TARTRATE-RESISTANT ACID PHOSPATASE 5b, CARBOXY TERMINAL TELOPEPTIDE OF TYPE I COLLAGEN AND CA15.3 AS PROGNOSTIC FACTORS FOR SUBSEQUENT METASTASES FROM PRIMARY BREAST CANCER

Thesis

Submitted to the Medical Research Institute

Alexandria University

For partial fulfilment of the

Master degree

In

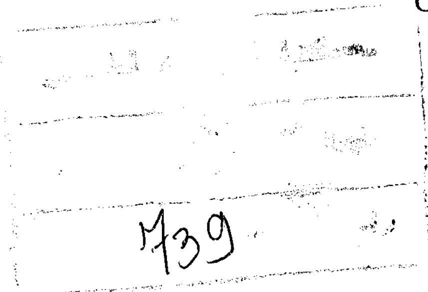
RADIATION CHEMISTRY

By

Marwa Sameh Ibraheim Abo-El-Eneen

B.Sc., Chemistry/Biochemistry (2000)
University of Alexandria

5741.15



March 2006

TARTRATE-RESISTANT ACID PHOSPATASE 5b, CARBOXY TERMINAL TELOPEPTIDE OF TYPE I COLLAGEN AND CA15.3 AS PROGNOSTIC FACTORS FOR SUBSEQUENT METASTASES FROM PRIMARY BREAST CANCER

Presented by

Marwa Sameh Ibraheim Abo-El-Eneen

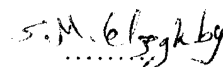
For the Degree of Master

In

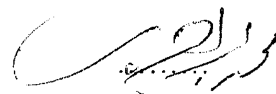
Radiation Chemistry

Examiners Committee: **Approved**

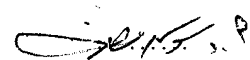
Prof. Dr. Safinaz Mahmoud El-Zoughby
Professor of Applied Medical Chemistry
Medical Research Institute - Alexandria University
(Supervisor and Head of the committee)



Ass.Prof. Dr. Mohamed Ibrahim Morsi
Assisstant Professor in Radiation Sciences Department
Medical Research Institute - Alexandria University
(Supervisor and Member)



Prof. Dr. Sawsan Moustafa Mousa
Professor in Radiation Sciences Department
Medical Research Institute - Alexandria University
(Member)



Prof. Dr. Salah El-Din Abd-El-Moneim Ibrahem
Professor of Clinical Oncology
Faculty Of Medicine - Alexandria University
(Member)

Date 4/3/2006

TARTRATE-RESISTANT ACID PHOSPATASE 5b, CARBOXY TERMINAL TELOPEPTIDE OF TYPE I COLLAGEN AND CA15.3 AS PROGNOSTIC FACTORS FOR SUBSEQUENT METASTASES FROM PRIMARY BREAST CANCER

Presented by

Marwa Sameh Ibraheim Abo-El-Eneen

For the Degree of Master

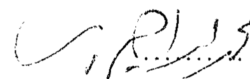
In

Radiation Chemistry

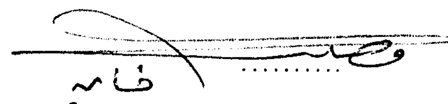
Examiners Committee: **Approved**

Prof. Dr. Safinaz Mahmoud El-Zoughby
Professor of Applied Medical Chemistry
Medical Research Institute - Alexandria University

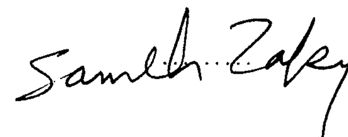
Ass. Prof. Dr. Mohamed Ibrahim Morsi
Assisstant Professor in Radiation Sciences Department
Medical Research Institute - Alexandria University



Ass. Prof. Dr. Wagdy Ibrahim Fayed
Assisstant Professor in Surgery Departement
Medical Research Institute - Alexandria University



Ass.Prof.Dr. Sameh Ibrahem Zaki
Assisstant Professor in Clinical Oncology and Nuclear medicine
Medical Research Institute - Alexandria University



Date 4/3/2006

Acknowledgement

Before all, thank GOD,
who gave me the strength and patience to fulfill this work.

I would like to express my gratitude to Prof.Dr. Safinaz El-Zoghby, professor of Applied Medical Chemistry, Medical Research Institute, Alexandria University, for her precious advise, constructive guidance and valuable support during the course of the work.

I am deeply thankful and indebted to Prof. Dr. Mohamad I.Morsi, Assistant Professor in Radiation Sciences Department, Medical Research Institute, Alexandria University, for his kind supervision, continuous guidance and support, constant encouragement and generous unlimited help from the beginning to the very end of this work.

I would like to express my gratitude to Dr. Wagdy Fayed , Assistant Professor in Surgery Department, Medical Research Institute,, Alexandria University, for his support and effort in carrying out the clinical part of the study.

I cannot express my gratitude to Dr. Sameh Zaky, Assistant Professor in clinical oncology unit, Medical Research Institute, Alexandria University, for his precious cooperation, willing assistance and support, which made this work possible.

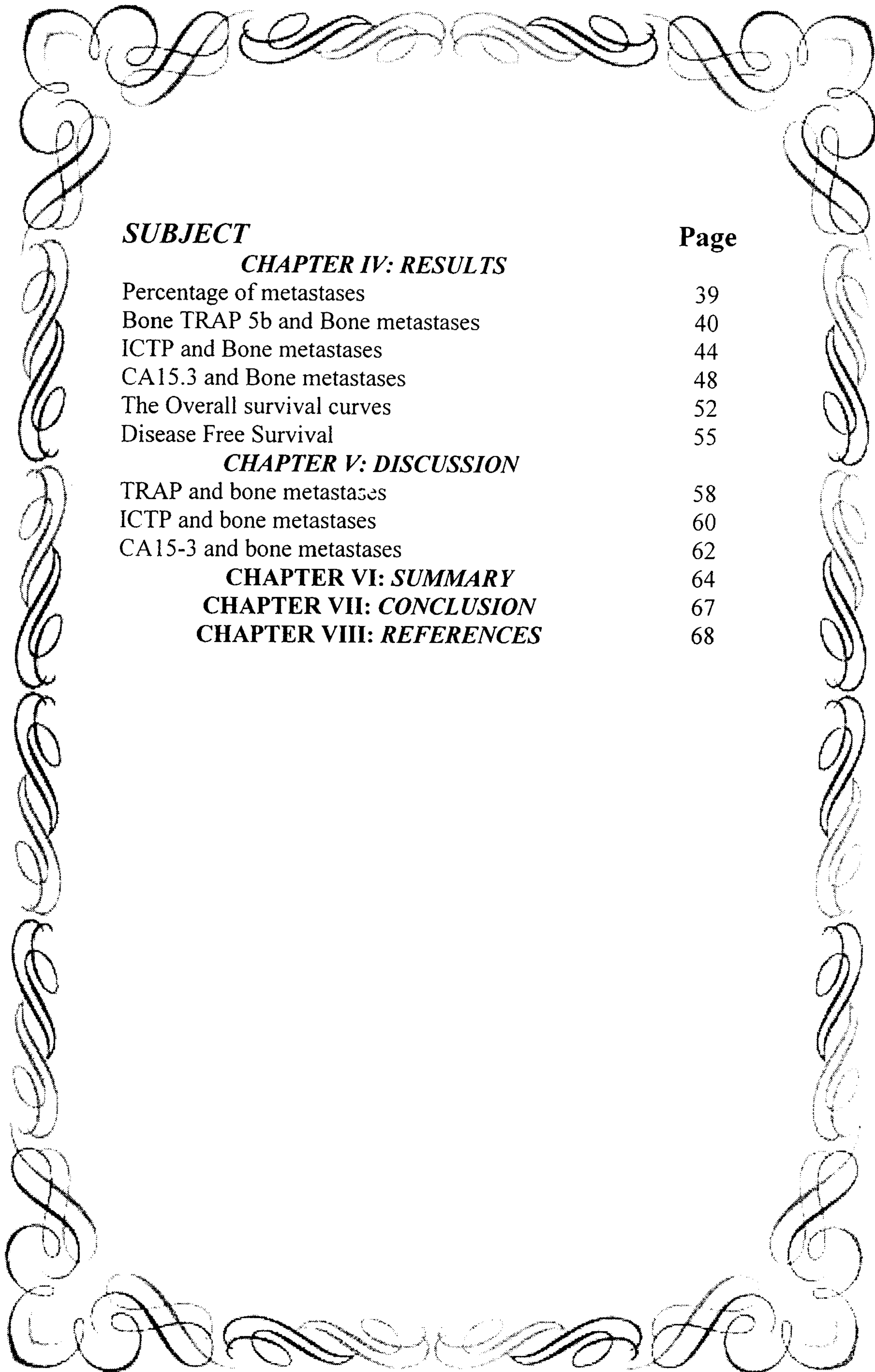
I would like to thank Dr. Mervat Moustafa, assistant professor in Applied Medical Chemistry, Medical Research Institute, Alexandria University, for her sincere help and guidance during the study.

I would like to thank all staff and members of the Radiation Sciences Department, for their unlimited help and support through this work.

Finally, I would like to express my deepest gratitude to my husband
for his precious advice, constant encouragement,
generous unlimited help and support through the course of this work.

CONTENTS

SUBJECT	Page
ACKNOWLEDGEMENT	I
LIST OF CONTENTS	II
LIST OF TABLES	III
LIST OF FIGURES	IV
CHAPTER I : INTREODUCTION	
Metastases	1
The bone tissue	3
Methods used to evaluate bone metastases	9
ACID PHOSPHATASES	13
Tartrate Resistant Acid Phosphatase (TRAP)	13
Isoforms of TRAP	14
Biological function of TRAP 5b	16
How does TRAP 5b contribute to the bone resorption process?	17
Clearance of TRAP 5b	18
COLLAGENS	20
The structure of collagen	20
Type I collagen	23
Carboxy Terminal Telopeptide of Type I Collagen (ICTP)	24
The clinical importance of ICTP	26
CA15-3	27
The biochemical properties of MUC1	27
The biochemical properties of CA15-3	27
CA15-3 concentrations in blood	28
The clinical utility of CA15-3	28
CHAPTER II: AIM OF WORK	29
CHAPTER III: PATIENTS AND METHODS	
Patients	30
Methods	30
I -Bone TRAP 5b Assay	30
II- ICTP Assay	33
III - CA15-3 Assay	35
Statistical method	38



SUBJECT	**Page**
CHAPTER IV: RESULTS	
Percentage of metastases	39
Bone TRAP 5b and Bone metastases	40
ICTP and Bone metastases	44
CA15.3 and Bone metastases	48
The Overall survival curves	52
Disease Free Survival	55
CHAPTER V: DISCUSSION	
TRAP and bone metastases	58
ICTP and bone metastases	60
CA15-3 and bone metastases	62
CHAPTER VI: ***SUMMARY***	64
CHAPTER VII: ***CONCLUSION***	67
CHAPTER VIII: ***REFERENCES***	68

LIST OF TABLES

NO.	Title	Page
1-	Number and percentage of different metastatic sites developed during follow-up in primary breast cancer patients	39
2-	Serum level of Bone TRAP 5b (U/L) in Control, Non-metastatic, Local Recurrence, Lung, Bone metastases sub-groups before surgery, after 10 and 20 months.	41
3-	The percent of elevation in serum Bone TRAP 5b in different sub-groups before surgery, after 10 months and after 20 months.	41
4-	Serum level of ICTP (μg/L) in Control, Non-metastatic, Local Recurrence, Lung, Bone metastases sub-groups before surgery, after 10 and 20 months.	45
5-	The percent of elevation in serum ICTP in different sub-groups before surgery, after 10 months and after 20 months	45
6-	Serum level of CA15-3(U/mL) in Control, Non-metastatic, Local Recurrence, Lung, Bone metastases sub-groups before surgery, after 10 and 20 months.	49
7-	The percent of elevation in serum CA15-3 in different sub-groups before surgery, after 10 months and after 20 months.	49

