Prealbumin In Rheumatoid Arthritis

Thesis

Submitted In Partial Fulfilment For

The Degree Of M.Sc. General Medicine

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1984

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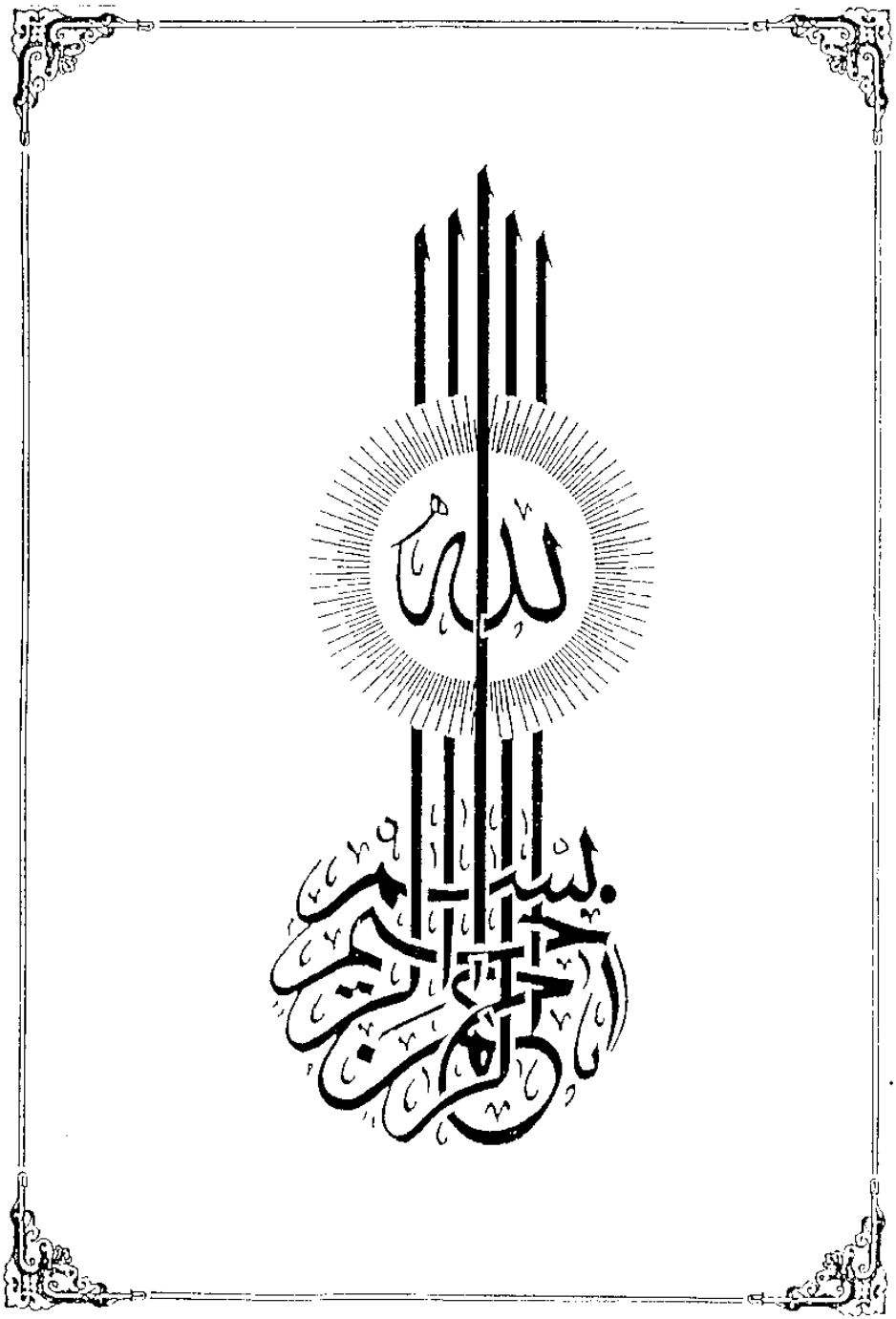

ACKNOWLEDGEMENT

I wish to take this opportunity to express my sincer appreciation to Dr. MOHAMED ABD EL FATAH TAHA, who gave me the honer of supervising this work and supplied me with a continuous and constant help.

