ALPHA-2-MACROGLOBULIN IN DIABETES MELLITUS WITH HEPATOMEGALY

THESIS

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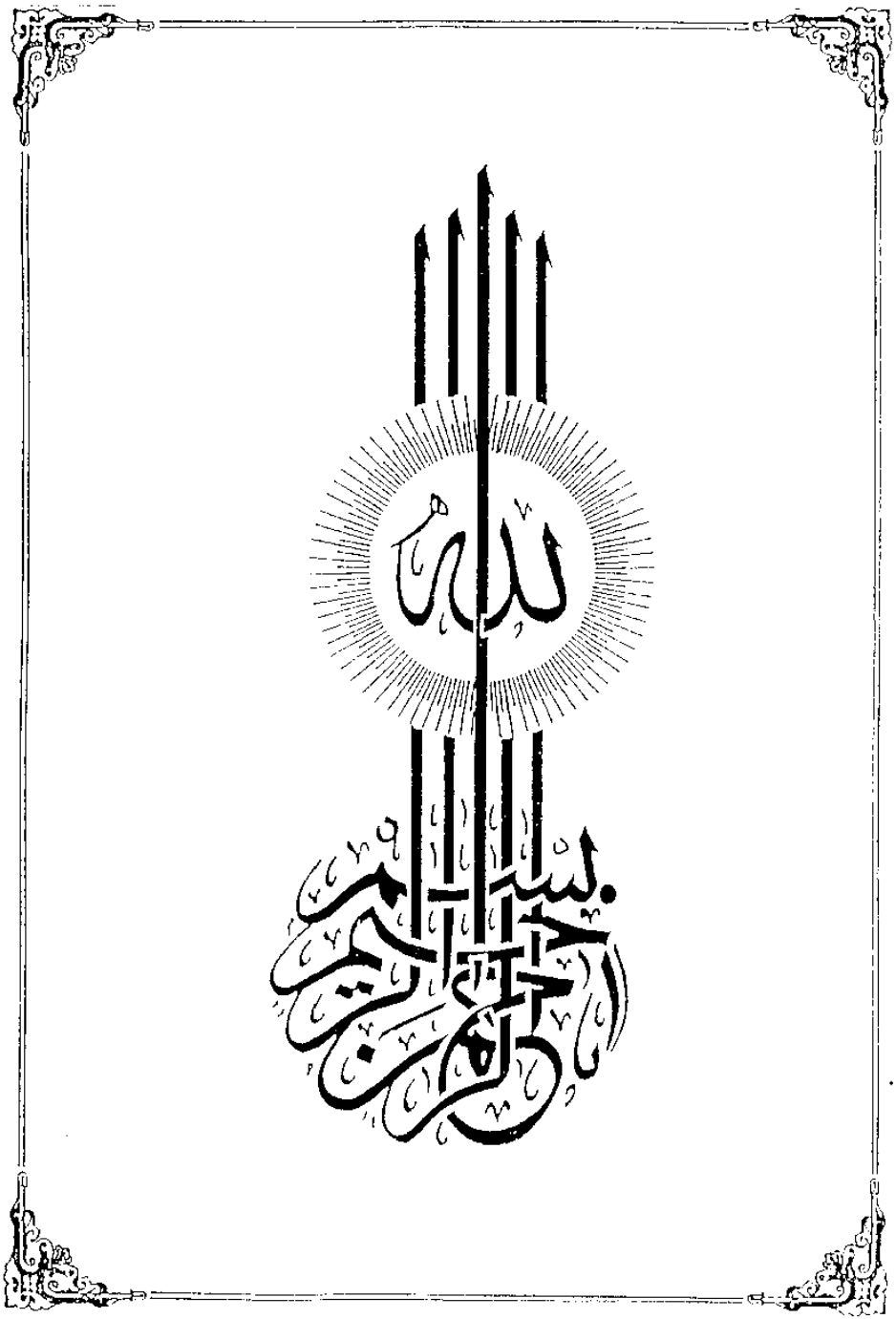

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INTRODUCTION

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Alpha-2-Macroglobulin was found to be increased in chronic liver diseases as Diabetes Mellitus which may be associated with hepatomegaly.

The aim of this work is to study the changes that may occur in the serum levels of alpha-2-macroglobulin in insulin dependent and non-insulin dependent diabetics to see whether it is related to the occurance of hepatomegaly. This study comprises twenty insulin dependent diabetics with and without hepatomegaly, and twenty non-insulin dependent diabetics with and without hepatomegaly in addition to ten control non-diabetic healthy subjects. All of these five groups will be subjected to full clinical examination, routine laboratory investigations including fasting blood sugar, liver function tests (S.G.P.T., Alkaline phosphatase), and finally estimation of serum level of alpha-2-macroglobulin done by radial immunodiffusion.