LIST OF FIGURES

NO.	Title	Page
1-	General mechanisms of tumor cell metastasis to bone. Multiple steps involved in tumor cell metastasis from a primary site to the skeleton	2
2-	Constituents of bone tissue	3
3-	Structure of osteoblast	4
4-	Structure of resorbing osteoclast	4
5-	An osteoclast shown in cross section	4
6-	Development of osteoclasts from hematopoietic stem cells	5
7-	Schematic representation of the bone remodeling cycle	7
8 -	Schematic representation of biochemical markers of bone remodeling	11
9-	The structure of a typical collagen molecule.	21
10-	Structure of collagen	22
11-	Cross-links formed between modified lysine side chains within a collagen fibril.	23
12-	Collagen molecules assemble in a regular staggered array to form fibrils.	24
13-	Schematic presentation of type I collagen	25
14-	Serum Bone TRAP 5b activity U/L in Control, Non-metastatic, Local Recurrence, Lung, Bone metastases sub-groups before surgery, after 10 and 20 months.	42
15-	The percent of elevation in serum Bone TRAP 5b in different sub-groups before surgery, after 10 and 20 months.	43
16-	Serum ICTP concentration μg/L in Control, Non-metastatic, Local Recurrence, Lung, Bone metastases sub-groups before surgery, after 10 and 20 months.	46
17-	The percent of elevation in serum ICTP in different sub-groups before surgery, after 10 and 20 months.	47

NO.	Title	Page
18-	Serum CA15-3 concentration U/ml in Control, Non-metastatic, Local Recurrence, Lung, Bone metastases sub-groups before surgery, after 10 and 20 months	50
19-	The percent of elevation in serum CA15-3 concentration in different sub-groups before surgery, after 10 and 20 months	51
20-	Overall survival curve according to serum bone TRAP 5b activity in patients with primary breast cancer after 10 months.	53
21-	Overall survival curve according to serum bone TRAP 5b activity in patients with primary breast cancer after 20 months.	53
22-	Overall survival curve according to preoperative serum ICTP concentration in patients with primary breast cancer.	54
23-	Disease free survival curve according to serum bone TRAP 5b activity in patients with primary breast cancer after 10 months.	56
24-	Disease free survival curve according to serum ICTP concentration in patients with primary breast cancer before surgery.	56

Introduction

INTRODUCTION

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer in women. Worldwide, it is a major public health problem where, it has now been estimated that 1 in 8 women will develop the disease in her lifetime. It accounts for approximately one-third of all cancer diagnosed and nearly about 18% of all cancer deaths in American women.[1]

Metastatses:

Once a diagnosis of cancer is established, the urgent question is whether it is localized or has already spread to regional lymph nodes and visceral organs.

Patients with cancer usually die, not as a result of their primary malignancy but due to the relentless growth of metastases that are resistant to conventional therapy. Surgical excision of primary neoplasm is not curative in many patients because, by time, metastases may well have occurred. Metastases can be located in different organs and in different regions of the same organ. [2]

It was suggested that the tumor invasion (metastases) might be similar to trophoblast invasion in pregnancy, which is also dependent on angiogenesis and adhesive cell interactions. So that, it was emphasized that the trophoblast may be an appropriate model for studying the mechanism of tumor metastasis. [3, 4,5]

Types of metastases:

1-Lung metastases:

Approximately 3% of all women with breast cancer develop a solitary pulmonary lesion detectable by chest X-ray. [6, 7, 8]

2-Liver metastases:

More than half of all patients with metastatic breast cancer will have liver involvement at some point. [8, 9] Typically, this is a late finding, when metastases are already present at other sites. Of all patients with metastatic breast cancer, approximately 5% will have metastasis confined to the liver with no evidence of extra hepatic disease.[8,10,11] Even with systemic chemotherapy, the median survival time for patients with metastatic disease to the liver only or with limited disease elsewhere is only 19 months .[8,12]

3-Brain metastases:

Brain metastases are diagnosed in approximately 10% of breast cancer patients.[8,13] Without treatment, the median survival for these patients is a about 1-2 months.[8,14] With whole brain radiation therapy , this increase to 3-6 months.[8,15]

4- Bone metastases:

Bone is one of the earliest and most common sites of breast cancer metastasis. [8,16] One-third of cancer patients will have metastases of their primary tumor to bone.[17,18] The metastatic spread of cancer to bone is common to many different malignancies, particularly breast (73%). Patients with breast cancer, for example, have a four- to five fold higher rate of vertebral fracture than age-matched women. [17]

A metastasis to the bone fig.(1) is a consequence of a cascade of events including:

1) Progressive growth at the primary site.
2) Tumor neo-vascularization.
3) Detachment of tumor cells from the primary tumour.
4) Invasion in the neighbouring tissues.
5) Intravasation into the blood stream.
6) Survival in the circulation.
7) Homing and arrest at the level of the bone marrow.
8) Extravasations.
9) Evasion of the host defence.
10) Growth and stimulation of the osteoclast mediated bone resorption.. [19.20]

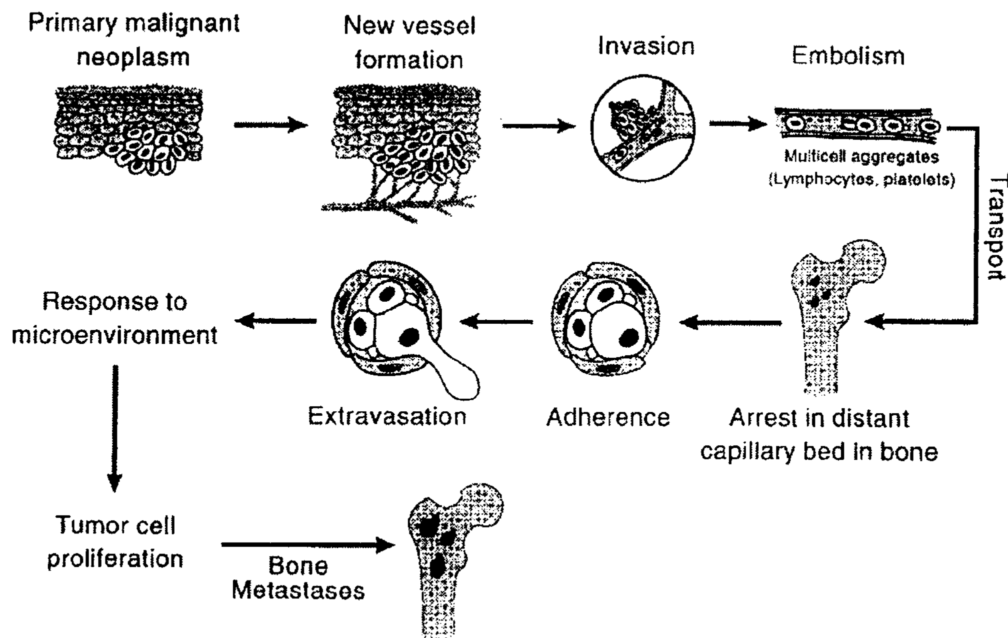


Fig (1): General mechanisms of tumor cell metastasis to bone. Multiple steps involved in tumor cell metastasis from a primary site to the skeleton. [18]

The bone tissue:

1- physiology:

Constituents of bone tissue are shown in FIG.(2).[21.22]

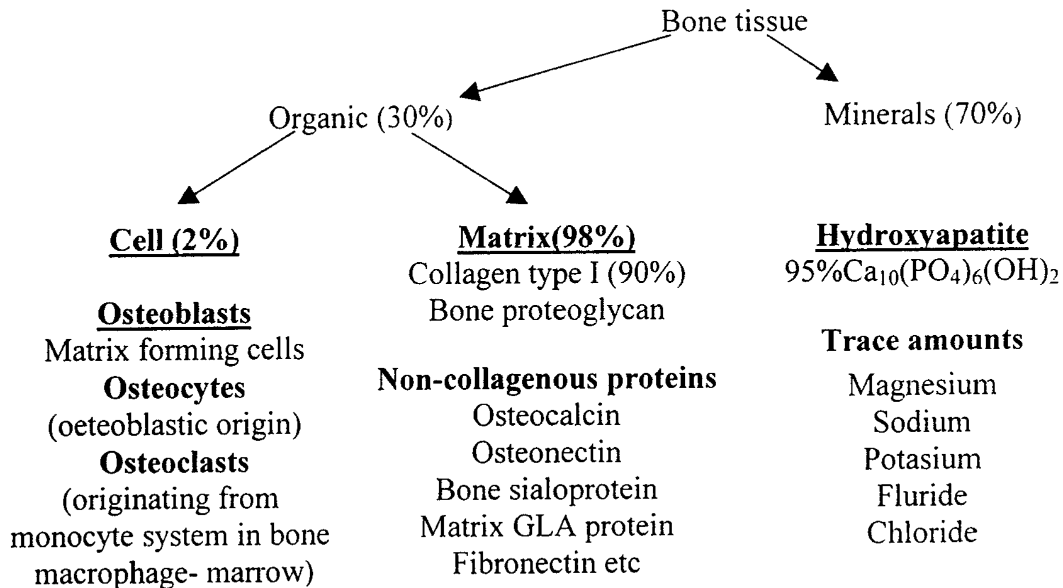


Fig. (2): Constituents of bone tissue.[21]

2-structure:

Bone consists of two physically and biologically distinctive Structures: cortical (compact) and trabecular (spongious). The outer cortical bone is hard-mineralized matrix in which cellular and metabolic activities are relatively low. Cortical bone makes up 85% of the total bone in the body and is most abundant mainly in the diaphysis of long bones and on the surfaces of flat bones of the appendicular skeleton.

Cancellous or trabecular bone constitutes the remaining 15% of the skeleton and is most abundant in the vertebral bodies. Trabecular bone is found in the distal ends of long bones and in the inner surfaces of flat bones. Cortical bone functions as a mechanical support, while trabecular bone is involved in metabolic processes.[5, 21]

3-Cellular part:

The cellular part of bone is composed of osteoblasts, osteocytes, and osteoclasts.

A-osteoblasts:

Osteoblasts are pleuripotent stromal cells originating from bone marrow. They are responsible for production and subsequent mineralisation of bone matrix. Osteoblasts Fig. (3) are derived from the stromal cell lineage synthesise type I collagen, growth factors. and non-collagenous proteins. In other words, these cells are responsible for bone formation. [23]

Fig (3): Structure of osteoblast. [23]

B-osteoclasts:

Osteoclasts Fig (4,5) are derived from precursors in the mononuclear- phagocyte lineage and are responsible for bone resorption. [24] Osteoclast differentiation fig.(4) has various characteristic features, such as multinucleation induced by the cell fusion of mononuclear osteoclasts to cover a larger area, synthesis of the vacuolar proton pump and acid to dissolve the bone mineral, formation of ruffled borders to secret protons and acid, and formation of a sealing zone to prevent proton and acid leakage.

