A STUDY OF COLLAGEN TYPE I CROSS-LINK ASSOCIATED C-TELOPEPTIDE: A NEW MARKER OF BONE RESORPTION IN METASTATIC BONE DISEASE

THESIS
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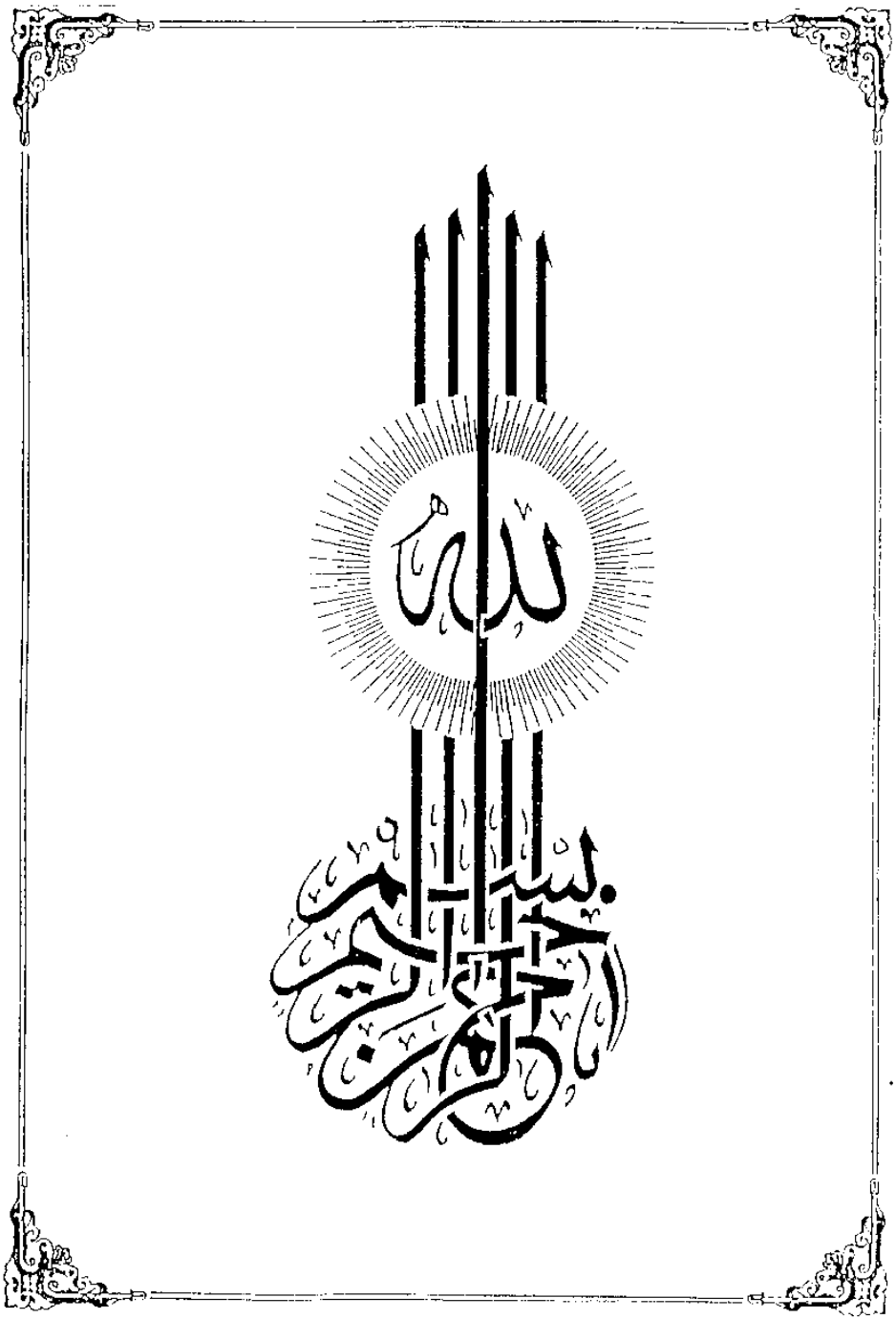
ABSTRACT

Type I collagen is synthesized by osteoblasts and accounts for about 90% of the organic matrix of bone. We have used a new specific immunoassay for the cross-linked carboxy-terminal telopeptide of type I collagen (ICTP) which allows assessment of degradation of type I collagen. Forty six patients having cancer lung, breast and prostate were investigated. Twenty two of them had metastases to bone, eleven had metastases to distant organs other than bone and thirteen patients had localized cancer. The whole group of patients with distant metastasis showed a highly significant increase in their total calcium, ALP and ICTP levels. However, the degree of elevation of ALP and ICTP was much higher in patients with bone metastases ($p<0.01$ and $p<0.001$ respectively) as compared to those with non-bone metastasis. Concerning patients with localized malignant disease, these showed a statistically insignificant difference in their calcium, phosphorous and ALP levels as compared to the control group ($p>0.05$ respectively). However, a highly significant increase was recorded in their ICTP levels ($p<0.001$). We concluded that ICTP is an excellent non-invasive biochemical marker of bone resorption. It is of great value in identifying patients with bone metabolism. At a cut off level of 7 µg/L, its diagnostic sensitivity was 100%, specificity 95.8% and diagnostic accuracy 95% as evidenced by ROC curve analysis.



