THE VALUE OF PLASMA ASCITIC FLUID FIBRONECTIN DIFFERENCE IN DIFFERENTIATION BETWEEN MALIGNANT AND CIRRHOTIC ASCITES

THESIS

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by

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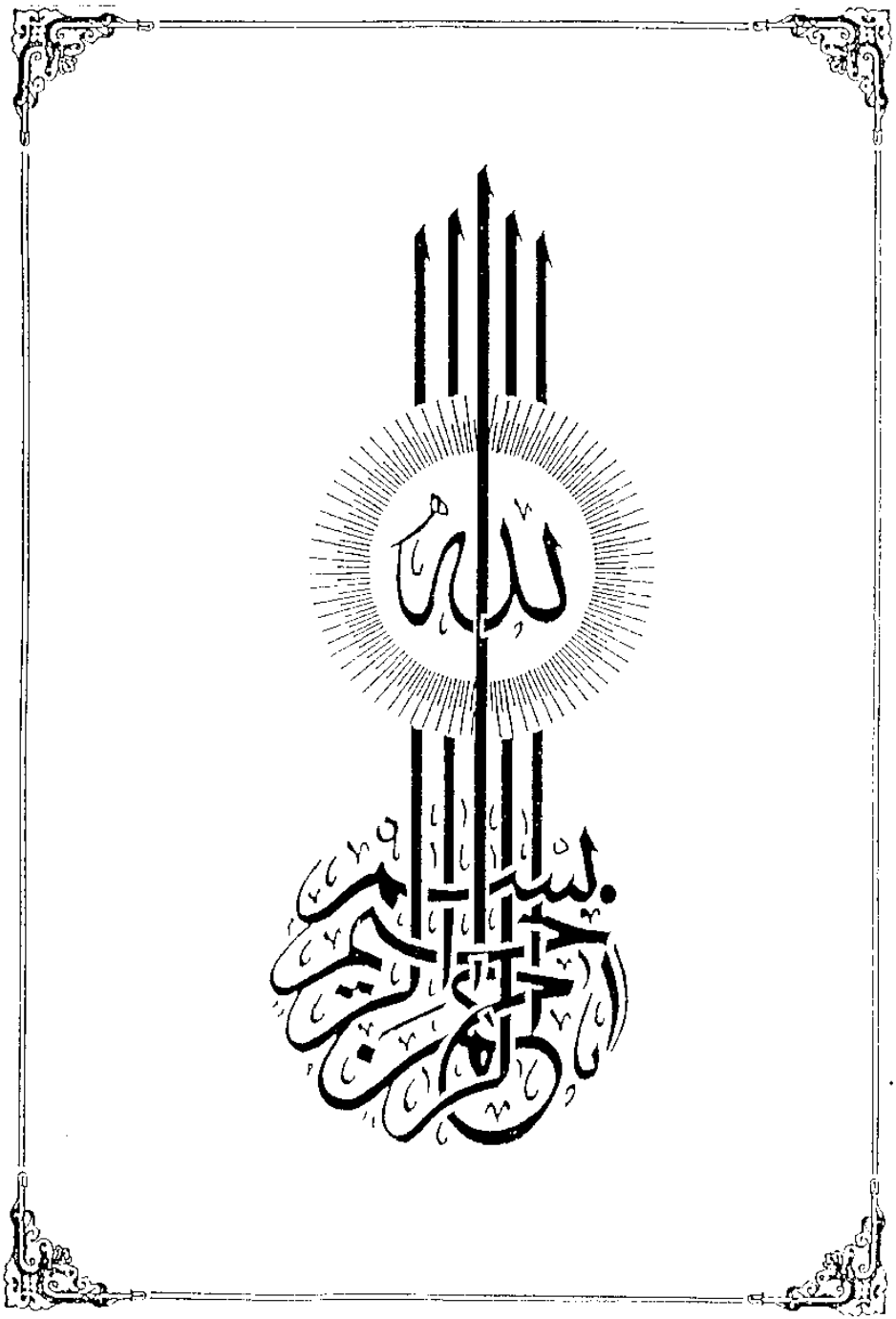

CONTENTS

INTRODUCTION AND AIM OF THE WORK

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INTRODUCTION :

Ascites is a common clinical problem in Egypt. It is most often caused by either chronic liver disease or malignancy.