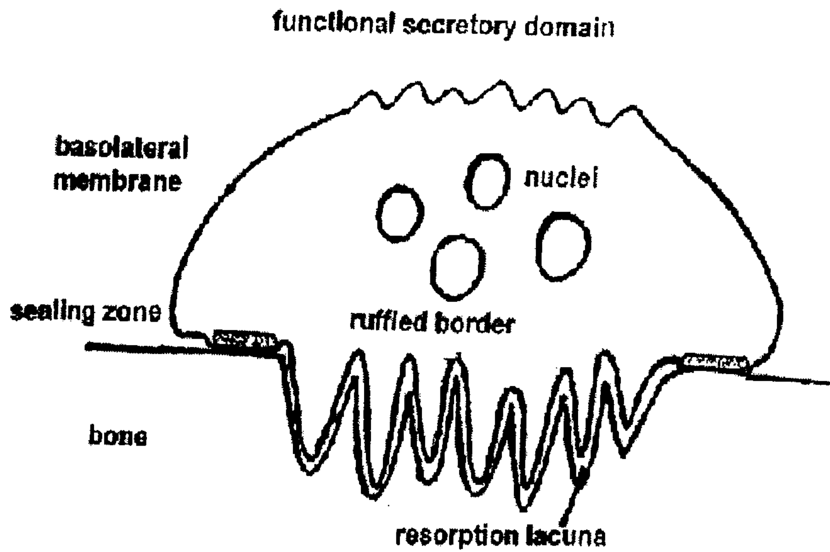


Fig. (4): Structure of resorbing osteoclast. [24]

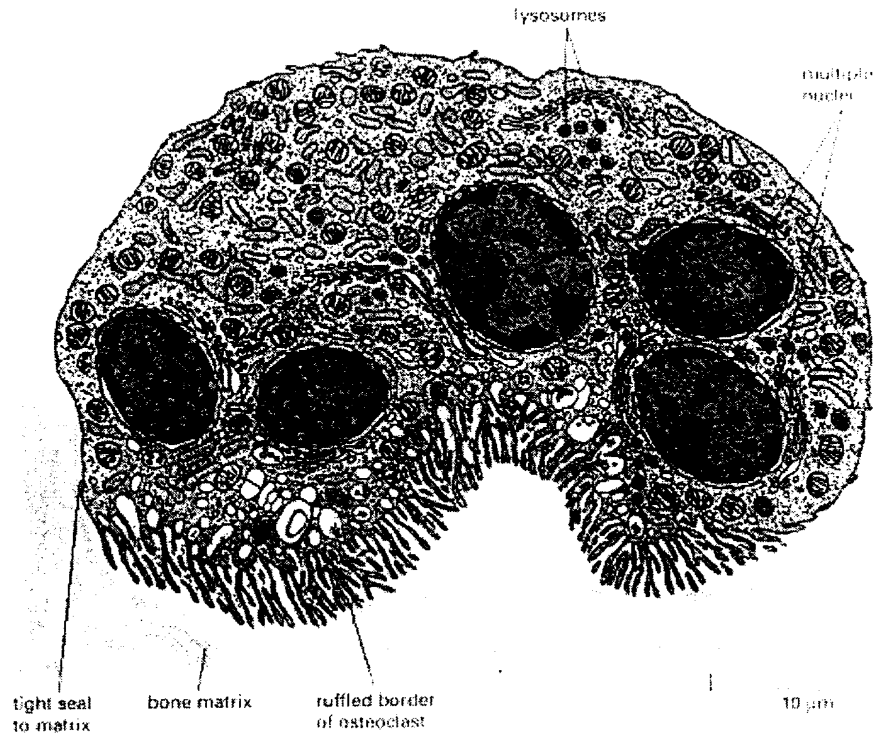


Fig (5): An osteoclast shown in cross section. [23]

Mononuclear osteoclasts tightly adhere to bone, and fuse with each other to form multinuclear osteoclasts. The fused cells are re-organized, polarized and construct osteoclast-specific structures. Transcytosis develops from the basal to the apical side of osteoclasts to enable the discharge of dissolved bone debris. [25, 26]

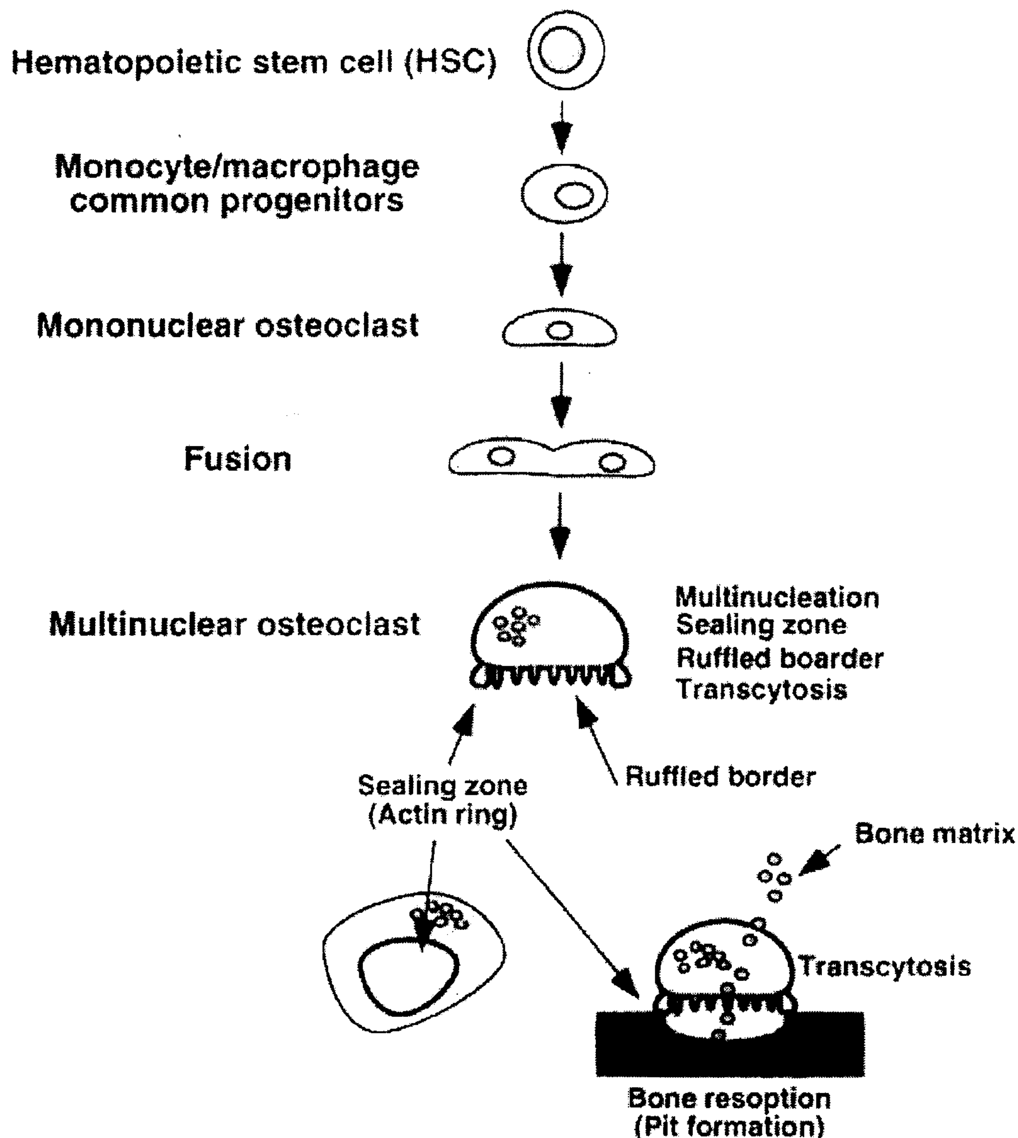


fig. (6) Development of osteoclasts from hematopoietic stem cells (HSCs). [25]

C-Osteocytes:

Osteocytes are flat small cells found in bone matrix, which are thought to play a part in the oestrogenic response to mechanical stimuli. [23]

Since bone formation and resorption are continuing processes, there is a dynamic metabolism in bone tissue. Bone resorption precedes the bone formation. New bone formation (bone remodelling) occurs both in the cortical and in the trabecular bone. [21]

Bone formation:

The bone matrix is secreted by osteoblasts that lie at the surface of the existing matrix and deposit fresh layers of bone onto it. Some of the osteoblasts remain free at the surface of the bone matrix, while others gradually become embedded in their own secretion. This freshly formed material (consisting chiefly of type I collagen) is called osteoid. It is rapidly converted into hard bone matrix by the deposition of calcium phosphate crystals in it. Once imprisoned in hard matrix, the original bone-forming cell,

now called an osteocyte, has no opportunity to divide, although it continues to secrete further matrix in small quantities around itself. [23]

Bone resorption:

The cell responsible for the resorption of bone tissue is the osteoclast. osteoclasts differentiate in a late phase from the monocyte/macrophage cell lineage to form a giant. multinucleated cell that can attach to mineralized bone tissue. Specific receptors are implicated in this attachment at the clear or sealing zone. The process of resorption itself takes place at the ruffled border with its highly infolded plasma membranes. The resorption of mineralized bone tissue is a two-step procedure involving the dissolution of bone mineral and the enzymatic degradation of the organic bone matrix.

For both processes, an acidic environment is needed, which is created in a sealed compartment between the osteoclast and the bone surface.[26,27] Osteoclasts, which are responsible for bone resorption, are rare cells with only 2–3 cells seen per 1 mm3 of bone.[25]

Bone remodeling:

At any particular time, most of the skeleton is quiescent. Something, as yet unidentified, leads to a signal (or combination of signals) as yet unknown that initiates a remodeling cycle fig. (7). The cycle begins with recruitment from bone marrow monocyte precursors of multinucleated bone-resorbing cells called osteoclasts, which attach to the surface of bone. A ruffled border develops beneath the osteoclast, sealing the space beneath the cell. Into this subcellular space, the osteoclast generates hydrogen ions, lactate, and proteolytic enzymes, which cause a breakdown of the protein matrix of bone and release of calcium and other bone mineral constituents. After the osteoclasts have excavated a resorption pit or lacuna, bone-forming cells called osteoblasts differentiate from connective-tissue precursors and begin the process of filling in the lacuna with a protein matrix, called osteoid, which subsequently becomes fully mineralized new bone. Resorption takes ~7–10 days, whereas formation requires 2–3 months. Overall, ~10% of bone is replaced each year.[28]

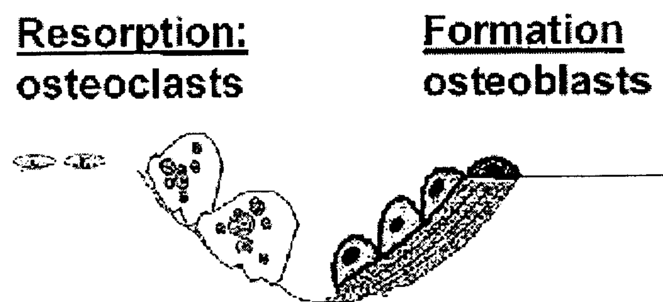


Fig.(7). Schematic representation of the bone remodeling cycle. [28]

Two balanced processes maintain the homeostasis of bone tissue throughout life: bone resorption by osteoclasts and bone formation by osteoblasts.

In early life, the bone formation predominates over the bone resorption until the so-called peak bone density is reached between the ages of 25 and 35 years. Later in life, the rate of bone formation cannot fully compensate for the bone lost by resorption, resulting in a net loss of bone tissue. [26]

In the adult, bone is constantly being turned over, old fatigued bone being replaced with new bone. The remodeling rate is between 2 and 10% of bone per year: approximately 80% of the cancellous bone is turned over in comparison with 20% of cortical bone. [29]

Any imbalance between bone formation by osteoblasts and bone resorption by osteoclasts causes bone abnormalities including osteopetrosis (due to a defect in osteoclastogenesis or the absence of functional osteoclasts), osteosclerosis (due to increased bone formation) and osteoporosis (due to increased bone resorption or a relative decrease in bone formation compared with bone resorption). Therefore, osteoclasts are indispensable to maintain bone homeostasis. [25]

The normal balance between resorption and deposition is disturbed by cancer. When the tumor has metastasized to bone, it can directly alter the bone and cause lesions that may be lytic (due to increased resorption), blastic (due to increased deposition) or a combination of both, causing a mixed lesion. [30, 31]

pathophysiology of bone metastases:

'Seed and soil' hypothesis:

In 1889, Paget proposed that metastatic growth in the bone was dependent on the characteristics of two factors: The 'seed' and the 'soil'.

Every "single cancer cell must be regarded as an organism, alive and capable of development. When a plant goes to seed, its seeds are carried in all directions; but they can only live and grow if they fall on congenial soil". [32]

The 'soil':

The bone microenvironment is unique and contains several growth factors, which encourage the growth of cancer cells. [29, 33]

Metastatic cells appear to disturb the bone microenvironment, altering its regulatory mechanisms and influencing the 'soil', thereby making it more conducive to the development of metastases. There is evidence for increased turnover of all elements of bone, both resorption and formation. Second, the amount of new bone formed does not always equal the bone resorbed, although the mechanisms for this are far from clear. Third, there is uncoupling between the osteoclasts and osteoblasts, resulting in both independent resorption of bone without formation and the deposition of new bone at sites of quiescent bone, not preceded by resorption. [29, 34]

The 'seed':

Not all metastatic cancer cells in the bone marrow develop into clinically detectable metastases. [29, 35] There are particular inherent characteristics of some cancer cells that contribute to the establishment and growth of metastases within the bone microenvironment.