REVIEW OF LITERATURE

ALPHA-2-MACROGLOBULIN

Alpha$_2$ macroglobulin is a glycoprotein which is composed of carbohydrate units covalently linked to peptide chains. The carbohydrate portion can make up as little as 1% or as much as 80% of the mass of the molecule (Spiro, 1969). Almost all glycoproteins contain sialic acids (N-acylneuraminic acid), while the other monosaccharides found include glucose, fucose, galactose and mannose, along with glucosamine and galactosamine. There may be present as monosaccharides or as polysaccharides.

Glycoproteins generally have only one type of carbohydrate unit, but may have two, the carbohydrate units are joined to the peptides through several types of bonds.

The function of the carbohydrate portion of the molecule is still doubtfull. It does seem that sialic acid residues, in particular, protect the molecule from too rapid breakdown in vivo.

This is of particular importance for the glycoproteins of the gut and for the plasma proteins. It has been suggested too, that the carbohydrate portion is involved in recognition of the molecule by cell surface (Morell et al., 1971) and also that it may facilitate transport of proteins out of the cell (Eylar, 1965).

In normal, the total plasma proteins concentration ranges from 6.2 to 8.4 g/100 ml. The fractionation of serum proteins by electrophoresis was initiated largely by Tiselius (1939) and Durrum (1948). They demonstrated that normal serum contains at least five electrophoretically distinguishable bands, which are albumin, alpha$_1$ globulin, alpha$_2$ globulin, beta globulin and gamma globulin starting with the band closet to the anode and moving towards the cathode. Another faint band, called pre-albumin, can also be identified beyond the albumin fraction.

If plasma is separated, we obtain six prominent bands, an additional band representing fibrinogen and migrating between the beta and gamma globulin fractions.

Alpha$_2$ globulin is the third in its anodal migration, and appears to be the most heterogenous of all the standard fractions. It is possible to distinguish up to ten alpha$_2$ globulin components among them. There are four major components represent over 80% of this fraction and they are alpha$_2$ macroglobulin, alpha$_2$ lipoprotein, haptoglobin and alpha$_2$ HS glycoprotein. The normal adult range of alpha$_2$ globulin was found to be 5.2 - 10.4 % with a mean value of 9.0% of the total protein level. It is almost always pathologically increased and alteration in alpha$_2$ macroglobulin and haptoglobin generally account for the changes in its pattern .

The alpha$_2$ macroblobulin was isolated by Schultze in 1955. It is one of the two heavy constituents of serum, its molecular weight is found to be 820.000 (Schultze et al., 1966). It is one of the main plasma proteins, representing about 3% of the total mammalian plasma proteins with an average concentration between 200-300 mg/100 ml, a mean level of 265 mg ± 55 mg/100 ml (James et al., 1966).

Hepatocytes synthesize the protein even in the early foetus. Because of the protein's large size, it is found primarily in the intravascular spaces but lower levels can also be found in various body fluids as the cerebrospinal and synovial fluids. Its presence at concentrations above 30 to 50 % of serum levels in these spaces suggests major leakage as a result of disturbed membranes.

Physical and Chemical Properties:

(a) Solubility: Alpha$_2$ macroglobulin is very soluble in distilled water at neutral pH. but their solubility decreases following a decrease in pH. (Bourrillon et al., 1968).

(b) Electric properties: The isoelectric point of alpha$_2$ macroglobulin thus for investigated is in the 4.5 - 5.4 pH range.

Alpha$_2$ macroglobulin has the chemical characteristics of a glycoprotein with a carbohydrate content of 8 - 11% (Heimburger et al., 1964, Picard et al., 1965, Demaille et al. 1965, Bourrillon et al., 1968). The sugar components present are characteristic of those observed in other serum glycoproteins.

There are 31 carbohydrate units per molecule of alpha$_2$ macroglobulin with average composition of three mannose to two galactose. In addition to these two hexoses, alpha$_2$ macroglobulin is the only hexosamine and one 6-deoxyhexose (Fucose). The N-acetyl-neuraminic acid is the only form of sialic acid present.

The chemical analysis of the human alpha$_2$ macroglobulin carbohydrate reveals the presence of 156 - 220 hexoses (62 Residues of galactose & 94 mannose), 13 - 35 fucose, 130-150 N-acetyl glucosamine at 48 - 50. N-acetyl neuraminic acid residues.

Each molecule consists of eight polypeptide chains with dimers of the chain forming very stable quarter molecules. The peptide portion of alpha$_2$ macroglobulin contains about 120 methionine 70 tryptophan and 530 basic amino acid residues, permitting the prediction that the molecule may be cleaved into 120 fragments by cyanogen bromide, and into 530 peptide fragments by trypsin.

