PATHOLOGICAL STUDY OF DIFFERENT TYPES OF LUNG FIBROSIS APPLYING HISTOCHEMICAL TECHNIQUES

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Thesis

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By

TAREK MOHAMMED HASHEM

M.B.,B.Ch., M. Sc.(Pathology)

Supervised by

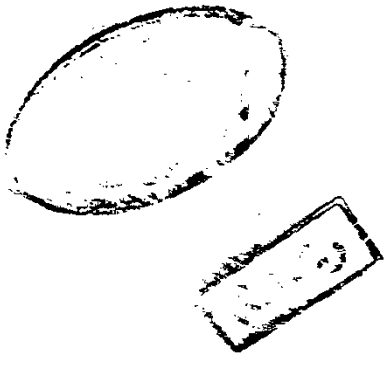
Prof.Dr.Adly Farid Ghaly
Prof. & Head, Dept. of Path.
Faculty of Medicine,
Ain Shams University

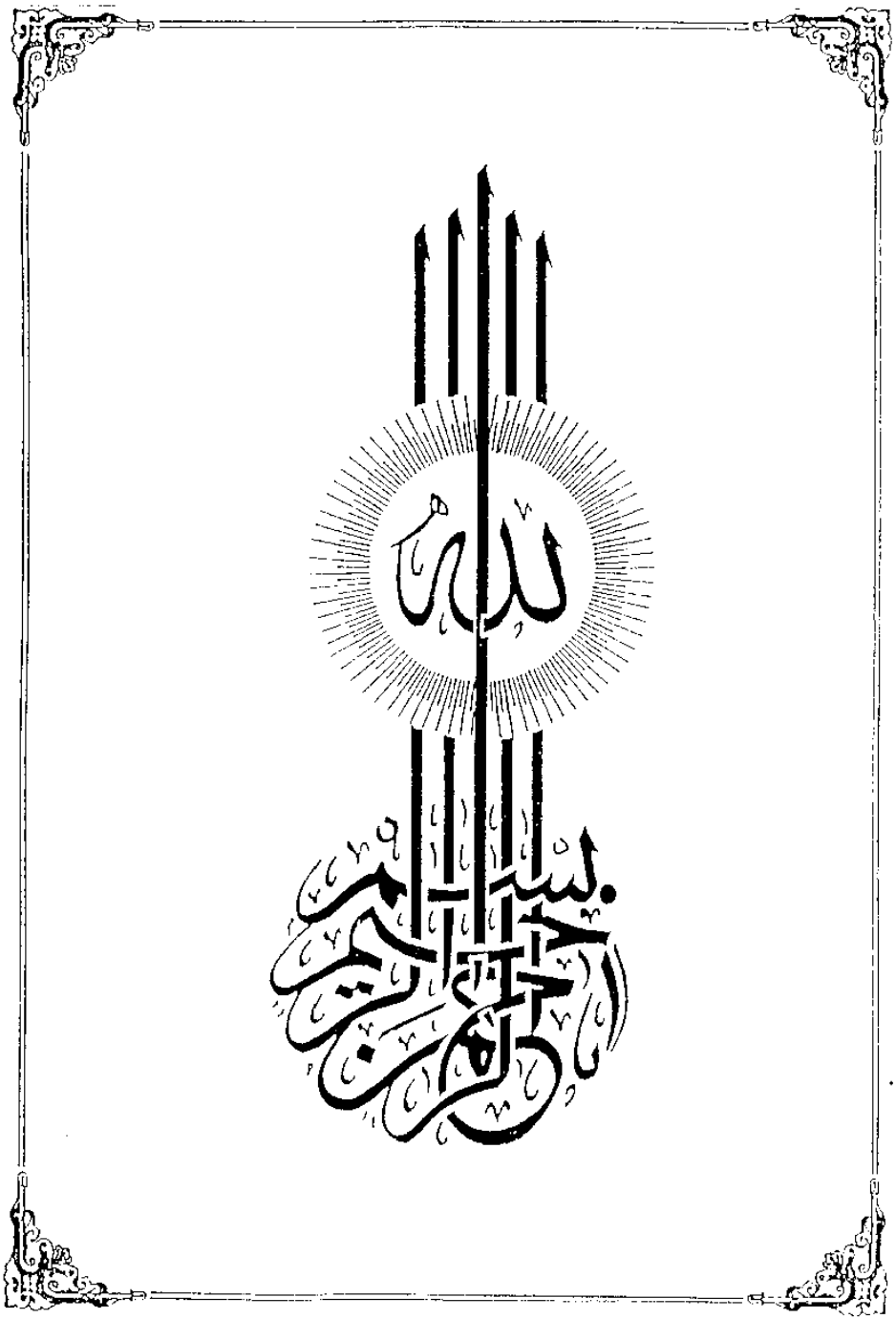
Prof.Dr.Hassan Housny Youssef
Prof. of Internal Medicine and
Chest Diseases
Faculty of Medicine, Ain Shams U.