Ascitic fluid parameters valuable for differentiation between the two conditions have long been sought. Ascitic fluid total protein has been used widely as a laboratory test in this differential diagnosis. However high protein ascites, although a consistent finding in malignant ascites, has been reported in up to 25% of patients with ascites due to chronic liver disease (Sampliner and Iber, 1974).

Other parameters such as ascitic fluid lactate dehyrogenase, carcinoembryonic antigen or fibrinogen degradation products have been investigated but a complete separation between both groups has not been achieved (Jungst et al., 1986).

Fibronectin is a high molecular weight glycoprotein present in soluble form in the blood and in insoluble form in connective tissue matrix (Agostini Colli et al., 1986).

It is beleived that the determination of ascitic fluid fibronectin concentration together with some of the parameters (as protein content and cholesterol) are of value in differentiation of infected from non infected ascites. This approach could make differential diagnosis of ascites easier and less uncomfortable for the patient with advanced disease (Scholmerich et al., 1984)

AIM OF THE WORK :

The aim of this work is to illustrate the value of estimating plasma fibronectin and plasma-ascitic fluid fibronectin concentration difference in differentiating malignant from cirrhotic ascites in Egyptian patients.

REVIEW OF LITERATURE

FIBRONECTIN

HISTROICAL PRESPECTIVE :

Fibronectin is a family of structurally and immunologically related high molecular weight glycoprotein. Fibronectin present in two forms : soluble form in blood and other body fluids and insoluble form which is present in the surface of cells, in extracellular spaces of connective tissue and as a component of basement membrane (Mosesson & Amrani, 1980 and Ruoslahti et al., 1981).

Fibronectin was firstly isolated by Morrison et al., at 1948, they described a protein component of fibrinogen containing fraction that was insoluble and present in precipitate that forms when plasma stands in the cold, hence the first name "cold insoluble globulin" or CIG. More physicochemical studies by Edsall et al. 1955, gave indication that the molecule of cold insoluble globulin was, large and suggested that it was a domer composed of two fibrinogen molecule.

Smith and Von Korff (1957) isolated and described a fractionof human plasma which is characterized by cold insolubility in te presence of heparin. In ultracentrifugation, two components were found the major one resembling

fibrinogen while the minor one resembling cold-insoluble globulin.

At 1968, Mosesson, Colman and Sherry discovered the similarities between naturally occurring cold insoluble globulin and the non clotable component of pathological plasma cryoprecipitates (cryofibrinogen) present throughout the illness of a patient with DIC (disseminated intravascular coagulation syndrome) and cryofibrinogenaemia.

Mosesson and Umfleet (1970) prepared highly purified form of CIG as unique and a major plasma protein. They prooved by immunochemical, immunoelectrophoretic and electrophoretic studies that CIG was not a form of fibrinogen.

In early of 7th decade of this century, the cellular form of fibronectin was discovered and purified from fibroblast. During that time, a number of investigations had been directed towards a protein associated with the surface of fibroblast grown in culture and was frequently lost on transformation by oncogenic viruses (Grahamberg et al., 1973; Ruoslahti & Voheri, 1974 and Ymada & Weston, 1974).

Nomenclature :

The name fibronectin derived from the latin word fibre which mean fibre and nectin which mean to link, bind or

connect (Roreslahti et al., 1981). It was created to emphasize the propensity of the protein to bind to fibrous protein like collagen (Engvall & Rouslahti, 1977 and Engvall et al., 1978) and fibrin (Rouslahti & Vaheri, 1975 and Kaplan & Snedeker, 1980).

ISOLATION, STRUCTURE AND PROPERTIES OF FIBRONECTIN :

Fibronectin isolation :

The early methods for isolation of plasma fibronectin depend on the cryoprecipitate phenomenon, this mean the presence of fibronectin in the precipitate that forms when plasma stand in cold. They, by differential priciptation and ion exchange chromatograpy fibronectin can be separated from proteins present in cryopericipiates (Mosher, 1975 & Mosseson & Umfleet, 1970).

Highly purified plasma fibronectin can be isolated using the affinity chromatography on gelatin sepharose (Engvall and Ruoslahti, 1977).

Fibronectin structure and properties :

Fibronectin is a glycoprotein of high molecular weight (Ruoslaht et al., 1981; Amrani et al., 1985). All fibronectins are composed of 200,000 : 250,000 molecular

weight subunits. All forms are composed of two similar polypeptide chains of about 250 (KDal) mass connected close to their carboxy terminal ends by two disulfide bonds. Each subunit may be divided into several functional domins with specific binding properties so that simultaneous interactions of fibronectin with other moleculs and with cell become possible (Jan Skrha, et al., 1988).

