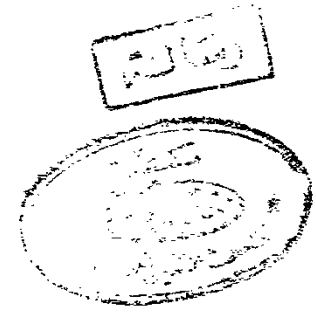
Study of serum Osteocalcin levels In Diabetic Patients

Thesis
Submitted for Partial Fulfilment
of master degree in Clinical Pathology

By
Amany Ahmed Osman
M.B., B. Ch.



Supervisors
Prof. Dr. Laila Mohamed Abou El-Magd
Prof. of Clinical Pathology
Ain Shams University

Dr
Nadia Aly Abd El-Sattar
Lecturer of Clinical Pathology
Ain Shams University

Dr
Ola Hamdy Demerdash
Lecturer of Clinical Pathology
Ain Shams University

Faculty of Medicine
Ain Shams University
1991

بسم الله الرحمن الرحيم

قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم

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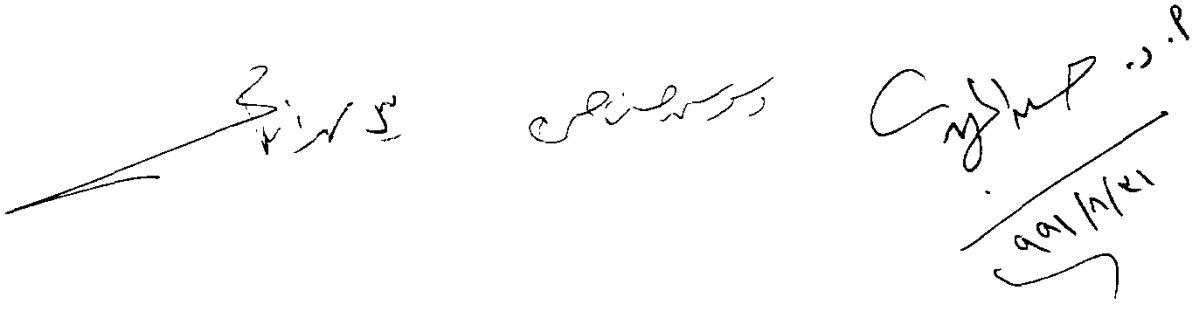