Also my profound gratitude to Dr. HANY SOUBHY, who supervised the work and reviewed it with patience and with a constructive criticism.

I would like also to express my thanks to Dr. HODA NASSER, who encouraged me and supplied me with references .

Finally, my thanks go to all patients who were the subjects of this thesis .

YASSER A. H. ALLAM

I N T R O D U C T I O N

INTRODUCTION

Rheumatoid arthritis is an autoimmune reaction which can be frequently encountered in clinical practice. The erythrocyte sedimentation rate is one of the most reliable indications of inflammatory joint disease but it is, of course; quite non specific. Rarely an anomalous normal erythrocyte sedimentation rate may persist despite active disease(Mason ,1979 and Davidson 1970). Tests for rheumatoid serum factor are in common use. They are positive in 80 per cent of patients with classical rheumatoid arthritis and in all patients with rheumatoid nodules. In about 20 per cent of patients, tests of lupus erythematosus cells or antinuclear factor will be positive, usually in the more severe examples with a greater tendency for multisystem involvement, their presence does not necessarily invalidate the diagnosis, although clearly they call for reappraisal. Rheumatiod factor and antinuclear factor could be detected in the synovial fluid but not in the serum of several patients suggesting either selective sequestration or local synthesis of antinuclear and rheumatoid factors in the affected joints (Ghose and Woodbury, et al, 1974, 1975).

Anaemia and leucocytosis are common and may reflect disease activity, but have no diagnostic features.
From these previous studies, the aim of this work came to our mind. The aim of this work is to study the serum concentration of prealbumin in rheumatoid arthritis and to assess the diagnostic and prognostic values of its determination, if any.

Prealbumin has been neglected in routine work despite several reports suggesting its diagnostic and prognostic

value.

low levels of prealbumin were found to be associated with liver disorders including cirrhosis of all types both active and quiescent, chronic active hepatitis, and acute viral hepatitis, and inflammatory disorders including the collagen disorders, acute and chronic bacterial infections and other diseases of an inflammatory nature, such as ulcerative colitis and Crohn's disease, and malignant disease including the myeloproliferative disorders and the reticulosis, and burns and congestive cardiac failure (Harris and Kohn, 1974).

One of the techniques required for visualization of prealbumin is radial immunodifusion which is simple and does not require any special apparatus or skill.

R E V I E W

REVIEW

Prealbumin is a protein of molecular weight 61,000 (Schultze, 1966), with a half-life in the serum of 1·9 days (Oppenheimer and Smith , 1965).
It has similar functions to the main albumin fraction but is of special importance in the binding of tetra-iodo thyronine and tri-iodothyronine (Ingbar, 1958). Two prealbumin fractions may be seen. One of these is the so-called tryptophan-rich prealbumin (Schultze and Schwick', 1956), and the other is said to be an alpha-1-acid glycoprotein (Schultze and Hermans, 1966) . For the purpose of this study, the prealbumin is considered as a single fraction .
The synthesis of albumin and prealbumin is synthetically linked (Oppenheimer, 1963). Because of the high concentration of albumin in serum, the absolute quantity of tryptophan present in the albumin fraction is many times that found with prealbumin (Ingbar, 1960).

This protein is found to migrate faster than albumin in electrophoresis (Harris and Kohn, 1974).
In the case of prealbumin, no significant age or sex related variations are seen(Lyngbye and kroll, 1971).
Few studies examining prealbumin levels in serum have been published, however, it is known that levels moderately low at birth rise rapidly to adult levels during the first few weeks of life, Malnutrition and inflammatory processes adversely affect serum prealbumin levels. It is of relatively high cocentration in cerebrospinal fluid (Davidson et al, 1979).

One of the intermediate level plasma constituents,it

plays a role in thyroxine metabolism and in the physiology of vitamin A or retinol (Peterson, 1971). This protein is unusual in that it is specifically responsible for the transport of two plasma costituents (Nilsson, 1971 and 1975).

Prealbumin is filtered through the glomerular membrane at low levels, however, the smaller associated retinol binding protein is readily filtered at the glomerulus and only partially reabsorbed through the renal tubules (Davidson et al, 1979).