It is clear that at least three different types of internal homolgy occur in fibronectin. At present, nine regions of type I homology, two of type II homology and four of type III homology have been identified (Petersen et al., 1983).

When fibronectin deposited on a surface and analysed by rotary shadowing electron microscopy, the dimer looks like a V, each arm of the V is approximately 2 n.m X 6 n.m (Tooney et al., 1983).

Spectroscopic and hydrodynamic studies of fibronectin in solution indicate that fibronectin can exist in both the extended form seen on electron microscopic grid and in compact form in which flexible arms presumably fold back on themselves (Erickson and Carrel, 1983).

Amino terminal region, composed of 5 blocks of type I (homology), bind to fibrin, heparin and Staph. aureus. The adjacent regions composed of 4 blocks of type I homology and

2 blocks of type II homology bind to gelatin and collagen. The carboxy terminal regions composed of 3 blocks of type I homology bind to fibrin. Several blocks of type II homology bind to heparin. The fragment which contain amino acids sequence. Argnine-glycine-asparatate-serine in the tenth block of type II homology mediate cell adhesion (Mosher, 1987).

Yamada et al. (1977) had found that the amino acid composition of fibronectin from different sources is very similar. Moreover, Furie and Rifkin, (1980) showed that no difference between the two polypeptide chains of plasma fibronectin separated on sodium dodecyle sulfate gel electrophoresis. On the other hand there are significant differnces in the carbohydrate part of different fibronectins (Ruoslaht et al., 1981).

Both plasma and cell surface fibronectins have similar amounts of carbohydrate, about 5%, but diffrences in the sugars have been present. The major sugars in plasma fibronectin molecule are mannose, galactose, N-acetyl glucosamine and sialic acid. The cellular form contain more glucose and much less sialic acid (Fukuda and Hakamori, 1979).

The carbohydrate moeity of fibronectin molecule is not needed in promoting normal fibroblast morphology, nor in

mediating cell attachement to collagen but it may have a protective role against catabolic degradation in culture (Olden et al., 1979).

Metabolism :

Radiolabeled fibronectin is cleard from circulation in a complex manner after intravenous injection. Half lives of 24 to 62 hours have been estimated. The fates of fibronectin leaving the circulation are obscure (Sherman and Lee, 1982).

Sources and distribution :

Fibronectin was first identified in fibroblasts (Gahmberg and Hakomori, 1973; Hynes, 1973). Several types of epithelial cells have been found to produce fibronectin. These include epithelial cells from the liver and kidneys (Ohen et al., 1976), Gut (Quaroni et al., 1978), breast (Smith et al., 1977) and amniotic membranes (Crouch et al., 1978). Other cells producing fibronectin are endothelial cells (Jaffe and Mosher (1978), macrophages (Alitalo et al., 1980), myoblast (Hynes, 1976), undifferentiated chondrocytes (Dessau et al., 1978) and glomerular cells (Oberly et al., 1979). Glial cells also synthesize fibronectin when cultures in vitro (Vaheri et al., 1976) platelets have been found to contain fibronectin (Zucker et al., 1979).

Fibronectin produced by megakaryocytes is packaged into

alpha granules of platelets and is secreted and bound to platelets following their activation with thrombin (Ginsberg, 1980; Hawiger, 1987).

Cell culture contain fibronectin in soluble form in medium while insoluble fibronectin present in cell layers where it distributed in 3 different components :

a. Intracellular.

b. At the cell surface associated with plasma membrane.

c. In extracellular matrix.

(Furchet et al., 1978).

Hedman (1980) reported that intracellular fibronectin is situated in structures concerned with protein synthesis and secretion namely rough endoplasmic reticulum, intracytoplasmic vesicles and Golgi apparatus.

The distribution of fibronectin follows that of the loose connective tissue with particular abundance in association with basement membrane (Linder et al., 1978; Quaroni et al., 1978).

The soluble form of fibronectin is present in : plasma 150 : 800 ug/ml (Mosher and Williams, 1978); amniotic fluid 10 : 300 ug/ml (Chen et al., 1976); cerebrospinal fluid 1-3 ug/ml (Kwisula et al., 1978); urine 100 ug/ml (Ruoslahti et al., 1981); seminal fluid 204 mg/litre (Gressner and Wollraf,