ALTERATION IN PLASMA FIBRONECTIN DURING CARDIOPULMONARY BYPASS SURGERY

THESIS

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بسم الله الرحمن الرحيم

" وقل إعملوا فسيرى الله عملكم ورسوله والمؤمنون
وستردون إلى عالم الغيب والشهادة فينبئكم بما كنتم تعملو

صدق الله العظيم

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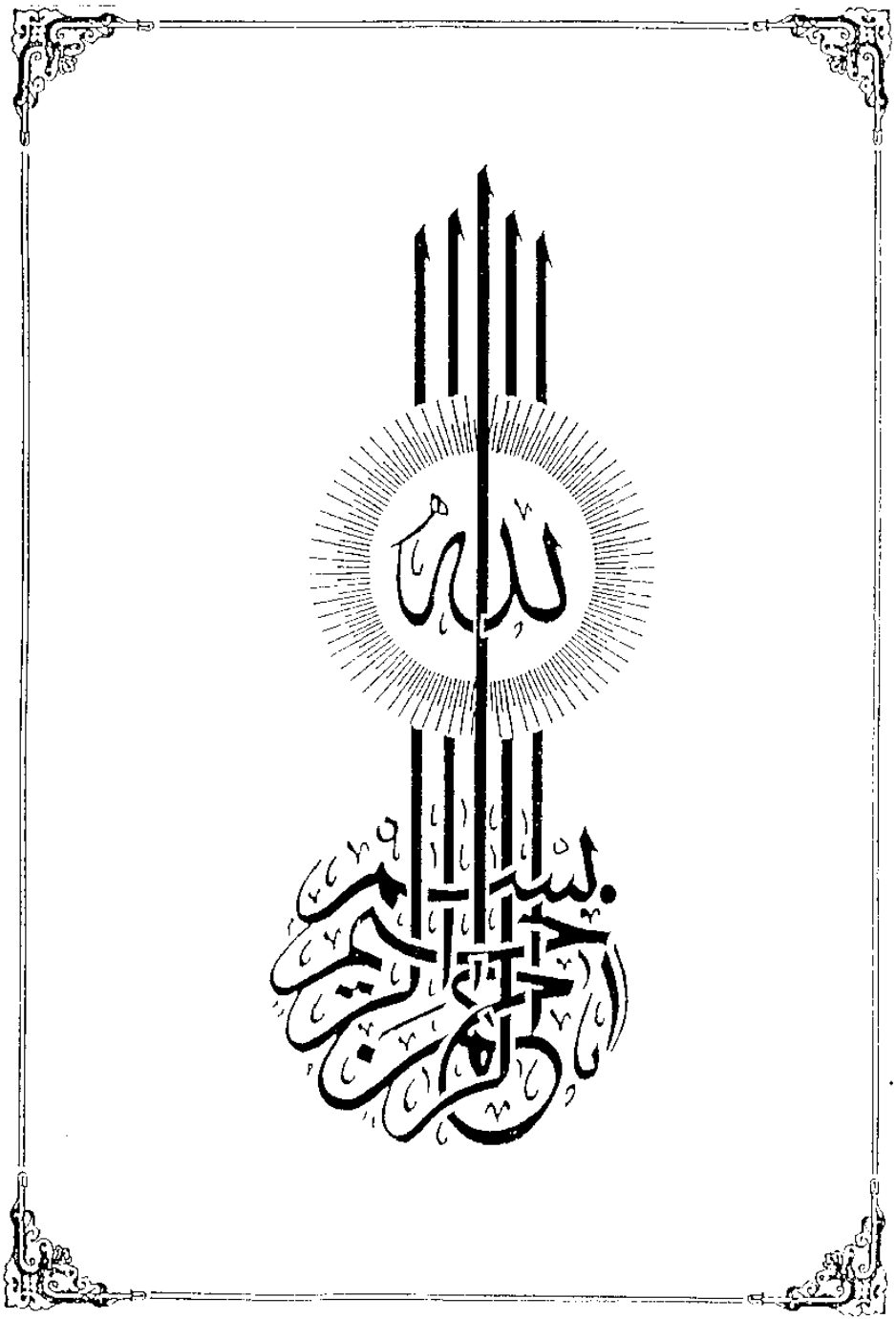

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Introduction:

Fibronectin is a glycoprotein which has an important role in the reticulo-endothelial host defense system. It promotes opsonization of bacterial particles , cell debris and fibrin aggregates thus promoting their clearance by the monocyte-macrophage system (Mossesson M. and Amrani D., 1980).

