PLASMA FIBRONECTIN CONCENTRATIONS IN PATIENTS WITH BILHARZIAL HEPATIC AFFECTION

THESIS

SUBMITTED FOR THE PARTIAL FULFILMENT

OF THE MASTER DEGREE IN

(TROPICAL MEDICINE)

BY

Nabila Sobhy Shenouda

M.B., B.Ch.

SUPERVISED BY

Dr. Effat Abd El Moneim El Fekhfakh

Ass. Prof. of Tropical Medicine

Ain Shams University

Dr. Moubark Mohamed Hussien

Ass. Prof. of Tropical Medicine

Ain Shams University

Dr. Zenab Mohamed Tawfeek

Assist. Prof. of Clinical Pathology

Ain Shams University

FACULTY OF MEDICINE

AIN SHAMS UNIVERSITY

1987

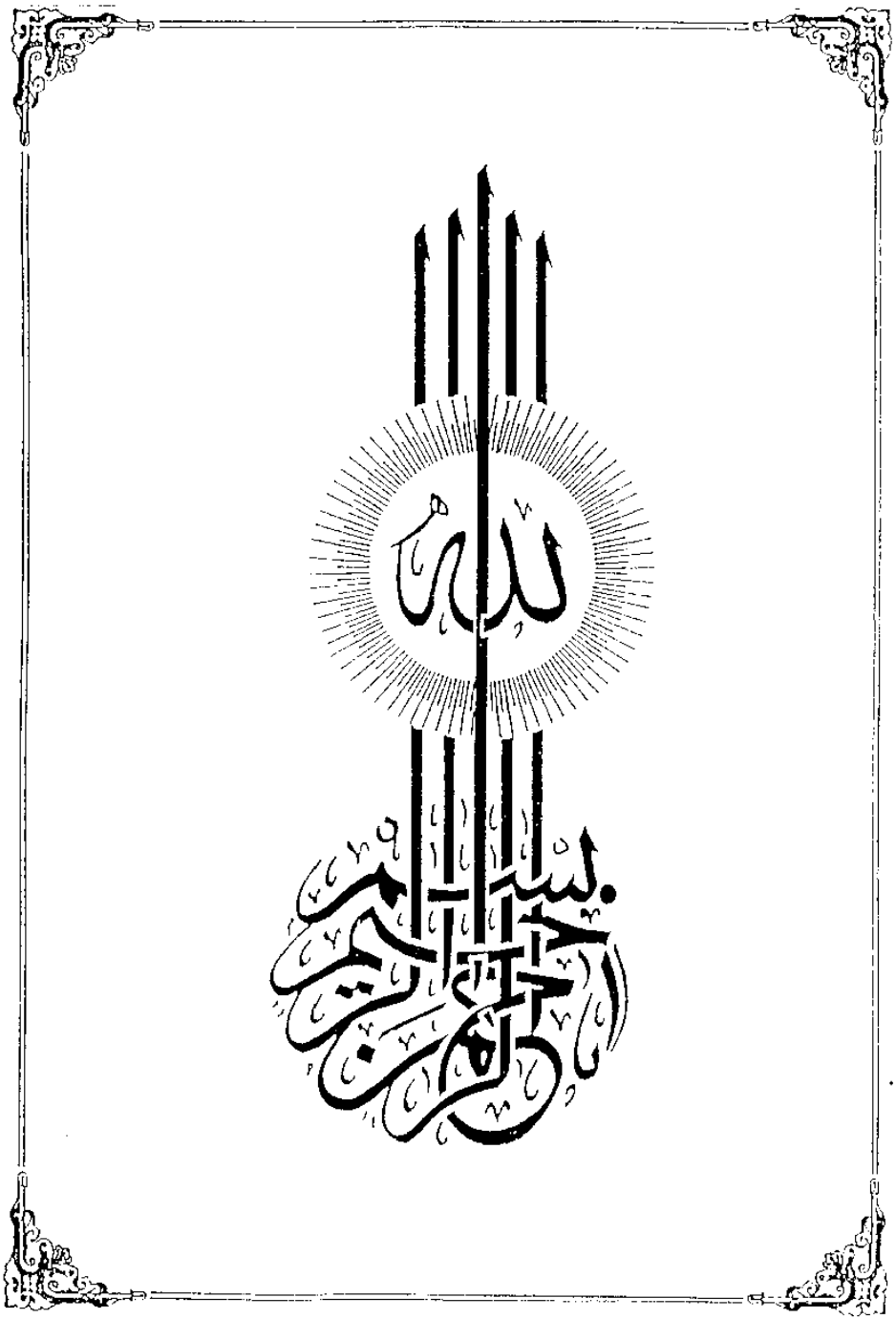
ACKNOWLEDGEMENT

I wish to express my profound gratitude to Dr. EFFAT ABD EL MOHEIM EL FEKHFAKH Assis. Prof. of Tropical Medicine, Ain Shams University, for her valuable guidance, constant support and encouragement. It is an honour to work under her supervision.