DEDICATION

♥ TO MY FAMILY

&

MY HUSBAND ♥

Layla

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BONE RESORPTION

LIST OF ABBREVIATIONS

α1(I):	Alpha 1 of type I collagen
α2(I):	Alpha 2 of type I collagen
ALP:	Alkaline phosphatase
AUP:	Aminomethyl propanol buffer
BGP:	Bone Gla protein
BSA:	Bovine serum albumin
C1q:	Complement 1q
Ca_T:	Total serum calcium
EIA:	Enzyme immunoassay
ELISA:	Enzyme linked immunosorbent assay
FN:	False negative
FP:	False positive
HPLC:	High performance liquid chromatography
ICTP:	Type I collagen carboxy terminal telopeptide
IRMA:	Immunoradiometric assay
NSB:	Non specific binding
NTx:	Amino-terminal telopeptide
PBS:	Phosphate buffered saline
PEG:	Polyethylene glycol
Pi:	Inorganic phosphorus
PICP:	Procollagen carboxy-terminal propeptide of type I collagen
PINP:	Procollagen amino-terminal propeptide of type I collagen
PTH:	Parathyroid hormone
RIA:	Radioimmunoassay
ROC:	Receiver Operating Characteristic
TN:	True negative
TP:	True positive

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INTRODUCTION
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AIM OF THE WORK

Introduction:

Bone is constantly being remodeled with resorption of old bone by osteoclasts and the formation of new bone by osteoblasts. The rate of these processes can be assessed by measuring bone matrix components of enzymes released into the circulation during breakdown and renewal. Biochemical markers of bone formation which are alkaline phosphatase, osteocalcin, and the carboxy-terminal propeptide of type I procollagen can be measured in blood samples, but at least until recently, urine samples have been needed for valid measurement of the biochemical markers of bone resorption which are hydroxyproline and pyridinoline cross-links *(Delmas, 1991)*.

The assay of pyridinoline cross-links is not specific for type I collagen. Moreover, the assay requires a relatively tedious high performance liquid chromatography analysis of urine samples. Clearly, there is a need for a simple, quantitative test of type I collagen degradation based on analysis of serum samples. The carboxy-terminal pyridinoline cross-linked telopeptide of type I collagen (ICTP) in serum has however, been proposed as a possible new serum marker of bone resorption *(Risteli et al., 1993)*.

Aim of the Work:

The aim of the present work is to give a detailed account on the various biochemical markers of bone turnover with special reference to alkaline phosphatase and collagen cross-links associated C-telopeptide in an attempt to investigate their diagnostic significance for the presence of bone metastasis in cancer patients as compared to bone scans.

REVIEW OF LITERATURE

I. BONE STRUCTURE

Bone are specialized connective tissue composed of intracellular calcified material, the bone matrix, and different cell types namely the osteoprogenitor cells, osteoblasts, osteocytes, osteoclasts and bone lining cells. All bones are lined on both internal and external surfaces by layers of tissue containing osteogenic cells, endosteum on the inner surface and periosteum on the outer surface ***(Holtrop, 1975)***.

Microscopically, bones are composed of cellular and non-cellular elements:

A. Cellular Elements of Bone:

1. Osteoprogenitor Cells:

The osteoprogenitor cells are undifferentiated stromal cells having the capacity to differentiate by mitotic division, thus, developing osteoblasts, or bone forming cells ***(Owen, 1970)***.

2. Osteoblasts:

Osteoblasts are mononuclear cuboidal bone matrix synthesizing cells with basophilic cytoplasm and high alkaline phosphatase activity. They are derived from stromal fibroblast-like cell precursors ***(Warshwsky, 1982)***. Osteoblasts are associated with bone formation and are formed on the surface of growing bones where they produce mineralized bone matrix. They are also responsible for the synthesis of the organic components of bone matrix such as collagen and mucopolysaccharide ***(Marie, 1982)***. Alkaline phosphatase, a product of osteoblast, is believed to be involved in the synthesis of procollagen which may be important in the process of mineralization ***(Boskey, 1981)***.

3. Osteocytes:

Osteocytes arise from the osteoblasts. Initially, osteoblasts are present on the surface of the bone, then they become entrapped within the