A fall in plasma fibronectin level was found in burns , trauma , major surgeries (Lanser and Saba , 1982).

In open heart surgery , the use of cardio-pulmonary bypass machine , although is an indispensible technique , yet itself is probably associated with temporary organ and system dysfunction following its use. Despite improvement in surgical technique, technological advances , pre-operative patient evaluation , and current antibiotic therapy in patients undergoing cardiac surgery , infection is still an important factor in post-operative morbidity.

Aim of the work:

The aim of this work is to estimate fibronectin level before and after cardio-pulmonary bypass in order to assess the extent of change as a base for consideration , for replacement therapy (Gershon et al. , 1985).

FIBRONECTIN

Fibronectin is a high molecular weight glycoprotein that exists in a soluble form in plasma and in an insoluble form in tissues. Plasma fibronectin can modulate phagocytic function as well as incorporate into the tissue matrix where it is believed to influence microvascular integrity and tissue repair (Cohler et al. , 1985).

Interaction with certain discrete extracellular substances such as fibrin , collagen , and with cell surface structures accounts for its biologic activities (Mossesson et al. , 1980).

Prior to the suggestion of the name fibronectin , it has been known by variety of terms including opsonic protein (Saba , 1970) , soluble fibroblast antigen (Rouslahi et al. , 1974) , cell surface protein (Yamada et al. , 1974) , cold insoluble globulin (Chen et al. , 1976), and cell adhesion factor (Pearlstein , 1976).

At present , there is a general acceptance of the term fibronectin. The word itself was created to emphasize the propensity of the protein to bind to fibrous protein like collagen and fibrin (fibra , fibre , and nectere , to bind).

Historical events:

In 1948 , Morrison and co-worker described a protein component of fibrinogen which was cold insoluble and unlike fibrinogen was not thrombin coagulable. It had a more rapid anodal electrophoretic migration rate and higher sedimentation coefficient than did fibrinogen.

Later in 1957 , Smith and Van Korff described a protein with properties similar to those of the cold insoluble protein that they had found in heparin induced cold precipitate plasma.

In 1968 , Mossesson and co-worker investigated patients with chronic intravascular coagulation secondary to an occult neoplasm. The illness was characterized by the persistence of cold induced plasma precipitate termed "cryofibrinogen". The solubilized cryofibrinogen was partially coagulable by thrombin , thus proving that fibrinogen was present . Another major component resembling cold insoluble globulin was also found in the fractions and was shown to be immunochemically identical with a normal serum protein unrelated to fibrinogen. Follow up investigation by Mossesson and Umfleet in 1970 presented a method for isolation and purification of cold insoluble globulin and provided clear evidence that it was a unique and major plasma protein (300 $\pm$ 100 ug/ml).

In the early 1970 , a number of investigations had focused on the changes that occurred in cell surface protein of fibroblast as a consequence of transformation by oncogenic viruses. Particular attention was paid to a large transformation-sensitive glycoprotein that was released from the fibroblast cell surface into culture medium (Hynes et al. , 1974).

The report by Ruoslahti in 1975 that cold insoluble globulin was antigenically identical to the transformation sensitive glucoprotein brought to light the uniqueness of fibronectin as both a cell-surface / matrix protein and a blood protein and has stimulated numerous investigations on all forms of the protein.

Structure:

Structural studies of fibronectin derived from cell surfaces , tissue culture medium , or extracellular fluids pointed to the presence of molecular heterogencity (Chen et al. , 1977).

Despite this , the outline of basic molecular architecture can be sketched (Fig. 1).

Plasma fibronectin molecules have a molecular weight of 450,000 Daltons and composed of two similar but nonidentical polypeptide chains with estimated molecular weights of 220,000 and 210,000 (Masher , 1975). The two chains are disulfide linked at the carboxyl - end of the molecule (Balian et al. , 1979).

Intrachain disulfide bridges are clustered in the terminal thirds of each chain , most of them located at the amine terminal end (Iwanga et al. , 1978).

There is also a significant number of free sulfhydryl groups (Pearlisten et al. , 1980). Blocking of these groups interferes with binding to the cell surface (Wagner and Hynes , 1979).