I would like to express my grateful thanks to Dr. MOUBARK MOHAMED HUSSIEN , Assis. Prof. of Tropical Medicine, Ain Shams University for his guidance, continous kind help, continous encouragement and advice throughout the work.

I would like to express my deep appreciation to Dr. ZENAB MOHAMED TAWFEEK, Assis. Prof. of Clinical Pathology, Ain Shams University, for her guidance, great help, assistance and advice throughout the practical part of this work.

Finally, I wish to thank all the staff of Tropical Medicine for their kind help.



C O N T E N T S

* * * *

Introduction and Aim of Work

INTRODUCTION

Fibronectin (cold insoluble globulin) is a high molecular weight glycoprotein found both in an insoluble form in many tissues, such as interstitial connective tissue, in many basement membranes, around smooth muscle cells and fibroblasts, and in the sarcolemma of striated muscle fibres (Linder et al., 1975; Stenman et al., 1978) and in soluble form in plasma and other body fluids (Mosesson et al., 1970; Kuusela et al. 1978; Yamada et al., 1978). Mesenchymal cells, including fibroblasts and vascular endothelial cells, are considered the main sites of synthesis of fibronectin (Wartiovaara et al., 1974; Jaffe et al., 1978; Mosher et al., 1980). Cultured rat hepatocytes have also been demonstrated to synthesize and excrete fibronectin (Voss et al., 1979).

Consequently parenchymal liver disease may lower plasma fibronectin either due to decreased synthesis or due to increased consumption of fibronectin. Repair processes following liver disease involve both proliferation of parenchymal liver cells and formation of fibrosis by mesenchymal cells. These repair processes may also influence plasma fibronectin concentrations. So far the problem has received little attention (Pott et al.,1980; Matsuda et al. 1982).

AIM OF WORK

The aim of the present study is to elucidate the concentration of plasma fibronectin in various stages of bilharzial hepatosplenomegaly as plasma fibronectin is a glycoprotein which has been associated with hepatic failure (Audhuy et al., 1984), sepsis (Rubli et al. 1983), and factors potentially related to the survival of cirrhotic patients (Naveau et al., 1985). Thus we undertook this prospective study to assess the prognostic value of plasma fibronectin in bilharzial cirrhotic patients.

REVIEW

Discovery and Nomenclature:

In 1948, Morrisen et al. isolated a partially purified fraction of human plasma which they term "Cold insoluble globulin" (CIG). Other workers independly described the various proteins or factors as anti-gelatin factor (Wolff, et al. 1967), microfibrillar protein (Ross et al., 1969), opsonic protein (Saba, et al., 1970), galactoprotein a (Gahmberg, et al., 1974), large external, transformation - sensitive "LETS" protein (Hynes, et al., 1974), fibroblast surface antigen "SFA" (Ruoslahti,et al., 1974), cell surface protein "CSP" (Yamada, et al., 1974), Zeta "Z" (Blumberg, et al., 1975), cell spreading factor (Grinnell, et al., 1976), cell attachment factor "CAF, C.CAP" (Pearlstein, et al., 1976). These factors are named according to sources or biological activities.

Recent evidence indicate that all of these proteins are closely related, and they are probably only one or two specific proteins: Cell surface (cellular) fibronectin, and plasma fibronectin. Cell surface fibronectin is a major constituent of the cell surface of many cultured cells, and was discovered when cell surface proteins or carbohydrates were labelled radio-isotopically or immunologically. This glycoprotein is also known as large, external, transformation sensitive "LETS" protein or cell

surface protein "CSP" (Yamada et al., 1974). Plasma fibronectin although known as cold insoluble globulin, purified plasma fibronectin is relatively soluble in the cold unless it is complexed with fibrin and fibrinogen (Morrison et al., 1948).

Current information suggests that although the two forms of fibronectin are very similar, they are probably not identical weather they are products of different genes or of one gene and are modified post-transcriptionally, is not yet known (Yamada et al., 1978).

Distribution and Biologic activities of tissue forms of fibronectin:

Fibronectin is synthesized by a variety of different cells: fibroblast (Rouslahti et al., 1973), Astroglial cells (Vaheri et al., 1976), certain epithelial cells including those derived from carcinoma cell lines (Chen et al., 1977), Chondrocytes (Dessau et al., 1978), myoblasts (Furcht et al., 1978), endothelial cells (Jaffe, et al., 1978), Schwann cells (Kurkinen et al., 1979), peritoneal macrophages have also been reported to synthesize and secrete fibronectin (Johansson et al.,1979), although Pearlstein, et al., (1978) did not find the protein on the surface or within resident or activated preitoneal macrophages. Also isolated hepatocytes have

been reported to synthesize and secrete fibronectin (Voss et al. 1979) as well as to incorporate it into extracellular matrices (Voss et al. 1979).