Highly motile metastatic breast cancer cells are more likely than cells with low motility to become established in the bone in vitro; this feature is independent of their osteolytic capacity. [29, 36] Another important feature is the ability of cells to adhere to specific components of bone. such as collagen or endothelial cells. Adhesion molecules and their relevant substrate have been isolated from several cancer cell lines. [29, 35]

Types of bone metastases:

Bone metastases are generally characterized as **osteolytic**, leading to bone destruction, or **osteosclerotic** (**osteoblastic**), leading to new bone formation. Breast cancer metastases are usually osteolytic. and prostate cancer metastases are usually osteoblastic.

The type of metastasis is a reflection of the local interaction between tumor cells and the bone remodeling system. Thus, the development of osteolytic and osteoblastic lesions results from a functional interaction between tumor cells and osteoclasts or osteoblasts, respectively. As a result of the dynamic nature of bone remodeling and the potential heterogeneity of metastatic lesions, patients can have both osteolytic and osteosclerotic lesions or mixed lesions containing both elements. In addition, due to the regulatory interactions between osteoclasts and osteoblasts, secondary bone formation occurs in response to bone destruction. [24, 37]

Complications of bone metastases:

Several complications give rise to the substantial morbidity from metastases. They include pain, impaired mobility, pathological fracture, spinal cord compression, cranial nerve palsies, hypercalcemia, and suppression of bone marrow function. [18, 38]Back pain is a frequent symptom in patients with disseminated carcinoma and in 10% is due to spinal instability. This can cause excruciating pain, which is mechanical in origin. The patient is only comfortable when lying absolutely still, and any movement reproduces sever pain. Consequently, the patient may not be able to sit, stand, or walk even with the use of a spinal support. Because the pain is due to the instability, radiation therapy or systemic treatment will not alleviate it. As with pathologic fractures of long bones, stabilization is required for pain relief. This involves major surgery, which is associated with significant morbidity and mortality. [38]

Therapeutic decisions to treat metastatic bone disease rely on the presence of bone metastases, which are usually difficult to determine radiologically. In addition, therapy in these patients is quite difficult to gauge because radiological changes are generally slow to occur and difficult to detect even with bone scan measurements. [17]

Methods used to evaluate bone metastases:

1-Techniques:

The state of the skeleton can be evaluated by a variety of techniques, including histomorphometry, densitometry, and measurement of calcium fluxes. Histomorphometry is invasive, expensive, has a long turnaround time, and is limited to a single skeletal site (iliac crest). Densitometry is precise and noninvasive but slow to reveal changes. Measurement of calcium fluxes is technically difficult. [28]

The diagnostic work-up of the patient with suspected metastatic bone disease primarily relies on imaging techniques such as plain radiographs, bone isotope scans, computer tomography, magnetic resonance imaging, or 18 F-fluorodeoxyglucose positron

emission tomography. Although all of these methods are valuable tools in case-finding studies, their usefulness is often limited when it comes to the early detection of bony lesions or to the monitoring of disease progression and therapeutic response. For example, depending on the type of tumor and its origin, changes in skeletal morphology or radionuclide uptake may be discrete or even missing. Abnormal findings are often nonspecific and may reflect malignant as much as inflammatory or degenerative changes. Finally, repeated studies are costly and associated with radiation exposure. [39]

2-biochemical markers:

A-Bone formation markers:

Bone formation markers are direct or indirect products of active osteoblasts. The oldest bone formation marker is total alkaline phosphatase. It is thought to participate in the initiation of bone mineralization. It has a low sensitivity and specificity.

Bone specific alkaline phosphatase measurements can be used as a bone formation marker in order to improve sensitivity and specificity.Serum osteocalcin, also known as bone GLA protein, is another bone formation marker. It is a bone specific non-collagenous protein. Osteocalcin is synthesized by osteoblasts, and deposited mainly in the extracellular matrix of bone, but a small amount enters blood. Serum osteocalcin is a sensitive and specific marker of osteoblastic activity and its serum levels reflect the rate of bone formation. Osteocalcin is filtered by the kidneys, so serum levels are dependent on renal function.

Extension peptides of procollagen type I are other bone formation markers, which are removed by specific proteases from procollagen type I during the process of conversion to collagen. These peptides have amino and carboxy terminals, which are named accordingly as either procollagen type I carboxy terminal propeptide (PICP) or procollagen type I amino terminal propeptide (PINP). When the conversion of procollagen to collagen is increased, these peptides are released into the circulation, so their serum levels may be used as a bone formation marker. These two peptides are less valuable bone formation markers than osteocalcin. Importantly, these components are expressed during different phases of osteoblast development and therefore reflect different aspects of bone formation.[21, 39]

B-Bone resorption markers:

Urinary calcium measurement is the cheapest bone resorption marker, but its sensitivity is low. Another marker is urinary hydroxyproline, which is an amino acid in the collagen structure.

Urinary hydroxyproline excretion will increase when collagen degradation is accelerated during bone resorption. However, the majority of the hydroxyproline derived from the breakdown of collagen is reabsorbed by the renal tubules and degraded in the liver. Only 10% of hydroxyproline is excreted in the urine. Ingestion of collagen rich foods such as meat may also increase the level of urinary hydroxyproline. As a result, hydroxyproline is not an ideal marker of bone resorption, but when measured correctly it can provide useful information.

Hydroxylysine is another amino acid in the collagen structure. Galactosyl-hydroxylysine appears to be specific for bone collagen degradation. It is neither affected by

diet nor reused by the body, so it seems to be a more sensitive marker compared with urinary hydroxyproline.

Pyridinoline (pyr) and deoxypyridinoline (D-pyr) are the most promising markers of bone resorption. Peptide chain of collagen has amino (N) and carboxy (C) terminals from which N-telopeptides and C-telopeptides are covalently bound to collagen by pyr and D-pyr cross-links. When collagen is degraded, pyr and D-pyr are released into the circulation, where 20% is free and the remaining is protein bound. The protein bound fraction binds to N-telopeptide, as collagen type I either cross-linked N-telopeptide (NTx) or to C-telopeptide, as collagen type I cross-linked C-telopeptide (CTx). The noncollagenous protein such as bone sialoprotein (BSP) has also been demonstrated to reflect bone resorptive processes. [21, 39]

High blood levels of TRAP 5b (Tartrate-Resistant Acid Phosphatase) are usually associated with active bone remodeling remodeling and increased serum levels are observed during normal bone growth among healthy children. serum TRAP 5b activity is a specific and sensitive marker of bone resorption. It may be the only marker known that has serum levels reflect specifically the bone resorption rate, because it may be derived exclusively from osteoclasts, and it is the only known resorption marker whose serum levels are not affected by renal or hepatic failure. The elevation in TRAP 5b activity predicts future bone loss.[40]

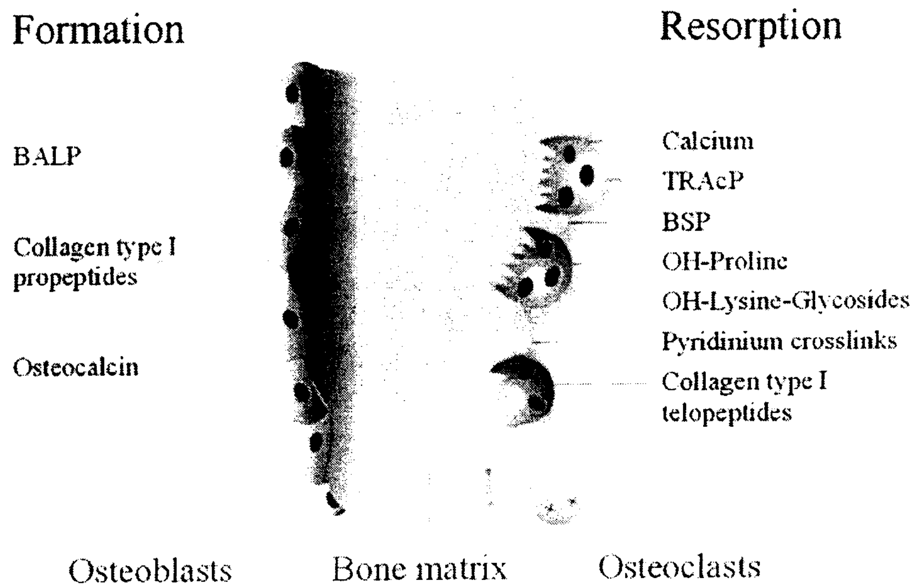


Fig. (8): Schematic representation of biochemical markers of bone remodeling. [39]

These biochemical markers fig (8) are mostly useful for the prediction of bone loss in the early stage of developing osteoporosis before the actual loss of bone, and for monitoring antiresorptive treatment. However, the use of biochemical markers in animal models has not been extensively investigated. [40]

Bone marker measurements are noninvasive, inexpensive, and can be repeated often. Major changes occur in a short time. Markers are derived from both cortical and trabecular bone and reflect the metabolic activity of the entire skeleton. [28]

Conventional tumor markers such as carbohydrate antigen (CA)15-3, tissue polypeptide specific antigen, carcinoembryonic antigen (CEA), or prostate-specific antigen (PSA) are useful to monitor tumor behavior, but they usually do not provide information on skeletal involvement. In contrast, biochemical markers of bone metabolism specifically reflect bone resorption or bone formation rates and are strongly affected by the processes active in metastatic bone involvement. Therefore, bone markers may be able to bridge the gap between classical tumor markers and imaging techniques when it comes to the diagnosis and monitoring of skeletal metastases and their complications. [39]

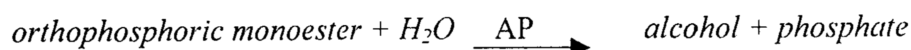
ACID PHOSPHATASES

Phosphatases can be classified according to several frameworks. [41] They can be divided into groups based on their substrate type. Protein phosphatases prefer phosphoproteins or phosphopeptides as substrates, whereas non-specific phosphatases catalyze the hydrolysis of almost any phosphate ester and can be divided into alkaline and acid phosphatases based on their optimal pH for catalysis. Non-specific alkaline and acid phosphatases recycle phosphate in metabolic reactions.

Acid phosphatases :

Acid phosphatases (AP) (EC 3.1.3.2) form a group of four isoenzymes with different origins at the structural level of the gene: prostatic, lysosomal, erythrocytic and macrophagic APs. APs can also be distinguished based on their molecular weight into low molecular weight APs and high molecular weight APs, or on the basis of their resistance to inhibition by tartrate into tartrate-sensitive and tartrate-resistant APs. [42]

Reaction catalyzed by APs:



orthophosphoric monoester + H_2O $\xrightarrow{AP}$ *alcohol + phosphate*

Human APs are normally found at low concentrations. However, pronounced changes in their synthesis occur in particular diseases, where unusually high or low enzyme expression is seen as part of the pathophysiological process. This observation suggests that APs could be diagnostically useful as serological and histological markers of disease, and could also be of use in the investigation of the pathophysiology of the associated disease. [43]

Tartrate Resistant Acid Phosphatase (TRAP):

The Committee of Scientific Advisors of the International Osteoporosis Foundation has endorsed the name type 5 acid phosphatase (AP 5) for mammalian purple APs. The name is based on the relative electrophoretic motility of APs, when these enzymes are being sorted electrophoretically under nondenaturing conditions. Type 5 AP migrates fastest. Purple APs or type 5 APs are not yet listed in Enzyme Nomenclature.

Type 5 APs are resistant to inhibition by tartrate, and are therefore called also tartrate-resistant acid phosphatases. Resistance to inhibition by L+tartrate distinguishes type 5 acid phosphatases from acid phosphatases of lysosomal or prostatic origin.