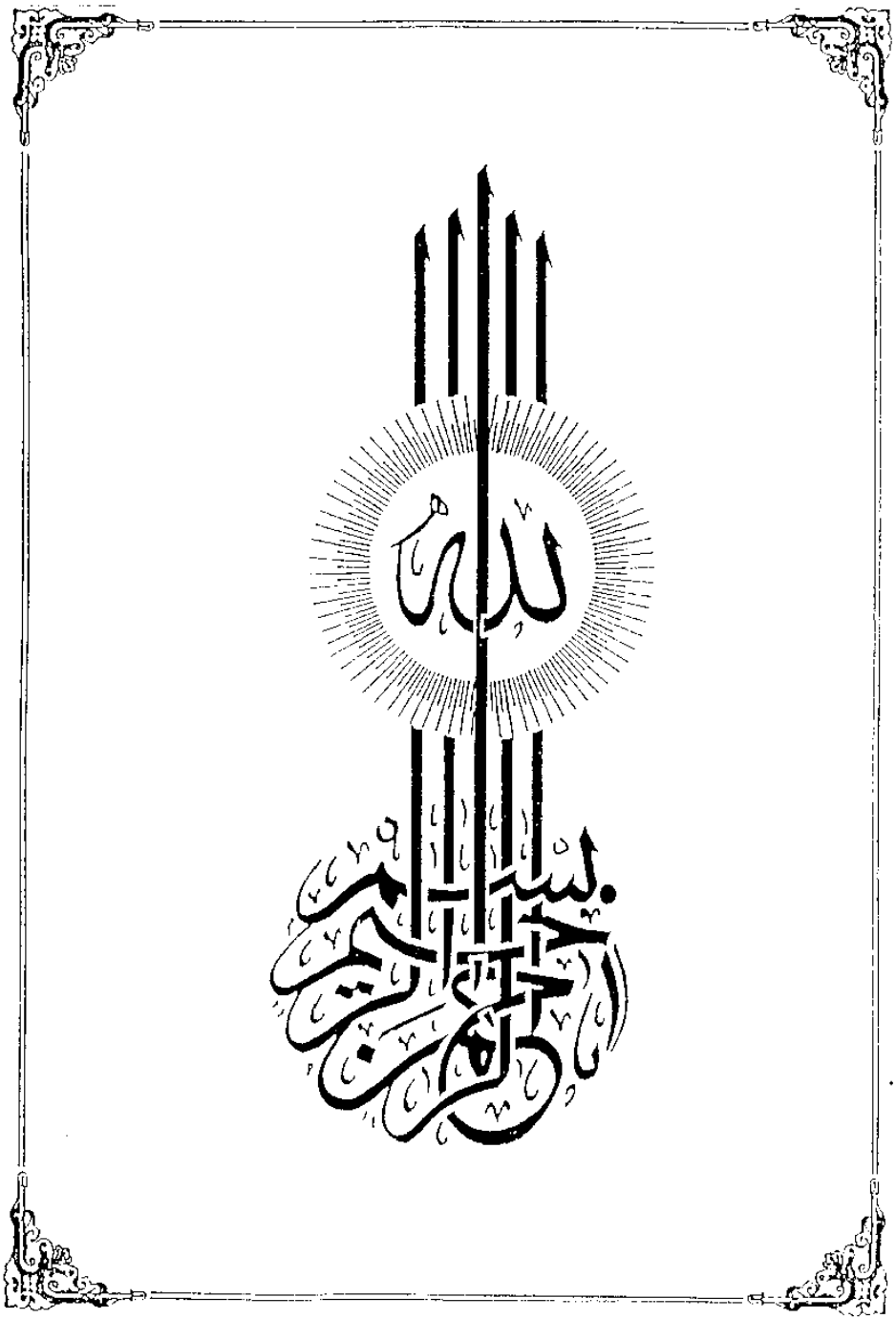

ACKNOWLEDGEMENT

First And Foremost Thanks are to GOD, the Most Beneficial and Merciful

It is a great pleasure to express my sincerest appreciation and deepest gratitude to Professor Dr. Laila Mohammed Abou El-Magd, Professor of Clinical Pathology, Ain Shams University for her precious advice and kindness. She has generously offered me a lot of her time and experience to accomplish this work.

I am also greatly obligated to Dr. Nadia Ali Abd El-Sattar, Lecturer of Clinical Pathology, Ain Shams University for her generous cooperation and fruitful suggestions to put this work in its complete form.

My heartful thanks are to Dr. Ola Hamdy Demerdash, Lecturer of Clinical Pathology, Ain Shams University for her extreme patience, meticulous supervision and moral support. She offered me much of her time and effort through out the whole work.

I am indebted to my parents and my husband for their kindness and fruitful encouragement. It is to them all this work is dedicated.

Another word of gratitude to my colleagues and to Biochemist Ahmed Kamal for his unforgettable help in computer print out of this work.

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Introduction And Aim Of Work

INTRODUCTION :

Osteocalcin is a vitamin K-dependent calcium-binding protein which is synthesized by osteoblasts. It is found primarily in bones, where it is present as the most abundant form of non-collagenous proteins. Osteocalcin contains three residues of the amino acid gamma-carboxyglutamic acid (GLA) which bind strongly to hydroxyapatite in the presence of calcium. Thus followed by its subsequent accumulation in bone matrix *(Hauschka and Carr, 1982)*.

Recent studies suggest that serum osteocalcin is a highly specific and sensitive marker of bone turnover. Moreover, measurements of its serum level makes it possible to evaluate osteoblastic bone formation without biopsy. Hence, it is rapidly becoming a clinically important diagnostic parameter of bone pathology *(Delmas et al. ,1986)*.

Diabetes mellitus is primarily a disturbance of carbohydrate metabolism characterized by hyperglycemia with or without glycosuria, with secondary disturbance of protein and fat metabolism *(Seth, 1981)*.

A series of recent reports suggest the existence of altered bone metabolism in diabetes mellitus in the form of diabetic osteopenia *(Ishida et al., 1988)*. The underlying mechanism is still unclear and may involve defective osteoblastic activity *(Rico et al., 1989)*.

AIM OF WORK :

The aim of the present work is to study serum osteocalcin levels in type II diabetic patients (non-insulin dependent) in order to investigate the possibility of occurrence of diabetic osteopenia (Bone mass reduction) and to clarify its underlying mechanism. Also we will correlate between serum osteocalcin level and the duration of the disease.

2

Review Of Literature

I. BONE STRUCTURE

Bone is a specialized connective tissue with a mineralized collagenous framework for skeletal support of the body. It is either spongy (cancellous) or compact in structure.

A] Components of Bone:

1) *Bone Cells:*

a. Osteoblasts:

They are fusiform cells relatively large, measuring about 15 to 20 μ in diameter, each contains a large nucleus and one fairly large nucleolus. It is characterized by abundant cytoplasm which is deeply basophilic. Ultrastructures of osteoblasts show a cytoplasm with a characteristic well developed rough endoplasmic reticulum (RER), Golgi apparatus and secretory vesicles *(Tureк, 1984)*.