Fibronectin is a major component of the connective tissue matrix, immunofluorescent studies of normal tissues have demonstrated that it is present in many basement membranes, around smooth muscle cells, and in the sarcolemma of striated muscle fibers, in the sinusoidal walls of the liver, in the stroma of lymphatic tissue, and in loose connective tissues (Stenman et al., 1978).

Cellular expression of fibronectin is linked with cell differentiation and organogenesis. During embryogenesis fibronectin is first detectable on cells of the blastula inner cell mass (Wartiovaara,et al., 1978). At later developmental phases, fibronectin is lost or becomes redistributed concomitant with differentiation of mesenchymal cells into muscle, cartilage and renal tubular epithelium (Linder et al., 1975).

In tissue culture, fibronectin usually appears as fibrillar matrices that are situated on cells, between cells, and between cells and the substratum (Yamada et al., 1978). This pattern is consistent with the role

it evidently plays in mediating the phenomenon of spreading and adhesion that takes place among cells and the substratum (Pearlstein, et al., 1976).

Studies of the effect of fibronectin addition on transformed cells have helped to clarify the role that this protein plays in regulating cellular functions. Adding fibronectin to cultures of transformed fibroblasts, which lack the extra cellular fibronectin matrix (Furcht et al., 1978) results in partial resumption of more normal behaviour and appearance (Hynes, et al., 1978). The normalizing effects on the cell include restoration of a more flattened and elongated shape, improved adhesion to substratum, and reconstitution of prominent intracellular actin microfilament bundles (Yamada, et al. 1976). Fibronectin treated cells also display more rapid cellular locomotion (Yamada, et al., 1978).

Structure and Molecular interactions:

All fibronectins are composed of 200,000 - 250,000 molecular weight subunits. Plasma fibronectin is a disulphide-bonded dimer of two such subunits (Mosesson et al., 1975), cell surface fibronectin is found as disulphide bonded dimers and multimers (Hynes et al.1977).

The amino acid composition of fibronectins from different sources is very similar, but there are significant differences in carbohydrate composition (Mosher,et al., 1980).

Earlier observations suggested plasma and cell surface fibronectins to be immunohistochemically indistinguishable (Yamada et al., 1978), but a monoclonal antibody with specificity for cellular fibronectin has recently been described (Atherton et al., 1981). Recognition for fibronectin by this antibody was not dependent on carbohydrate residues, and it was concluded that structural differences must exist between plasma and cellular forms. Some studies have indicated that plasma fibronectins have smaller apparent subunits than cell culture fibronectins (Yamada et al., 1979). These authors also noted that cell-surface fibronectin is 50 times more active than plasma fibronectin in restoring a more normal morphology to transformed cells. This property has been attributed to the multimeric form of the cell surface protein (Mosher et al., 1980).

Fibronectin interacts with a variety of molecules including fibrin, collagen, glycosaminoglycans and DNA. In the presence of activated factor XIII (fibrin stabilising factor), it is bound convalently to fibrin and to itself (Mosher et al., 1975). It binds more avidly to denatured collagen (gelatin) than to native collagens, but of the latter it has greatest affinity

for collagen type III (Engvall et al. 1978). It binds to heparin, hyaluronic acid and dextran sulphate, but not to dermaton sulphate or chondroitin sulphate (Yamada et al., 1980). Affinity of gelatin or collagen for fibronectin is increased in the presence of heparin, heparin sulphate or hyaluronic acid. It has been suggested that these interactions are involved in formation of extracellular matrices (Jilek et al., 1979). Fibronectin can also bind to actin (an intracellular protein) (Keski-oja, et al., 1980), to staphylococcus (Kuusela et al., 1978) and to cell surfaces.

A model of fibronectin molecule has been proposed (Fig.1) based on binding properties of polypeptide fragments produced by proteolytic cleavage. The glutamine residue required for cross-linkage to fibrin, collagen and the surface of staphylococci is present at the N-terminal end. The gelatin-binding site is close to, but seperated from the N-terminal end, and the glycosaminoglycans - binding site is at the C-terminal end. The cell attachment site is between gelatin and heparin - binding sites. (Mosher et al., 1980).