TRAP (EC 3.1.3.2) can easily be distinguished from other APs by their purple color in solution. The color is due to the presence of a binuclear iron center and arises from tyrosine-to-iron charge transfer transition. [42]

TRAP circulates in the serum as part of a complex with a molecular weight of more than 250 kDa. The complex also contains Ca2+, and TRAP is buried inside the complex, making it impossible for antibodies to bind TRAP. [44]

TRAP is expressed by bone-resorbing osteoclasts and activated macrophages. [45] Histochemical, cytochemical and immunocytochemical studies reveal that TRAPs are localized in lysosomes and lysosome-like organelles or intracellular transcytotic vesicles in osteoclasts, and in antigen presentation route in activated macrophages. [42, 46-52]

The clinical importance of TRAP:

High blood levels of TRAP 5 are usually associated with active bone remodeling and increased serum levels are observed during normal bone growth among healthy children. Elevated serum TRAP levels have been detected in diseases characterized by increased bone resorption; Paget's disease of the bone, hemodialysis, primary hyperparathyroidism, metastatic malignancies involving bone resorption, multiple myeloma and bilaterally ovariectomized women. Post-menopausal women have higher levels of serum TRAP than post-menopausal women on estrogen replacement therapy. [53] Also TRAP expression is increased in some pathological conditions: in Gaucher's disease, which is a lysosomal storage disease, in leukemic reticuloendotheliosis [54], in HIV-induced encephalopathy [55], in osteoporosis and metabolic bone diseases. [42, 56-58]

The difference between TRAPs:

It has been suggested that TRAPs are synthesized as latent proenzymes and activated by cleavage into subunits. [42, 59, 60]

TRAP activity is present in erythrocytes, plasma, osteoclasts, and hairy cells. The erythrocytic isoenzyme is characterized by its low relative molecular mass (<20000 KDa) and lack of activity toward 1-naphthyl phosphate. These characteristics are quite different from those of the isoenzyme 5 of plasma, osteoclasts, and hairy cells. AP activity of the hairy cell is confined to subcellular granules and does not leak out into the blood; in contrast, isoenzyme 5b of osteoclasts is released into the blood during physiological bone growth and in malignancies metastasized to bone. [61]

Isoforms of TRAP:

1-TRAP 5a:

Serum type 5 TRAP [EC 3, 1, 3, 2] exists as two related isoforms: 5a and 5b. Only isoform 5b is osteoclast-derived; the origin and significance of isoform 5a has hardly been explored. It is important to be able to distinguish TRAP 5a from 5b. There are four criteria by which to do so:

(1) TRAP 5a bears sialic acid residues while TRAP 5b does not.

(2) The optimum pH for TRAP 5a is 5.2 while that for TRAP 5b is 5.8.

(3) The specific activity of TRAP 5a is significantly lower than that of TRAP 5b.

(4) TRAP 5a is an uncleaved polypeptide, whereas TRAP 5b is a proteolytically nicked disulfide-linked "heterodimer."

There are differences in both biochemical properties and disease-specific expression of TRAP isoforms 5a and 5b, and that suggest that they are regulated differently and perform separate functions in a tissue-specific manner. [62] In serum from healthy subjects, 5a accounted for 87% of the enzyme protein but only 55% of the activity. [63]

2-Osteoclastic TRAP (TRAP 5b):

There is an association between osteoclasts and the TRAP 5b enzyme. Osteoclasts are well known for containing a large amount of TRAP activity, [43, 64] and this phenomenon

has been used for many years to identify osteoclasts in tissue samples using histochemical techniques. Further evidence of this association is abundant. For example.

(1) osteoclasts cultured in the laboratory on cortical bone slices or dentine show a progressive accumulation of TRAP 5b in the culture medium. This corresponds to the development of resorption lacunae on the bone surface. [43, 65]

(2) Antibodies directed to the active site of TRAP 5b prevent the enzyme carrying out its normal catalytic role. This triggers a decrease in bone resorption. [43, 66]

(3) TRAP "knockout" mice, which do not carry the gene for synthesizing TRAP, develop mild osteopetrosis (excessive bone growth); the balance in bone remodelling is allowed to tilt towards osteoblast activity.[43] Whereas overexpression of TRAP leads to accelerated bone turnover; increase in osteoclast activity. Therefore, TRAP has an important role in bone resorption. [42,67]

(4) TRAP occurs in much higher concentrations in the serum of people with skeletal disease than in normal control subject. [43, 58, 68, 69] Furthermore, it increases with the rate of resorption taking place. [43, 70]

There is a direct relation between excessive osteoclast facilitated bone resorption and the arrival of increased amounts of TRAP 5b in the circulation. Therefore, serum TRAP 5b has been indicated as a disease associated marker for the clinical diagnosis of excessive bone resorption and for quantitatively monitoring the rate and progression of metabolic bone disorders. [43, 64, 71]

Recent evidence suggests that serum TRAP 5b activity is a specific and sensitive marker of bone resorption. [72, 73] It may be the only marker known that has serum levels reflect specifically the bone resorption rate, because it may be derived exclusively from osteoclasts, and it is the only known resorption marker whose serum levels are not affected by renal or hepatic failure. The elevation in TRAP 5b activity predicts future bone loss. [40]

Structure of the TRAP:

TRAPs are all monomeric iron-containing glycoproteins with Mr 30-40 kDa. TRAPs have basic isoelectric point and optimal enzymatic activity at acidic pH. [74, 75]

Each molecule of TRAP binds two iron atoms in a binuclear cluster. There are two types of iron, a chromophoric tyrosine coordinated species that remains ferric and a colorless species those cycles between ferric and ferrous states depending on the reduction-oxidation state. [42]

The purple form of the enzyme is inactive and contains two ferric ions. Mild reduction activates it to a pink form containing one ferric and one ferrous ion. Instead, strong reduction removes the iron content, resulting in a colorless, inactive enzyme.

Halleen et al. [76] describe the spontaneous activation of purple form to pink form upon incubation at 37°C; further incubation results in slow inactivation of the enzyme color change to yellowish. The enzyme purified from osteoclasts is a mixture of the purple and pink forms, but the enzyme purified from serum represents yellowish form. So, it is expected that the newly synthesized form is purple and reduced in the cell to functionally active pink form. After fulfilling its biological function in the cell, the enzyme is further reduced to yellowish form and secreted into the circulation. In the serum, further reduction would dissociate the iron content.

The fully active enzyme not only requires proteolytic processing and a slightly acidic environment but also reducing conditions to maintain the iron cluster in the mixed valent state. The advantage of regulation at different post-translational levels would be to prevent unscheduled dephosphorylation during transport of the enzyme to its site of action.[77]

Substrate specificity, inhibitors and activators:

TRAP activity is inhibited by phosphate, molybdate, zink, copper, fluoride, vanadate, arsenate, and tungstate. [42, 52, 56, 58, 78-83] Oxidation by e.g. hydrogen peroxide causes inhibition of the enzyme activity. [42, 52, 54, 56, 79, 82, 84] Reaction product phosphate and its analogs vanadate, arsenate and molybdate cause competitive inhibition. [78, 82] Fluoride, tungstate, copper and zinc cause noncompetitive inhibition. [42, 56, 79, 81] The enzyme is activated by mild reducing agents, such as β- mercaptoethanol. [42, 54, 56, 79, 82] Strong reducing agents, such as dithionite, lead to removal of iron and thus cause inhibition of the activity. [42, 54, 79] .

Manganese and magnesium are reported to have an activating effect on TRAP. [42, 79] The TRAP enzyme can hydrolyze a variety of phosphomonoesters; among the known substrates for the enzyme that could be physiologically relevant are acidic phosphoproteins such as β-casein, the bone matrix phosphoserine-containing proteins osteonectin, osteopontin (OPN), and bone sialoprotein (BSP), and in addition, nucleotide tri- and diphosphates. It was shown that TRAP dephosphorylates OPN and BSP . OPN and BSP are matrix phosphoproteins.

Proteolytic cleavage:

TRAP purified from bone tissue exists primarily as two smaller fragments. [42, 56, 59, 79, 85] The size of the smaller fragment varies between Mr 15-16 kDa and that of the larger fragment between Mr 18-23 kDa. Purified recombinant bone-derived enzyme exists as one polypeptide chain form. [42, 85, 86] A small portion of the recombinant enzyme appears after disulfide reduction as two smaller fragments with Mr 16 kDa and 20 kDa. [42, 86]

Biological function of TRAP 5b:

The physiological function(s) of TRAP are not known. Diverse functions for mammalian TRAPs have been proposed, including AP activity, reactive oxygen species (ROS) generating activity and iron transport. These activities may have different roles in different cells and cell compartments. [42]

TRAP 5b has an important role in bone metabolism. It has been reported that TRAP is able to regulate the attachment of osteoclasts to the bone surface. [42, 46, 85] In osteoclasts the physiological function of TRAP has therefore been hypothesized to be the destruction of endocytosed matrix degradation products by highly destructive reactive oxygen species. [42, 50]

The observation that TRAP is found in bone marrow derived dendritic cells and macrophages that function as antigen-presenting cells suggests that it participates in processing of macromolecular antigens. TRAP may participate in degradation of cell membrane phosphoproteins in phagocytosed red blood cells. [42, 48]

TRAP may have an important biological function in the defense mechanism of macrophages by generating intracellular reactive oxygen species (ROS) which would be targeted to destroy phagocytosed foreign material.[87]

How Does TRAP Contribute to the Bone Resorption Process?

The rate of TRAP synthesis in osteoclasts and its accumulation in the body fluids has a quantitative association with the rate at which bone resorption is taking place.[43, 64] This is noted both for normal bone remodelling and the exaggerated activity associated with bone resorption diseases. Therefore, the enzyme is inducible by some kind of trigger and appears to have a direct and/or indirect functional link with the resorption process.

TRAP is localised within the transcytotic vesicles of osteoclasts, but not at the ruffled border or resorption lacunae.[43,50] At these sites, TRAP was colocalised with the organic products of bone degradation that had been released from bone matrix during resorption and endocytosed into the osteoclast cells. When matrix degradation products transport from the ruffled border to the functional secretory domain (FSD) of osteoclasts, the enzyme is thought to be secreted out of the cells, together with the matrix degradation products. After this stage, both entities leak into the circulation at a rate that corresponds to the amount of resorption activity being undertaken by the osteoclast.[43]

1-Extracellular role of TRAP 5b:

An extracellular role for TRAP during bone resorption is suggested by its extracellular accumulation in the bone matrix immediately next to the ruffled border of resorbing osteoclasts.[42, 43, 47] However; the precise nature of its catalytic contribution at this site is still unclear.

TRAP can remove phosphate groups from (OPN), an event that consequently disrupts adhesion of osteoclasts to the bone.[42, 43, 46] This suggests that the enzyme might regulate osteoclast adhesion to the bone and also enable migration of osteoclasts to adjacent sites of resorption. The ability of TRAP to degrade phosphoproteins in bone by dephosphorylation may illustrate a preliminary stage in the degradation of the bone matrix. Of further interest, the bone matrix is rich in pyrophosphate, a known inhibitor of bone resorption. TRAP can hydrolyse and therefore liberates pyrophosphate from the bone matrix. This hydrolysis event would enable bone resorption activity by osteoclasts to begin.[43]

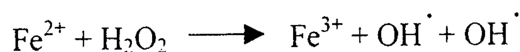
2-Intracellular role of TRAP 5b:

Osteoclasts resorb bone by secreting acid and proteases into the resorption lacuna through Ruffled Border (RB). After initial degradation in the lacuna, matrix components, including type I collagen, are endocytosed into the osteoclast, transcytosed, and finally released through a functional secretory domain in the basolateral membrane. The large cytoplasmic TRAP containing vesicles have been identified as the transcytotic vesicles, as degradation products of bone collagen are transported in these same vesicles. In addition, it has been suggested that TRAP may directly participate in bone resorption by generating ROS that would further destroy organic bone matrix components in the transcytotic vesicles.[50, 88-90]

Formation of reactive oxygen species (ROS):

The ROS-generating activity of TRAP requires hydrogen peroxide as substrate, and superoxide to regenerate the ferrous ion needed for ROS production. This means that if TRAP generates ROS in the transcytotic vesicles, they must contain large quantities of superoxide and hydrogen peroxide.