The binding sites in the molecule of prealbumin for retinol binding protein-retinol are separate and distinct from the site for thyroxine, giving this protein its unique dual transport function (Rask, 1972).

Plasma prealbumin has been recognized as one of the three proteins responsible for the transport of L- thyroxine in the blood stream (Robbins and Rall 1960, Ingbar 1963, and Oppenheimer 1968). It is distinctly less important for this function than thyroxine binding alpha globulin (Oppenheimer,1968). The proportion of endogenous thyroxine normally bound to prealbumin in man is probably of the order of 15% (Ingbar, 1968).

Moreover, less than 1% of the total plasma prealbumin molecules normally circulate in the form of a comlex with thyroxine. These data indicate that prealbumin plays only a physiologically minor role in the transport and metabolism of thyroid hormones in humans.

Recent reports have shown that prealbumin is also involved in the transport of vitamin A in plasma . This is done in association with a specific retinol binding protein

(Goodman, 1968,1969, 1970, and peterson, 1971). The prealbumin-retinol binding protein complex is able to combine with both thyroxine and retinol. This complex of hormone and vitamin binding protein is clearly of considerable interst. Prealbumin and retinol binding protein have now been purified and partly characterized (Raz and Goodman, 1969, and Peterson, 1971).

The major physiological function of human prealbumin appears to involve its role as part of the protein complex normally responsible for the transport of vitamin A in plasma. The normal concentration of prealbumin in plasma is about 250 ug per ml., and that of retinol binding protein is about 40-50 ug per ml. the normal mole ratio of prealbumin to retinol binding protein in plasma is 2.5 : 1 (Smith and Goodman, 1970, 1971, 1973).
Accordingly , retinol binding protein normally circulates almost entirely in the form of the retinol binding protein -prealbumin complex, and about 40% of plasma prealbumin molecules also circulate in this form. Plasma retinol binding protein and prealbumin levels are both significantly decreased in a variety of diseases, including liver disease (Goodman et al, 1971), hyperthyroidism, cystic fibrosis of the pancreas (Goodman et al, 1972), and protein-caloric malnutrition (Smith and Goodman , 1973).In all of these diseases the relative ratio of prealbumin to retinol binding protein is not very different from normal. In contrast, patients with severe chronic renal disease show markedly elevated levels of retinol binding protein but normal levels of prealbumin in plasma (Smith and Goodman, 1971). This finding reflects the fact that the kidneys normally play a major role in the catabolism of retinol binding

protein but not of prealbumin . The interaction of prealbumin with retinol binding protein serves the important physiological function of greatly restricting the glomerular filteration of the relatively small retinol binding protein molecule, and hence the renal catabolism and loss of retinol binding protein and vitamin A .

Neither the interaction of prealbumin with thyroxine nor with retinol binding protein, appeared to alter significantly the secondary structure of the protein(Goodman, 1972).

By equilibrium - dialysis studies of the thyroxine-prealbumin interaction, Raz and Goodman (1969) found a molecular weight of about 50,000. These authors found the same value by using the sedimentation-equilibrium technique and obtained results indicating that prealbumin consists of a single molecular species.

Peterson (1971), by studying the interaction between prealbumin and the retinol binding protein,found a molecular weight of about 17,000 for prealbumin by gel filteration on a Sephadex G-200 column equilibrated with 5-M - guanidine hydrochloride.

In a crystallographic study, Blake et al (1971),have obtained evidence for a tetrameric structure for the prealbumin molecule. Examination of the molecular symmetry made it possible to define the quaternary structure of prealbmin as two pairs of non identical subunits or four identical subunits.

Thus, in addition to its interactions with other molecules, prealbumin itself forms an associated system with a

defined quaternary structure.

The number and staining properties of the tryptic peptides indicate that the subunits are identical or closely similar. This conclusion is reinforced by comparing the sum of the aminoacid composition of the tryptic peptides with the aminoacid composition of the whole protein. All minor peptides that were isolated could be shown to be derived from major peptides. No evidence has been either from electrophoretic experiments or from aminoacid sequence determination, for any dissimilarity between the subunits(Gonzalez and Offord, 1971).