Biological Properties and Function of Alpha-2-Macroglobulin

(a) Genetics:

Knight et al (1968 a,b) have shown the existence of two specific allotypes MTZ_1 & MTZ_2 associated with $alpha_2$ macroglobulin. They are controlled by two allelomorphic genes. It is not sex-linked.

(b) Isolation:

The ultracentrifugation and the results from starch-gel electrophoresis in urea suggest that alpha-2-macroglobulin is composed of several subunits linked by different types of bonds, in particular disulphide and hydrogen bonds (Bourrillon et al. (1968).

Human alpha-2-macroglobulin was isolated in gram quantities using polyethylene glycol, ammonium sulfate, and ion exchange fractionation of outdated human plasma.

(c) Anti-Protease Activity:

Alpha-2-macroglobulin seems to be involved and play a complex role in a variety of enzymalogical processes. But the only well defined biological function is inhibition of proteolytic enzymes, thus these enzymes including trypsin,

alpha-chymotrypsin, thrombin, plasmin elastase are effectively inhibited. (Haverbuk, 1962; Schultze et al., 1963; Ganrot, 1966).

The ability of alpha-2-macroglobulin to form enzymatically active complexes with these proteolytic and esterolytic enzymes is now well established (Haverbuk, 1962). With Trypsin this complex was called "Trypsin proteinase esterase (TPE). The binding of trypsin to alpha-2-macroglobulin reduces the proteolytic activity of trypsin on high molecular weight substances (higher than 10.000 e.g. casein and fibrin)(Bourrillon, 1968). According to Ganrot (1966^b) the molar ratio alpha-2-macroglobulin trypsin is ½ i.e. a molar combining ratio of two moles trypsin per mole alpha-2-macroglobulin was obtained when the molecular weight of trypsin was taken as 24.000 and the molecular weight of alpha-2-macroglobulin as 820.000.

Alpha-2-macroglobulin also inhibits the proteolytic action of plasmin thus potentiates the antifibrinolytic action of synthetic inhibitors (Bourrillon, 1968) and acts on thrombin as a progressive anti-thrombin resulting in an inhibition of the clotting activity of thrombin.

This inhibition is reduced by plasmin. These three enzymes (Trypsin, plasmin and thrombin) seem to compete

for the same site on alpha-2-macroglobulin particularly plasmin and thrombin (Ganrot et al., 1967).

Alpha-2-macroglobulin fails to inhibit the esterase activity of trypsin, thrombin and plasmin, and thus can act simultaneously as an inhibitor of the proteolytic activity and as protector of the esterolytic activity of these proteases. This property allows alpha-2-macroglobulin to be distinguished from $alpha_1$ anti-trypsin and inter $alpha_1$ trypsin inhibitor (Heide et al., 1965).

Chymotrypsin, papain and asparto-transferase are also bound by alpha-2-macroglobulin (Boyde et al., 1968). In fact these enzymes migrate in electrophoresis. Alpha-2-macroglobulin also inhibit the elastolytic activity of elastase. The elastase/alpha-2-macroglobulin molar ratio was 1/1 (Baumstark, 1970). The inhibition of elastase by alpha-2-macroglobulin was of the "Temporary type" since alpha-2-macroglobulin was slowly digested by this enzyme.

Alpha-2-macroglobulin has the capacity to bind toxic substance from urine and to prevent them from increasing capiklary permeability (Schumacker et al., 1963).

The turnover of alpha-2-macroglobulin in normal man is about 10 days 8-11 percent per day which amounts to about 8 mg per Kilogram body weight per day (Reuge et al. 1966).

Alpha-2-macroglobulin may also has a transport function for various hormones and for metals. It binds the growth hormone and insulin also about one third of the Zinc present in the plasma is bound to it, as it is a Zinc metalloprotein and the trypsin - alpha-2-macroglobulin esterase activity increases in parallel with Zinc content (Parisi, 1970). Nickel is also bound to it at least in rabbits (James et al. 1967).

(D) Inhibition of Viral Haemagglutination.

The ability to inhibit the haemagglutination of the A_2 strain of influenza virus and to neutralize its infectivity was located exclusively in the alpha-2-macroglobulin fraction of horse serum (Pepper, 1968[b]). The influenza virus haemagglutination inhibitor isolated from human collustrum has been identified as alpha-2-macroglobulin (Shortridge,1970).

In recent years there have been reports indicating an association between alpha-2-macroglobulin and the development and function of lymphocytes (McNeill,1970, Havemann et al. 1970). There are some indications that it is present on the surface of a subpopulation of lymphocytes (Ford et al.1973); McCornick et al. 1973) and may be synthesized by peripheral blood lymphocytes (Tunstall et al.,1974).