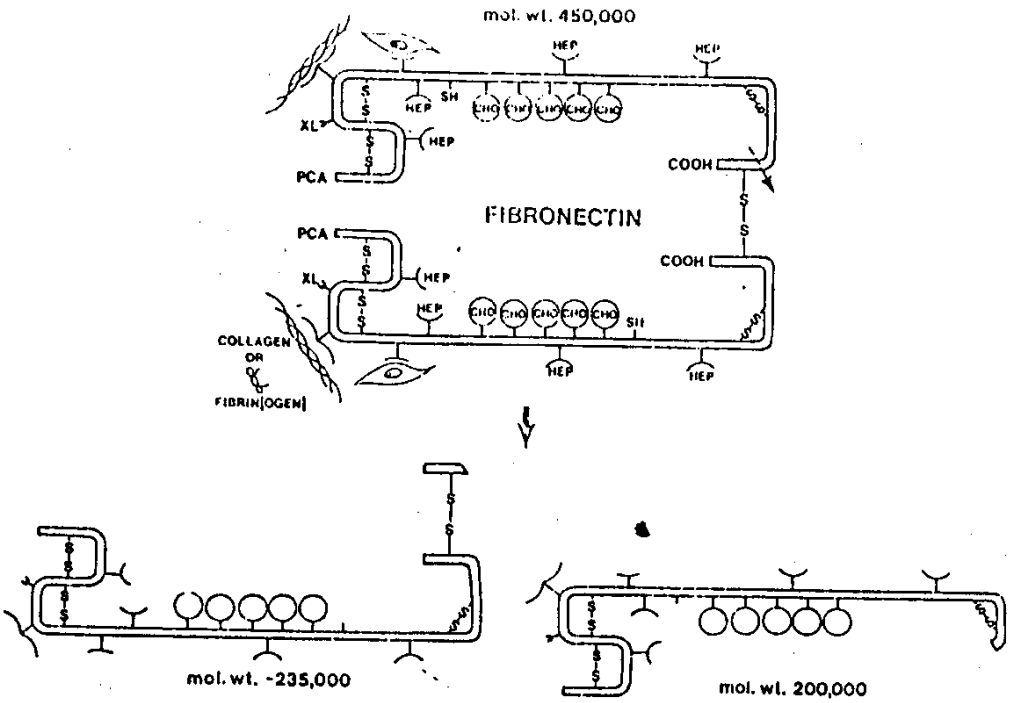


Fig. 1. Structural model of plasma fibronectin (Cfg). Most circulating molecules are composed of two more-or-less identical chains of approximate mol. wt. 220,000, each linked near the COOH-terminal end by disulfide bridging (S-S). The NH_2-terminus is designated PCA (pyrrolidone carboxylic acid). The general location of intrachain disulfide bridging is also depicted as are the approximate locations of sulfhydryl groups (SH), carbohydrate groups (CHO), and collagen-binding, fibrin(ogen)-binding, cell-binding, crosslinking (XL), and heparin (HEP)-binding sites. The dashed arrow indicates a region in the dimeric molecule which is cleaved during the course of hydrolysis by several proteolytic enzymes including plasmin,[79,83] cathepsin D,[84] or subtilisin.[85] The products of such attack are shown in the lower portion of the diagram.

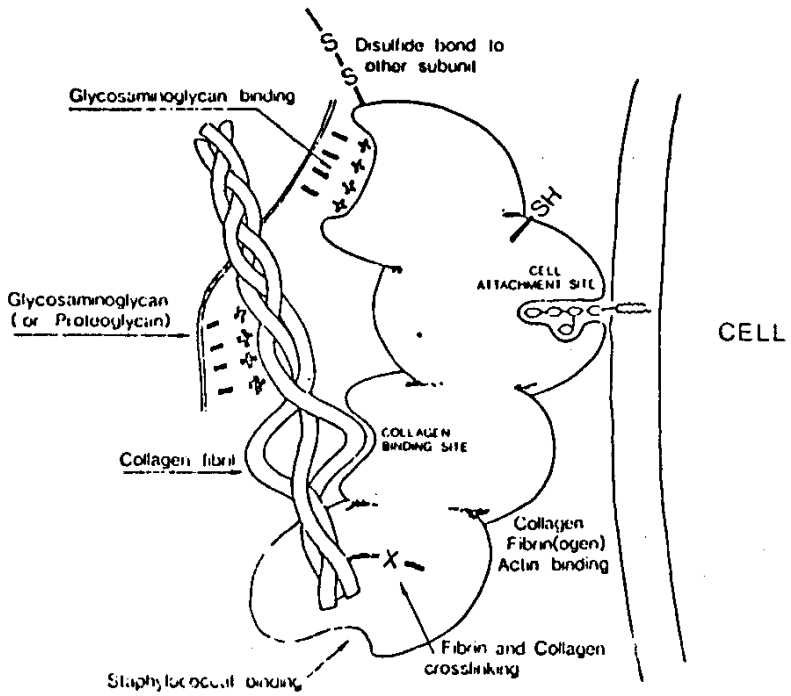


Fig. 1—A model of a fibronectin subunit. (Reproduced from Ruoslahti, Engvall and Hayman[29] with kind permission of the authors)