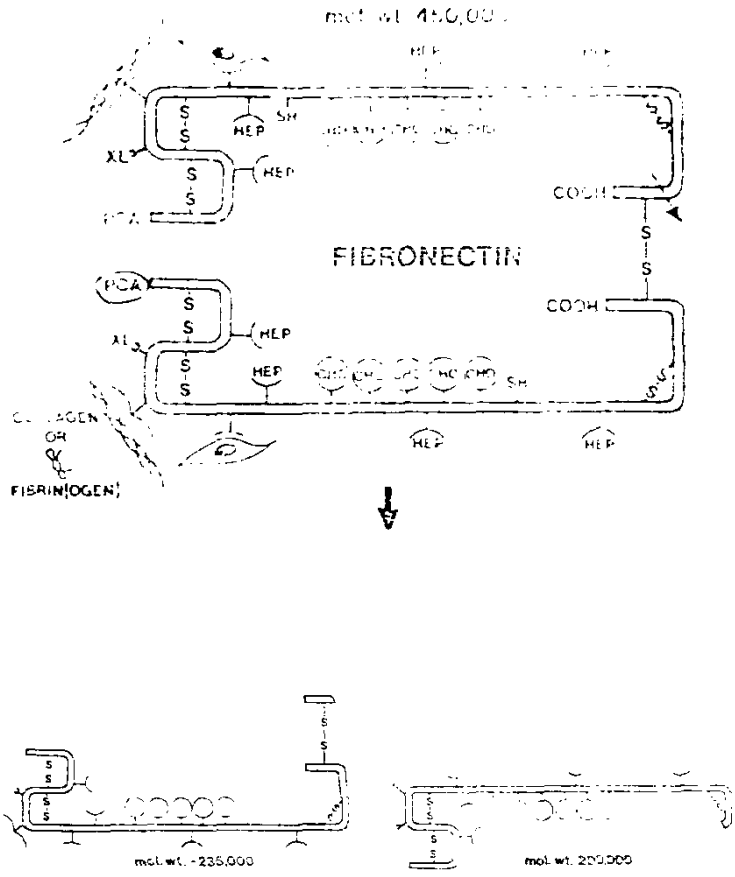

Fig. 1

Structural model of plasma fibronectin:

Most circulating molecules are composed of two more-or-less identical chains of approximate mol.wt. 220,000 Daltons , each linked near the COOH-terminal end by disulfide bridging (S-S). The NH2 terminus is designated PCA (Pyrrolidone Carboxylic Acid). The general location of intrachain disfluide bridging is also depicted as are the approximate locations of sulfhydryl groups (SH), Carbohydrate groups (CHO), and collagen-binding, fibrinogen-binding, cell-binding, Cross linking (XL), and Heparin (HEP) - binding sites. The dashed arrow indicate a region in the dimeric molecule which is cleaved during the course of hydrolysis by several proteolytic enzymes (Mossesson & Amrani , 1980).

Several minor fibronectin components of smaller size than the two chain structure have been identified in plasma molecules and most of them range in size from 235,000 to 146,000 Daltons. It may be derived by catabolic processes from large parent molecules (Chen et al. , 1977) , and it is not yet known whether the degradative processes ; they appear to reflect ; occur intravascularly (Mossesson , 1980).

Plasma fibronectin contains also 5 % carbohydrate (Fukuda and Hakomori , 1979). All oligosaccharide units are linked to the peptide backbone by asparagine residues at 4-6 sites along the middle portion of the peptide chain (Yamda et al. , 1977). The major sugars in plasma molecules are mannose , galactose , N. acetyl glucosamine and sialic acid (Fukuda and Hakomori , 1979). It was suggested that carbohydrate fraction may serve to increase fibronectin resistance to proteolysis (Fukuda and Hakomori , 1979).

Structural differences among fibronectin:

Many of the essential characteristics of fibronectin are shared by all forms including affinities for fibrin and collagen. Nevertheless , there is a structural difference between plasma fibronectin which is dimeric and cellular fibronectin which is multimeric. This multimeric form may indicate a higher degree of interchain disulfide bridging and could account for the lower solubility of the tissue fibronectin in aqueous buffer at neutral pH (McConnel et al. , 1978). The relationship between plasma and cell fibronectin has not been well established and it seems likely that a dynamic equilibrium exists between circulating and cell associated fibronectin and that a variation in plasma concentration may reflect important changes at the tissue level (Masher et al. , 1984).