The elution position of a protein on a sephadex columnmay give an approximate measure of the molecular weight (Andrews 1964). In the absence of urea, prealbumin was eluted at a position cerresponding to a molecular weight of 57,000. In the presence of urea, prealbumin and carboxymethyl-prealbumin eluted at a position corresponding to molecular weight either 30,000 or 57,000 depending on the mildness of the previous treatment of the protein.Remarkably, the lower molecular weight peak predominated in these samples that had been mildly treated whereas the higher molecular weight peak predominated after the more severe treatment(such as brief boiling or boiling in saturated urea).Under the disaggregating conditions,the molecular weight of prealbumin was decreased to about 14,000.This is about one quarter of the value determined by gel filteration and by x-ray crystallography(Blake, 1971) and is consistent with the existence of four subunits for the prealbumin molecule.

As a result of aminoacid analysis of prealbumin

(Goodman and Morgan et al, 1974), the molar ratios are given on the basis of 16 histidine residues, a value which brings the total molecular weight (56,000) neerest to that begining to become apparent for the whole molecule(Offord, 1971). Values are also given for a total chain length of one quarter of this value, which is suggested for the subunit size by the gel electrophoresis experiments. It appears, therefore, that urea is inadequate for the complete disaggregation of the molecule (Gonzalez, 1971).

These previous reports indicated that prealbumin molecule is a stable tetramere (Goodman and Morgan, 1974).

The N-terminal aminoacid of prealbumin was identified; by using the dansyl method, the reaction was performed in the presence of urea in an attempt to maximize the access of the reagent to the terminal part of the polypeptide chain. The result showed glycine to be the sole aminoacid in prealbumin with a free alpha-amino group (Offord and Gonzalez, 1971).

Taken with all the other evidence, the summation indicates that the molecule must be made up of four subunits and that, if the subunits differ in sequence, they can do so only slightly. If there were slight differences between subunits one might expect to find peptides with almost identical sequences, or alternatively a difference could occur that might not be sufficient to permit separation of two forms of a peptide. The results presented above indicate that prealbumin is composed of four polypeptide chains each having a molecular weight of about 14,000. There is no evidence inconsistent with the view that the four chains have an identical aminoacid sequence but the possibility

that there are small dissimilarities can not be eliminated until the complete sequence is determined.

Each prealbumin subunit contains 127 aminoacids, including one cysteine(position 10), one methionine(position 13), four arginine, and eight lysine residues . Thirteen tryptic peptides were aligned after studies which included the tryptic digestion of maleylated, reduced,and carboxymethylated prealbumin (to yield five peptides),and subsequent tryptic digestion of these peptides after removal of the maleyl groups(Goodman and Morgan, 1974).

The sequence of aminoacid of each subunit is ;

NH_2-- gly - pro - Thr - gly - Thr - Gly - Glu - Ser - Lys-
Cys - pro - leu - Met - val - lys -val - leu - Asp - Ala - val-
Arg - Gly - Ser - pro - Ala - Ile - Asn - Val - Ala - val-
His - val - phe - Arg - lys - Ala - Ala - Asp - Asp - Thr-
Trp - Glu - pro - phe - Ala - Ser - Gly - lys - Thr - Ser-
Glu - Ser - Gly - Glu - leu - His - Gly - leu - Thr - Thr-
Glx - Glx - Gln - phe - val - Glu - Gly - Ile - Tyr - Lys-
val - Glu - Ile - Asp - Thr - lys - Ser - Tyr - Trp - lys-
Ala - leu - Gly - Ile - Ser - pro - phe - His - Glu - His-
Ala - Glu - val - val - phe - Thr - Ala - Asn - Asp - Ser-
Gly - pro - Arg - Arg - Tyr - Thr - Ile - Ala - Ala - leu-
leu - Ser - pro - Tyr - Ser - Tyr - Ser - Thr - Thr - Ala-
val - val - Thr - Asn - pro - lys - Glu - CooH.

Each of the four identical subunits of prealbumin contains one methionine residue, and cleavage with cyanogen bromide produced two fragments . The smaller fragment corresponded to the NH_2- terminal thirteen residues and the larger fragment to the remainder of the subunit polypeptide chain (Morgan and Goodman, 1971).