Prof.Dr.Leila ElShabrawy
Prof. of Pathology
Faculty of Medicine,
Ain Shams University

Dr.Mahmoud Abdel Aleem
Lecturer of Pathology
Faculty of Medicine,
Ain Shams University

Faculty of Medicine
Ain Shams University
1989





CONTENTS

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INTRODUCTION

The major function of the lung is to effect the transfer of oxygen from the atmosphere to blood and carbon dioxide from the blood to the atmosphere. To accomplish this, the lung must constantly replenish the air within the alveoli and bring blood and air into close apposition. Thus, gas exchange requires that the lung have the unique structure that both maintains the organization of its constituent parts and permits the dynamic mechanical properties of respiration. To a large extent, these properties of the lung are defined and regulated by the extracellular connective tissue matrix specially collagen. By virtue of both its mechanical properties and structural organization, collagen plays a critical role in defining lung structure and function in health and disease.

Lung fibrosis is a peculiar and diverse disease entity which may be of idiopathic etiology or an end stage condition complicating other lung disease as inflammatory lesions, vascular or circulatory conditions and industrial dust inhalation. Certain connective tissue diseases, adverse reaction to some drugs or exposure to ionizing radiation may also induce lung fibrosis. The problem of fibrogenesis of lung has

undergone a tremendous involution over the last decade regarding etiology and collagenesis.

AIM OF THE WORK

This study aims at investigating the different types of collagen fibers encountered in the various fibrotic lung diseases by using the available histochemical techniques. Total lung collagen and the relative proportions of the different collagen types as well as their morphology and sites of deposition are investigated in each diagnostic entity. Inflammatory cellular infiltrate and its relation to the fibrotic process are also considered.

REVIEW OF LITERATURE

COLLAGEN

The word collagen is derived from the Greek, 'Kolla' meaning glue and the term 'glue former' or 'collagen' was originally used in the 19th Century for the component in skin and bone, cartilage, and tendon, which when the tissues were boiled in water and the extracts evaporated produced glue. Collagens are now recognized as a class of relatively insoluble fibrous proteins which are found in species of all phylla of multi-cellular organisms and are the most abundant of mammalian proteins (Weiss and Ayad, 1982).

Function of collagen

Collagen is distributed throughout the body and it provides strength and integrity. Therefore, collagens serve as a structural component in organs that bear weight as bone and cartilage, transmit force as in tendons and ligaments, protect and compartmentalize as in dermis and fascia, distribute fluids as in blood vessels and glandular ducts (Piez, 1976).

According to Bornstein and Sage (1980), collagen does not only serve as a static structure, but there is a dynamic turnover during normal morphogenesis in embryonic development. Collagen forms a scaffold on

which structures such as the cornea are built. In addition, collagen affects hemostasis through interaction with platelets and other biologically active factors in various disease processes in the body, such as blood coagulation, repair and healing and inflammation (Weiss and Ayad, 1982).

For collagen to fulfil its different functions, it may assume various arrays or may be associated with other molecules that contribute to produce the desired property. Miller (1976), stated that, in tendons, collagen fibrils are arranged in large parallel fiber bundles that confer great tensile strength. In the skin, the fibers are irregular and loosely woven, permitting extension in all directions within the plane of the tissue. In tubular structures, such as blood vessels, the fibers form circumferential networks that are flexible, strong and resistant to rupture. Again, through the addition of proteoglycans or glycosaminoglycans, cartilage is equipped to imbibe water and to present a resiliant surface for the joint.

Components of collagen

Any discussion of the structural composition of collagen should include its three basic components; namely collagen fibers, ground substance and cells

associated with collagen. The wide variety of connective tissue types in the body represent modulation in the degree of expression of these components (Deyl and Adam, 1981).