Osteoblasts are associated with bone formation and are found on the margin of growing bones where osseous matrix is being deposited. They secrete bone collagen and the uncalcified matrix around osteoblasts is termed osteoid. Osteoblast secrete alkaline phosphatase as well as non-collagenous components concerned with calcification and mineral homeostasis *(Saunders, 1988)*.

b. Osteocytes:

When osteoblasts synthesize and secrete the organic intercellular substance of bone with which they surround them selves, they lie inside lacunae in the intercellular substance and become osteocytes. The osteocyte has a faintly basophilic cytoplasm, a large oval nucleus with large chromatin granules, and one or more nucleoli. Ultrastructures show less rough endoplasmic reticulum and less cytoplasm *(Turek , 1984)*.

c. Osteoclasts:

They are multinucleated giant cell varying in size and number of nuclei. The cytoplasm is pale acidophilic and foamy. The nuclei are poor in chromatin with a prominent nucleoli. It is supposed that osteoclast are formed by fusion of several osteoblasts or from stromal cells of the marrow. Its function is concerned with bone resorption and they are always associated with mineralized bone and not with osteoid tissue. They contain several enzymes including β-glucuronidase. Ultrastructures show rough endoplasmic reticulum (RER) that may or may not be developed, well developed Golgi apparatus, abundant mitochondria and various vesicles that contain lysosomes and hydrolytic enzymes *(Turek , 1984)*.

2) Intercellular Substance:

It consists mainly of matrix, inorganic salts and water.

a. Matrix:

It is the organic framework consisting of a unique type of collagen (type I collagen) approximately 90% and that provide strength and structural integrity of bone. There are other components of different nature termed non-collagenous proteins *(Triffitt, 1987)*.

The non-collagenous proteins such as sialoproteins, phosphoproteins, acid-containing proteins such as osteocalcin and a small sulfated polysaccharides provide the highly acidic nature of the amorphous ground substance. The high acidity is associated with high calcium-binding properties and aggregation tendencies and may influence the mineralization process *(Saunders, 1988)*.

b. Inorganic Salts:

It is responsible for hardening and rigidity of bone. It include calcium phosphate (about 85 percent), calcium carbonate (10%) and small amount of calcium and magnesium fluoride. The bone mineral present in the form of submicroscopic crystals of hydroxyapatite $Ca_{10}(Po_4)_6(OH)_2$. It presents between the collagen fibers. It forms about 65 to 75 percent of adult dry bone *(Saunders, 1988)*.

c. Water:

It occupies spaces inside the bone.

3) Periosteum and Endosteum

They are layers of connective tissues on the external and internal bone surfaces, respectively. Their cells resemble fibroblasts. They are responsible for nutrition of osseous tissue through blood vessels *(Saunders, 1988)*.

B] Bone Calcification:

The initial stage in bone production is the secretion of (type I collagen) and ground substance by osteoblasts. The collagen polymerizes rapidly to form collagen fibers and form osteoid tissue. As the osteoid is formed, some of the osteoblasts become entrapped within lacunae and then are called osteocytes. Within a few days after the osteoid is formed, calcium salts are deposited inside the holes of the collagen fibers and later on fill the rest of the space *(Glimcher, 1981)*. Calcium precipitated in the form of hydroxyapatite crystals. Many factors help calcium precipitation in bone such as concentration of calcium and phosphate ions in extracellular fluid and the high acidity of bone ground substance that increase calcium binding properties. The latter is mediated by the non-collagenous bone proteins *(Boskey and Posner, 1984)*. The non-collagenous bone proteins regulate bone mineralization and have a role in skeletal homeostasis *(Romberg et al., 1986)*.

C] Bone Remodelling:

Bone is not a static tissue. Through out life bone is constantly being resorbed and reformed, thereby developing and preserving the structure and size of the bone as well as providing a mechanism for maintaining calcium ionic homeostasis in body fluid *(Lacroix, 1972)*.

Normally, except in growing bones, the rates of bone formation and resorption are equal to each other so that the total bone mass remains constant. Bone resorption is mediated by osteoclasts while bone formation is mediated by osteoblasts. During bone formation, bone is deposited in successive layers for several months till completed.The new bone formation ceases due to encroachment on blood vessels. On the other hand, bone resorption is increased by parathyroid hormone and 1,25-dihydroxycholecalciferol which also promotes bone calcification. Calcitonin inhibits bone resorption *(Turek, 1984)*.

II. NON-COLLAGENOUS BONE PROTEINS

INTRODUCTION:

There are many types of proteins of the bone tissue. They are of diverse characters. Recently a great deal of effort has been applied for the characterization of such proteins *(Triffitt, 1980)*. The main constituent of the organic matrix of bone tissue is collagen specially (type I collagen). The defective synthesis of this collagen has a dramatic effect on the skeleton *(Smith, 1986)*.

The lack of apparent tissue specificity of collagen has focused attention on the minor proteinaceous constituents of the bone tissue. These are known as non-collagenous proteins. These more specific proteins which are located mainly, if not solely, in bone tissue are synthesized by osteoblasts. The non-collagenous proteins constitute about 1% of total protein *(Triffitt, 1987)*.

Many of non-collagenous bone proteins have been identified and characterized. However, the most important ones are: Bone Gla protein (Gamma-carboxyglutamic acid protein) or osteocalcin, bone sialoproteins (BSP), matrix Gla-protein (MGP), proteoglycans, phosphoproteins, osteonectin and plasma proteins.