When the redox-active iron in the binuclear iron center of TRAP is in the ferrous form, it can react with H_2O_2 by the Fenton reaction to produce a ferric ion and $OH^{\bullet}$:



$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{\bullet}$$

The newly formed ferric ion is still redox-active and able to react with H_2O_2 to form $O_2^{\bullet -}$ and a ferrous ion:

$$Fe^{3+} + H_2O_2 \longrightarrow Fe^{2+} + 2H^{+} + O_2^{\bullet -}$$

The formed ferrous ion is again able to react by the Fenton reaction etc. Thus, a sequence of reactions generating both $OH^{\bullet}$ and $O_2^{\bullet -}$ occurs with continuous oxidation and reduction of the redox-active iron, making it possible for one enzyme molecule to generate a high number of these ROS as long as H_2O_2 is available.

The TRAP derived ROS was seen in vitro to degrade type I collagen to fragments, whereas the TRAP enzyme protein itself was not degraded. If this oscillation of the iron state took place repeatedly, it would enable a single TRAP molecule to generate a large amount of ROS, providing H_2O_2 was present. [43, 50]

The activity of producing ROS has been detected in three different laboratories with three different methods:

(1) By using electron spin resonance spectroscopy

By using luminol peroxidation

(2) By measuring formation of malondialdehyde acetal from degradation products of deoxyribose to verify ROS generation by TRAP.

The ROS generated by TRAP are capable of destroying type I collagen, the main protein in bone matrix. Macrophages overexpressing TRAP have increased amounts of intracellular ROS, suggesting that the enzyme is capable of producing ROS in vivo. [91- 94] The ROS generating activity has a neutral pH-optimum, distinguishing it from the phosphatase activity that has an acidic pH-optimum. This suggests that the two activities may function in different intracellular compartments, and the pH of the environment may determine which activity is used. Mutant TRAP enzymes that are completely inactive as phosphatase are capable of producing ROS, suggesting that the two activities are functionally independent. [50, 91]

Clearance of TRAP 5b:

Once secreted, the enzyme is exposed to physiological influences present in the body fluids. TRAP has a tendency to bind to and complex with a high molecular weight molecule in serum, namely α_2 macroglobulin. [43, 92] Although the role of macroglobulin is

not clear, it may serve as a carrier and a regulator of cytokine activity. [43, 93] Therefore, it is feasible that it is also a carrier molecule for TRAP that mediates the clearance of the enzyme from areas of bone resorption and then the circulation.

Ultimately, TRAP has the fate of all circulating enzymes. Its structure becomes compromised, leading to its inactivation as a catalyst. It loses its binuclear iron centre, which is then recycled, and the iron free enzyme protein is broken down by proteases in the plasma and the liver. The fragments that result from these events are eventually metabolised by the liver and/or removed in the urine. [43]

COLLAGENS

The collagens are a family of fibrous proteins found in all multicellular animals. They are secreted by connective tissue cells, as well as by a variety of other cell types. As a major component of skin and bone, they are the most abundant proteins in mammals. [23]

Collagen is the predominant constituent of skin, tendons, and cartilage as well as the organic component of bones, teeth, and the cornea. Indeed it constitutes approximately 25 to 33% of the total protein in mammalian organisms. It is found in the connective tissues of nearly all organs as insoluble fibers embedded in the mucopolysaccharides and proteins of the extracellular matrix and serves to give tissues their structure and strength. Consequently, any process that results in degradation or loss of integrity of this protein is likely to have significant implications for health. [23]

The structure of collagen:

Collagen is a long (approximately 300 nm), rod-like molecule which comprises three parallel polypeptide chains. Each chain is a left-handed helix, and the three helices twist around a common axis to form a major helix of slightly right-handed sense. [95, 96]

The primary feature of a typical collagen molecule is its long, stiff, triple-stranded helical structure, in which three collagen polypeptide chains, called *α chains*, are wound around one another in a ropelike superhelix Fig. (9). [23]

The triple helix domains of the collagens consist of repeats of the amino acid sequence Gly-X-Y. A glycine (the smallest amino acid, with a side chain consisting only of hydrogen) is required in every third position in order for the polypeptide chains to pack together close enough to form the collagen triple helix. Proline is frequently found in the X position and hydroxyproline in the Y position; because of their ring structure, these amino acids stabilize the helical conformations of the polypeptide chains. The unusual amino acid hydroxyproline is formed within the endoplasmic reticulum by modification of proline residues that have already been incorporated into collagen polypeptide chains fig. (10).

So far, about 25 distinct collagen α chains have been identified, each encoded by a separate gene. Different combinations of these genes are expressed in different tissues. Although in principle more than 10,000 types of triple-stranded collagen molecules could be assembled from various combinations of the 25 or so α chains, only about 20 types of collagen molecules have been found. The main types of collagen found in connective tissues are types I, II, III, V, and XI, type I being the principal collagen of skin and bone and by far the most common. These are the fibrillar collagens , or fibril-forming collagens, with the ropelike structure illustrated in fig. (9,10). After being secreted into the extracellular space, these collagen molecules assemble into higher-order polymers called collagen fibrils, which are thin structures (10–300 nm in diameter) many hundreds of micrometers long in mature tissues and clearly visible in electron micrographs . Collagen fibrils often aggregate into larger, cablelike bundles, several micrometers in diameter, which can be seen in the light microscope as collagen fibers. [97]

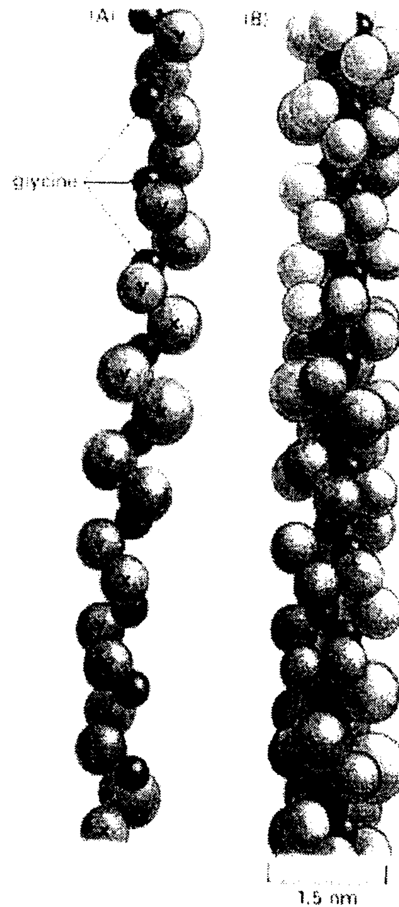


fig. (9) The structure of a typical collagen molecule. (A) A model of part of a single collagen α chain in which each amino acid is represented by a sphere. The chain is about 1000 amino acids long. It is arranged as a left-handed helix, with three amino acids per turn and with glycine as every third amino acid. Therefore, an α chain is composed of a series of triplet Gly-X-Y sequences, in which X is commonly proline and Y is commonly hydroxyproline. (B) A model of part of a collagen molecule in which three α chains, each shown in a different color, are wrapped around one another to form a triple-stranded helical rod. Glycine is the only amino acid small enough to occupy the crowded interior of the triple helix. Only a short length of the molecule is shown; the entire molecule is 300 nm long. (From model by B.L. Trus.) [97]

The different collagen types are generally associated with particular tissues, and this heterogeneity may reflect the different, site specific physiological functions of each collagen type. Furthermore, variation in the molecular structure of the different collagens may have consequences for the nature of the enzymes that are able to cleave each type. [96]

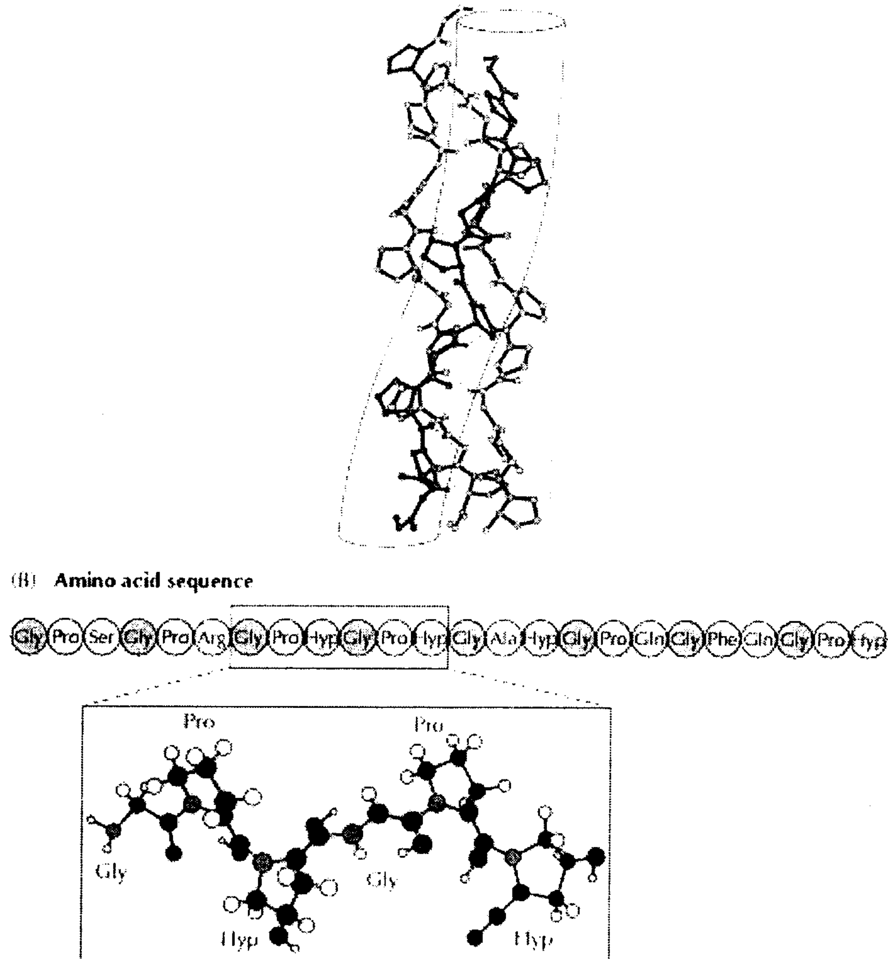


fig.(10) Structure of collagen (A) Three polypeptide chains coil around one another in a characteristic triple helix structure. (B) The amino acid sequence of a collagen triple helix domain consists of Gly-X-Y repeats, in which X is frequently proline and Y is frequently hydroxyproline (Hyp). [23]

Secretion of Collagens:

Individual collagen polypeptide chains are synthesized on membrane-bound ribosomes and injected into the lumen of the endoplasmic reticulum (ER) as larger precursors, called *pro-α chains*. These precursors not only have the short amino-terminal signal peptide required to direct the nascent polypeptide to the ER, they also have additional amino acids, called *propeptides*, at both their N- and C-terminal ends. In the lumen of the ER, selected prolines and lysines are hydroxylated to form hydroxyproline and hydroxylysine, respectively, and some of the hydroxylysines are glycosylated. Each pro-α chain then combines with two others to form a hydrogen-bonded, triple-stranded, helical molecule known as procollagen.

The cleave of Procollagen Molecules to Collagen Molecules:

After secretion, the propeptides of the fibrillar procollagen molecules are removed by specific proteolytic enzymes outside the cell. This converts the procollagen molecules to collagen molecules, which assemble in the extracellular space to form much larger

collagen fibrils. The propeptides have at least two functions. First, they guide the intracellular formation of the triple-stranded collagen molecules. Second, because they are removed only after secretion, they prevent the intracellular formation of large collagen fibrils, which could be catastrophic for the cell.

