranged as a common core sequence of Asn-NAcGlc-NAcGlc-Man-$(Man)_2$ with two or three antennae of (NAcGlc-Gal-Sia) coming off the terminal mannose to form bi-antennary and/or tri-antennary configurations. The side chains attached to the Asn^{46} and Asn^{247} are almost all bi-antennary, whereas the side chain attached to Asn^{83} are bi-antennary for 65% of α_1AT molecules and tri-antennary in 35%. This difference in carbohydrate side chains at position Asn^{83} is responsible for the two major bands of α_1-AT observed when serum is evaluated by isoelectric focusing ***(Carrell et al., 1981 & Vaughan et al., 1982)***.

Crystallographic analysis of α_1-AT molecule reveals that it has a globular structure 6.7nm by 3.2nm with the three carbohydrates on the external surface of the molecule. The internal structure is highly ordered with 30% in the form of alpha-helices and 40% in beta-pleated sheets. The first 20 N-terminal amino acids of α_1AT do not participate in any ordered structure. This peptide is accessible from outside of the molecule, and can be cleaved. Cleavage of the first five N-terminal amino acids results in some microheterogeneity of α_1-AT found in serum ***(Loebermann et al., 1984b and Duncan & Hutchison, 1988)***. There are salt

bridges within the molecule including Glu^{342}-Lys^{290} and Glu^{264}-Lys^{387}. Disruption of these salt bridges render the protein less stable and susceptible to intracellular proteolysis ***(Brodbeck et al., 1993)***.

2. Reactive centre:

The reactive site of α_1-AT is centered on methionine residue at position 358, 37 residues from the C-terminus of the molecule. The molecule has a single reactive site located in the cavity on its surface and available for SH-SS interchange reaction both in vivo and in vitro. For α_1-AT to act as a proteinase inhibitor, this reactive site must be held in a fixed conformation: the conformation of an ideal substrate ***(Kraut, 1977)***. Thus, the proteinase inhibition is accompanied by the formation of a 1:1 complex between α_1-AT and its target enzyme. The reaction mimics that between the enzyme and its substrate, with the reactive center of the inhibitor functioning as a bait for the active site of the proteinase ***(Carrell et al., 1982)***.

3. Homology of α_1-AT:

An interesting feature of the sequence of α_1-AT is its homology with those of antithrombin-III ***(Carrell et al., 1979)*** and ovalbumin ***(Hunt and Dayhoff, 1980)***.