I. Collagen Fibers

Primary structure of collagen

Collagen itself is a glycoprotein, somewhat unique in this classification since it contains no amino sugars and in fact has only two types of carbohydrate residues; glucose and galactose. Until fairly recently it was supposed that collagen was a single protein type but in the last decade it has become clear that it is polymorphic. Gay and Miller (1978), identified all mammalian collagen fibers as having the same basic molecular structure composed of 3 polypeptide chains (alpha chains), each of which comprises about 1,000 amino acid residues. The alpha chains are twisted one complete left turn for about every 3 residues. This conformation is peculiar to collagen and was only made possible by the absence of side chains. The 'tightness' of the alpha chain helix (ie. the consistent frequency with which the twisting occurs) would have been impossible if side-chains were present to interfer with

one another, as is the case with most protein chains. Three alpha chains are twisted in a right-handed helix to produce the triple helical collagen molecule. This very stable structure, which resists proteolytic degradation, forms the basic building unit of collagenous structures.

Perhaps because of its primary structural function in the body, collagen is peculiarly resistant to attack by neutral proteinases and other enzymes. Only one mammalian enzyme is known which is capable of cleaving the helical body of the major interstitial collagen molecules and this enzyme, collagenase, cleaves solely at one site along the whole length of the molecule. On the other hand, a bacterial enzyme, bacterial collagenase, is able to degrade native collagen into small fragments, although it has no action at all on other proteins. Furuto and Miller (1981), found that this piece of information provides a useful test in establishing the possibly collagenous nature of an isolated protein since if it is bacterial collagenase sensitive it is almost certainly collagen.

The remarkable strength of certain collagenous structures was investigated by Krause and Cutts (1983), who found that it results, in part, from the manner in

which the molecules are physically arranged. Another major contributing factor is the presence of inter-molecular cross links that add the tensile strength to collagenous structures. Cross links act to weld the building blocks (ie. the molecules). When this cross linking is disturbed either by drugs or in certain diseases, the mechanical properties of tissues are greatly altered.

Chemical composition of collagen fibers

In each polypeptide chain of collagen fibers, every third amino acid is glycine. The polypeptide chain can, thus, be regarded as a polymer of tripeptide units with the formula (X-Y-Glycine)n where (X) is frequently proline and (Y) is frequently hydroxy-proline. In fact, proline and hydroxy-proline constitute 20% to 25% of the total number of amino acids, ie. almost one third of residues in the X and Y positions. Proline and its hydroxylated derivative contribute two basic characteristics to the molecule. First, the imino-acid structure promotes a very rapid turning of the chain as it grows favouring the tightly turning left handed helix. Second, the hydroxyl groups of hydroxyproline form strong hydrogen bonds, which help to hold the three alpha chains together and thus stabilize the resultant

triple helix (Fietzek and Kuhn, 1976).

Light and Bailey (1980), stated that in types I and II collagens, glycine occurs invariably in the first position of the glycine-X-Y triplet. In type III colagen, glycine occurs in position X as well. The increased glycine content has a helix destabilizing effect that may be counterbalanced by an increased content of the helix stabilizing residue hydroxyproline.

Rauterberg et al (1981) and Last and Reiser (1984), noticed that glycine, proline, hydroxyproline, lysine and hydroxylysine are selectively distributed within the collagen chain. A number of other amino acids are also distributed non-randomly between positions X and Y. Phenylalanine and leucine, for instance, occur almost invariably in position X. Glutamic acid is usually found in position X while glutamine, arginine and threonine occur preferentially in position Y. This non-random distribution may be required for molecular stability.

Histology and staining of collagen fibers

Collagen fibers have several characteristic staining properties :

1. They stain pink with eosin.
2. By Van Gieson's stain (a mixture of picric acid and

acid fuchsin), the fibers are stained red.

3. By Mallory's stain (a mixture of aniline blue and phosphotun-gestic acid), the fibers are stained blue.

4. By Masson's stain (a mixture of hematoxylin and light green), the fibers are stained green.

5. By silver impregnation, the fibers are stained brown (Walter and Israel, 1979).

Types of collagen

According to Miller (1983), there are at least 5 types of collagens and these are named from I to V and are classified into 2 groups :

A. Interstitial collagens :

1. Type I collagen.
2. Type II collagen.
3. Type III collagen.

B. Basement membrane collagens :

1. Type IV collagen.
2. Type V collagen.

Minor (1980) and Miller and Gay (1982), described 9 unique and genetically distinct collagen alpha chains. These chains form the 5 different types of collagen. The following table was designed by Miller (1983), to describe the chemistry and biology of these 5 types of