After the fibrils have formed in the extracellular space, they are greatly strengthened by the formation of covalent cross-links between lysine residues of the constituent collagen molecules fig. (11). The types of covalent bonds involved are found only in collagen and elastin. If cross-linking is inhibited, the tensile strength of the fibrils is drastically reduced; collagenous tissues become fragile, and structures such as skin, tendons, and blood vessels tend to tear. The extent and type of cross-linking vary from tissue to tissue. [23]

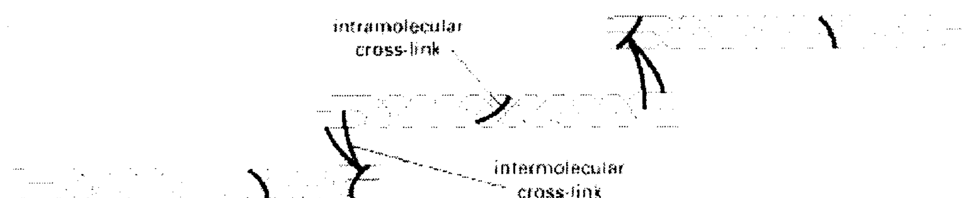


Fig.(11): Cross-links formed between modified lysine side chains within a collagen fibril. [97]

Type I collagen:

Type I collagen is the most abundant collagen type in the body and the only collagen type found in mineralised bone, [98] where both types of bone(cortical and cancellous bone) consist mainly of type I collagen, which comprises over 90% of the organic bone matrix. [99] In addition, type I collagen is found in loose connective tissues together with other collagen types such as types III, V and VI. Also in these locations the proportion of type I collagen is the largest. Type I collagen in tissues is present in fibers, the structure of which shows some variation according to tissue. In bone the type I collagen molecules are crosslinked via three residues of hydroxylysine, lysine or their derivatives which form a fluorescent, cyclic pyridinoline-structure and non-fluorescent unknown structures linking three different collagen polypeptide chains together. In loose connective tissues, such as skin, the major mature cross-link of type I collagen is non-fluorescent and contains histidine as one of the amino acid residues. [98]

Type I collagen is one of the fibril-forming collagens that are the basic structural components of connective tissues. The polypeptide chains of these collagens consist of approximately a thousand amino acids or 330 Gly-X-Y repeats. After being secreted from the cell, these collagens assemble into collagen fibrils in which the triple helical molecules are associated in regular staggered arrays fig. (12).

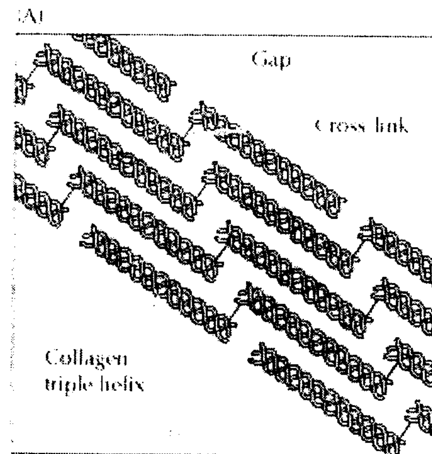


fig. (12): Collagen molecules assemble in a regular staggered array to form fibrils. The molecules overlap by one-fourth of their length, and there is a short gap between the N terminus of one molecule and the C terminus of the next. The assembly is strengthened by covalent cross-links between side chains of lysine or hydroxylysine residues, primarily at the ends of the molecules. [23]

The major difference in bone and other tissues collagen is that the first type contains hydroxylysylpyridinoline and lysylpyridinoline crosslinks. Crosslinking occurs between lysine and hydroxylysine residues on the nonhelical carboxyterminal and aminoterminal ends of mature type I collagen, termed telopeptides, and the helical portions of an adjacent collagen. This process results in forming the pyridoline and deoxypyridoline crosslinks, which provide rigidity and strength in the collagen molecule. [99]

Several degradation products of bone matrix are released in serum by osteoclasts when they resorb bone. The most critical molecular fragments of type I collagen that have clinical utility as sensitive and specific markers of bone resorption contain the nonreducible pyridinium crosslink, deoxypyridinoline (Dpd). [100]

Carboxy Terminal Telopeptide of Type I Collagen (ICTP):

ICTP is the carboxyterminal telopeptide region of type I collagen fig. (13), joined via trivalent cross-links and liberated during the degradation of mature type I collagen. This peptide is found in an immunochemically intact form in blood, where it seems to be derived from bone resorption and degradation of loose connective tissues. It has recently been shown, that the ICTP antigen is produced through the action of e.g. matrix metalloproteinases, which are enzymes involved in tissue destruction in various pathological conditions. [98]

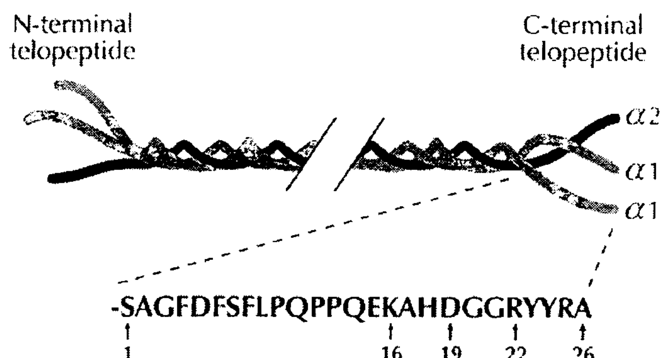


fig. (13): Schematic presentation of type I collagen showing the amino acid sequence of the human C-terminal telopeptide α I chain. Type I collagen consists of two α 1 chains and one α 2 chain. The intact C-terminal telopeptide α 1 chain consists of a 26-amino acid sequence. [101]

ICTP and CTX:

The degradation of type I collagen is necessary for bone resorption. [101, 102] During this process, type I collagen is cleaved into several fragments in the extracellular phagolysosomal-like resorption compartment of the osteoclast. Evidence based on bone developmental models showed that two types of proteinases may participate at this level: the cysteine proteinases, which act preferably at acidic pH, and the matrix metalloproteinases (MMP), which act at neutral pH. [101-104]

On the C-telopeptide end, two fragments have been characterized and include:

(1) The so-called ICTP (cross-linked carboxyterminal telopeptide of type I collagen), a cross-link-containing collagen peptide originally isolated by trypsin digestion of human bone collagen.

(2) CTX (C-terminal crosslinked telopeptide of type I collagen).

Actually, these two molecular markers of type I collagen degradation have been shown to respond differently according to the clinical situations and treatments. For example, serum and urinary CTX levels are markedly increased in postmenopausal women compared with premenopausal controls, and their values decrease markedly and shortly after initiation of antiresorptive treatment, including bisphosphonates in postmenopausal women with osteoporosis.[101, 105-107] These changes in CTX levels contrast with the only slight and nonsignificant changes of serum ICTP in the same conditions.[101, 107] Serum ICTP has however shown to be a valuable index of bone turnover in other pathological situations, including patients with bone metastases of breast, prostate and lung cancer cells and multiple myeloma.[105-112] Because the relative abundance of CTX and ICTP varies according to the pathology, one can speculate that these fragments are released through distinct collagenolytic pathways.[101]

ICTP is released from collagen through the actions of matrix metalloproteinases and is therefore also known as CTX-MMP. CTX is released through the actions of cysteine proteinases, including cathepsin K.[101, 113] ICTP and CTX are markers of type-I collagen breakdown and hence bone resorption.[113, 114] Furthermore, antigenicity of ICTP is destroyed by action of cathepsin K.[115, 116] These observations have been strengthened by studying bone resorption markers in pycnodysostosis, an autosomal recessive

osteosclerotic skeletal dysplasia characterized by cathepsin K deficiency; CTX and NTX levels were found to be low while ICTP was elevated.[115, 117]

However, the relative abundance of ICTP and CTX varies according to the type of bone pathology, suggesting that these two fragments are generated through distinct collagenolytic pathways. Cathepsin K released large amounts of CTX, but did not allow a detectable release of ICTP. Conversely, the matrix metalloproteinases (MMPs) released ICTP, but did not allow a detectable release of CTX. Next Garnero et al. analyzed the release of ICTP and CTX from bone explants cultured in the presence of well-established inhibitors of these proteinases and of matrix solubilization. An inhibitor of cysteine proteinases including cathepsin K, inhibited the release of CTX, but not the release of ICTP. MMP inhibitors inhibited the release of ICTP, but also that of CTX. Similarly the treatment of mice bearing bone metastasis with an MMP inhibitor led to a significant reduction of serum ICTP and CTX, and osteolytic lesions. Therefore, they concluded that the generation of ICTP and CTX depends on different collagenolytic pathways. This finding may explain why these two markers may discriminate between different bone pathologies.[101]

The clinical importance of ICTP:

Increased serum concentrations of ICTP are hence seen in conditions associated with increased lysis of bone, such as multiple myeloma, osteolytic metastases, rheumatoid arthritis and e.g. immobilisation. [99]

The measurements of ICTP is worthwhile as a serological diagnostic method of bone metastases from breast cancer, and also appears to be the leading marker of bone metastases from breast cancer.[113]

ICTP shows a direct relationship with the disease extent in bone, and correlates to the number of bone metastases on bone scan. It correlates inversely with the treatment response.[98]

The sensitivity was 70% and specificity was 32% in metastatic females. While the sensitivity was 63%, specificity was 25%.in bone metastases from breast cancer. In contrast Ulrich et al. showed in a study with 106 patients that the sensitivity for diagnosing bone metastases was 65%,[118,119] and high specificity of 91%. ICPT might be a useful marker for screening and monitoring bone metastases in breast cancer. [111,118, 120]

CA15-3

One of the most useful markers in breast caner is CA15-3; C means Carbohydrate or Cancer and A refers to the Antigen that will be detected by the antibody. [121,122] CA15.3 is most widely used and is regarded as the standard against which other assays are compared. Serum CA15.3 is elevated in 54–80% of patients with metastatic breast cancer. [123-128] The CA 15-3 assay measures the protein product of the MUC1 gene. [129]

The biochemical properties:

MUC1:

MUC1 is just one example of a class of molecules collectively referred to as mucins. [130] Mucins are the major components of mucous, the visco-elastic substance that protects and lubricates epithelial mucosa. They are highly glycosylated molecules; up to 80% of their mass consists of O-linked glycosyl residues. Mucins constitute an expanding family of proteins that can be grouped into "transmembrane mucins" and "secretory mucins". So far, different mucin apoproteins have been identified. The transmembrane mucins are emerging as signal transducer proteins that regulate the growth, adhesion, and motility of cells.

The best studied transmembrane mucin is MUC1 protein, also known as epithelial membrane antigen (EMA), polymorphic epithelial mucin (PEM) or episalin. MUC1 protein was first identified in human milk.

Although the core protein of MUC1 appears identical in different tissues, the extent of glycosylation can vary from organ to organ.8Thus, in the mammary gland, MUC1 has a relative molecular mass (Mr) of 250-500 kDa and contains approximately 50% carbohydrate by weight. [121]

MUC1 is highly heterogeneous owing to its polymorphic nature and the high degree of variation in glycosylation. A large number and diversity of distinct monoclonal antibodies have therefore been raised against these different epitopes. That is leading to which has been the basis for the development of numerous immunoassay kits such as cancer antigen 15.3 (CA15.3), CA27.29.[124]

Alterations in malignant tumor tissue:

In comparison with normal breast tissue, the MUC1 gene is frequently over expressed in malignant breast tumors. This altered glycosylation may expose epitope sites which are not present in the mucin from normal cells.[121]

In normal breast tissue, MUC1 is expressed on the apical surface of epithelial cells in the ducts and acini from where the molecule is shed via milk fat globules and in soluble form into the milk. In case of tumors, cell polarization is lost and this altered cell surface expression, coupled with the disruption of the normal tissue architecture caused by the growing tumor, allows MUC1 mucin to be shed into the circulation where it can be measured by means of immunoassays. [124]

CA15-3:

CA15-3 molecule is a mucin, being a product of MUC1 gene. CA15-3 is breast associated antigens defined by reactivity with two monoclonal antibodies, Ma695 as catcher antibody recognizing a sialylated carbohydrate epitope expressed on the MUC-1

antigen and Ma552 as tracer antibody targeting the PDTRPAPG (Pro- Asp-Thr- Arg- Pro-Ala-Pro- Gly) region of the protein core. [132]

CA15-3 concentrations in blood:

In healthy subjects, pregnancy and lactation did not affect CA15-3 concentrations. CA15-3 concentrations were similar in men and women. Elevations of CA15-3 concentrations are not organ specific. Serum CA15-3 measurements alone is of little use in identifying unknown primary cancers in patients with undifferentiated metastases.