Synthesis and kinetic:

Although many cell types have the capacity to synthesize fibronectin evidence suggests that most , if not all , circulating fibronectin is produced by hepatocyte (Gonzalez et al. , 1982).

Radiolabelled plasma fibronectin is cleared from the circulation in a complex manner after intravenous injection. Half lives of 24 to 72 hours have been estimated. The fates of fibronectin leaving the circulation are obscure. Immunofluorescence and tissue extraction studies, however , indicate that some of the protein is deposited in

extracellular tissue matrices (Sherman and Lee , 1982).

Fibronectin either in soluble or matrix form is sensetive to neutral tissue proteinases. Destruction of fibronectin may be critically involved after mast cell activation and in inflammatory conditions in vivo (Alan et al. , 1981).

Interactions of fibronectin:

1. Interaction with collagen and fibrinogen:

It is well established that fibronectin binds to denatured collagen and to several types of native collagen (Engvall et al. , 1978). The affinity of fibronectin for denatured collagen is greater than that for native collagen. This sort of affinity evidently is a factor in its ability to promote adhesion and spreading of cells on collagen-coated or plastic substrate (Grinell et al. , 1977). The collagen binding site is located in the terminal region of each polypeptide chain in a segment containing intrachain diulfide bridges (Balian et aal. , 1979). A region mediating cell attachment is close but separable from the collagen binding site (Grinell et al. , 1977).

The fibrinogen binding site on fibronectin is located in the same region as the collagen binding site , it may even be identical. Fibrinogen binding to fibronectin can be inhibited by preincubation of fibronectin with gelatin. Similarly , fibrinogen partially inhibits the binding of fibronectin to gelatin (Engvall et al. , 1978).

The fibrinogen binding site for fibronectin appears to be located in the carboxyl terminal region of the fibrinogen A alpha chain (Stathakis et al. , 1978). This region also contains a site that is utilized during factor XIIIa - catalysed cross linking of fibrin alpha chain. The presence of this fibronectin binding region in fibrinogen molecule may account in part for the ability of fibrinogen to bind to the surface of fibroblasts or to induce fibrin clot retraction (Colvin , et al. , 1979). It is suggested that fibronectin plays a major role in clearence of fibrin-fibrinogen complexes produced in blood under certain conditions because of the affinity between fibrinogen and fibronectin (Clemmensen , 1984).

2. Interaction with heparin:

At 4 degree centi grade heparin induces precipitation of plasma fibronectin (Yamada et al. , 1974). It changes the shape of fibronectin from globular to an elongated filomentary form (Jilek et al. , 1979). The binding of heparin to fibronectin does not depend on its anticoagulant activity and the fraction of heparin which is present in heparin precipitated fibronectin is not enriched or depleted in anticoagulant activity (Stathakis et al. , 1977). It has also been known that precipitation in plasma can be induced by a variety of sulfated mucopolysaccharides having little or no anticoagulant potential (Smith and Van Karff , 1957).

The precipitation of fibronectin by heparin is a contrubiting factor to the fall in plasma fibronectin which occurs soon after initiation of the extra corporeal by pass circulation during cardiac surgery (Gershon et al. , 1985).

3. Interaction with macrophages and granulacyte:

Fibronectin has a phagocytic function which is mediated through its interaction with macrophage and granulocyte. It has been shown that exposure of macrophage to fibronectin enhanced the phagocytosis of sheep erythrocyte. On the other hand , when these erthrocyte were allowed to act with macrophages alone , phagocytosis was less enhanced (Bohnsack et al. , 1985). The fibronectin enhanced phagocytosis can be inhibited by treating macrophage with a monoclonal antibody to C4b/C3b receptor (CR1). Many investigator have demonstrated that fibronectin acts as signal for CR1 activation and the molecular mechanism for this activation of complement receptors are as yet unexplained (Chadiwck et al. , 1986).

Studies have been made to demonstrate which domains in fibronectin structure are responsible for the enhancing effect of fibronectin on macrophage phagocytosis. John and co-worker at 1986 found that a unique tetrapeptide sequence in fibronectin composed of arginine - glycine - aspartic acid - serine (RGDS) is responsible for its phagocytosis enhancement (John et al. , 1986).