The preoperative CA15-3 concentrations are rarely elevated in patients with primary breast cancers. [121.133] Similarly, only a minority of patients with either regional or local recurrences exhibiting concentrations. In contrast, more than 70% of patients with distant metastases have elevated marker concentrations. Concentrations can be particularly high when either bone or liver metastases is present.

The clinical utility of CA15-3:

1-Screening and diagnosis of early breast cancer:

It is clear that preoperative of CA15-3 concentrations are of little value in early detection of breast cancer and thus cannot be used for either screening or diagnosis. If the concentration of CA15-3 is found to be elevated in a patient presenting with apparently localized breast cancer and this value remains high postoperatively, it almost certainly indicates either occult metastases, an additional carcinoma or possibly the presence of benign disease (e.g. of liver).[121]

2-Prognostic aid:

The ability to predict patient outcome using a circulating marker is thus desirable. Recent evidence suggests that patients with either high pre- or postoperative concentrations of CA15-3 have a worse outcome than those with low concentrations.[121. 133. 134]Duffy et al. found that postoperative values were more stronger indicator of prognosis than pre-operative concentrations.

3-Monitoring therapy:

As CA15-3 concentrations are elevated in the majority of breast cancer patients with distant metastases, it might appear reasonable to use this marker to monitor response to treatments. [121] After recurrence, there is a good correlation between the variations of CA 15.3 levels and the clinical response to treatment and all the guidelines recommend its use in that setting. [135]

AIM OF THE WORK

AIM OF THE WORK

The aim of this work was to determine serum level of Tartrate Resistant Acid Phosphatase 5b (TRAP 5b), Carboxy-terminal telopeptide of type I collagen (ICTP) and CA15.3 preoperatively in patients with primary breast cancer to asses their values as prognostic factors for the subsequent metastatic disease.

PATIENT & METHODS

PATIENTS AND METHODS

1-Patients:

This study included 41 female (35-80 years) divided into two groups:

Group I: (31 patients with primary breast cancer).

Group II: (10 normal healthy volunteers as control)

Patients were chosen from those admitted to Surgery Department and Clinical Oncology Unit, Radiation Sciences Department in the Medical Research Institute, Alexandria University.

2-Methods:

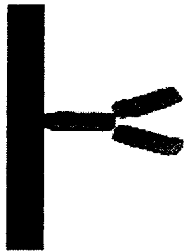
All patients were subjected to the following:

- Preoperative evaluation by FNAC to detect the presence of malignancy
- Full medical history.
- Clinical examination to detect the site and stage of the tumor and the presence of axillary node infiltration.
- Routine laboratory investigations including: Complete blood picture, liver, and kidney functions.
- Routine radiological studies including: Chest X-ray, Liver Ultrasonography, Abdomen Ultrasonography, and Isotopic bone scan.
- Radical mastectomy and axillary lymph node clearance were performed.
- Three blood samples were taken from each patient of Group I before surgery, 10 and 20 months after surgery. Also, a blood sample was taken from each control subject. Sera of all patients were kept frozen at -80°C until the time of assay to measure TRAP 5b, ICTP, and CA15-3.

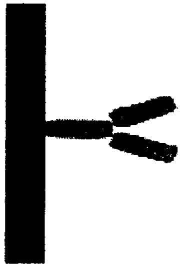
I- Bone Tartrate Resistant acid phosphatase 5b Assay:

It is quantitative Enzymeimmunoasssay (EIA) determination of the active isoform 5b of the tartrate-resistant acid phosphatase.

Principle of the test:

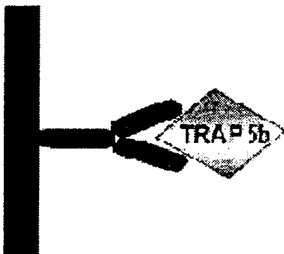


The plate is coated with anti-TRAP antibodies (monoclonal).

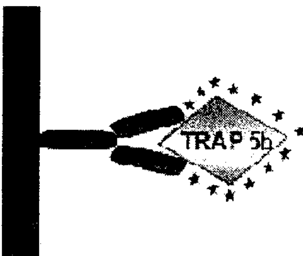


Calibrators, Control and patient samples are added.
Releasing reagent is added.

Dissociation of active TRAP 5b from the binding proteins.



TRAP 5b is bound by the anti-TRACP antibodies.



Incubation with pNPP (paranitrophenylphosphate) substrate (*).

The reaction is stopped by adding sodium hydroxide. The absorption is read photometrically.

Table for the test procedure:

	Blank	Calibrators	Control	Sample
Sample Diluent	100 µl	-	-	-
Calibrators	-	100 µl	-	-
Control	-	-	100 µl	-
Sample	-	-	-	100 µl
Releasing Reagent	50 µl	50 µl	50 µl	50 µl
Incubate for 60 min (± 5 min) at room temperature with constant shaking at 850–950 rpm.				
wash 4 times with 200 µl wash buffer.				
Substrate Solution	100 µl	100 µl	100 µl	100 µl
Incubate for 60 min (± 5 min) at 37 °C (± 1 °C).				
Stop Solution	25 µl	25 µl	25 µl	25 µl
Photometric reading at 405 nm				

Calculations of the results:

* Read optical density(OD) values at 405 nm.

* Subtract the average OD value of the blank from all other OD values.

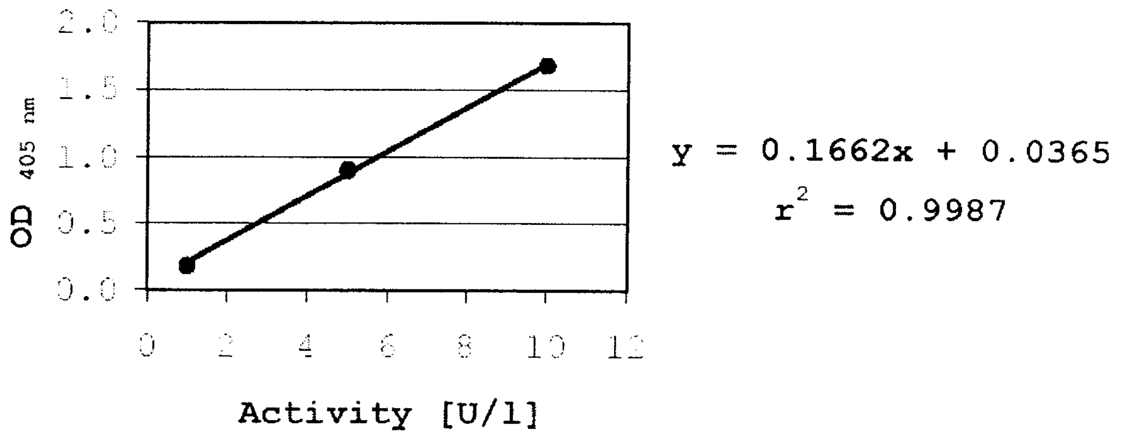
* The average OD values of the calibrators are plotted against the activity values.

The calibration line is calculated by linear regression.

* The measuring range spans from 1 to 10 U/l. Samples below the measuring range have to be interpreted as < 1 U/l. Samples with activities above the measuring range have to be interpreted as > 10 U/l. These values must not be extrapolated but the samples should be retested or diluted (up to 1:5).

* The TRAP 5b activities of the control and the samples can be read from the calibration line. If diluted samples have been used the dilution factor has to be considered.

calibration line:



The kit was obtained from Suomen Bioanalytiikka Oy SBA Sciences, Finland.

II-Carboxy terminal telopeptide of type I collagen Assay:

ICTP EIA is a quantitative enzymeimmunoassay designed for *in vitro* measurement of carboxyterminal cross-linked telopeptide of type I collagen concentration in human serum.

Principle of the test:

ICTP EIA kit is based on the competitive immunoassay technique. A known amount of peroxidase labelled ICTP and an unknown amount of unlabelled ICTP in the sample compete for the limited number of high affinity binding sites of the primary antibody. A secondary antibody, directed against the primary and coated to the wells, binds the antibody-antigen complex, which enables convenient separation of bound and free antigen. After washing away the free antigen, the amount of labelled ICTP in the well is inversely proportional to the amount of ICTP in the sample. The amount of labelled ICTP is measured by incubation with a substrate that produces a coloured product. The concentrations in unknown samples are obtained from a calibration curve.

Table for the test procedure:

	Blank	**Calibrator**	**Control/ Unknown**
Pipette sample		50 µ	50 µ
Pipette enzyme conjugate (red)		50 µ	50 µ
Pipette antiserum (blue)		50 µ	50 µ
Incubate on plate shaker for 2h at 18...25°C			
Wash 4 times			
Pipette substrate	100 µl	100 µl	100 µl
Incubate on plate shaker for 30 min at 18...25°C			
Pipette stopping solution	100 µl	100 µl	100 µl
Shake briefly to mix the reagents			
Read absorbances at 450 nm			

Calculations of the results:

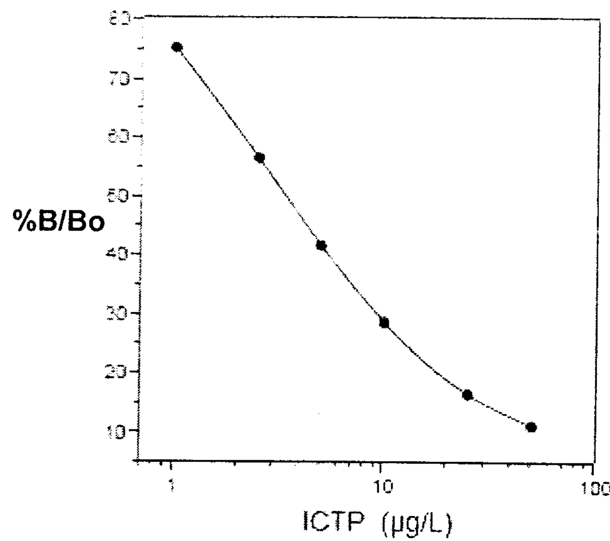
Results can be produced manually on semi-log graph paper.

Calculate the mean absorbances for all calibrators, samples and controls.

Calculate the %B/Bo from:

$$\%B/Bo = \frac{(\text{calibrator or sample absorbance - blank}) \times 100}{(\text{Zero calibrator absorbance - blank})}$$

Draw a calibration curve on semi-log graph paper with %B/Bo values on the ordinate and the ICTP concentrations (µg/L) of the calibrators on the abscissa.



The kit was obtained from Orion Corporation Orion Diagnostica Espoo, Finland

III- CA15-3 Assay :

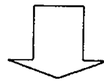
CA15-3 Immunoradiometricassay (IRMA) is intended for the quantitative determination of MUC-1 gene associated antigen (CA 15-3) in human serum.

Principle of the test:

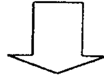
The MUC-1 gene associated antigen (CA15 - 3) assay is a two-step "sandwich" type assay in which two mouse monoclonal antibodies, directed against two different epitopes of the molecule, are employed. Samples or standards are incubated in tubes coated with the first monoclonal antibody, the contents of the tubes are then aspirated and the presence of CA 15-3 in the sample is revealed by incubation with a second, ^{125}I-labeled monoclonal antibody. The contents of the tubes are aspirated after this second incubation and unbound labeled antibody is eliminated by washing. The amount of bound reactivity measured in a gamma counter is proportional to the CA15-3 concentration. The unknown values are determinated by interpolation from a standard curve.

The test procedure:

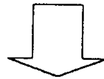
Mix 10 µl of sample and control with 500 µl of Sample Diluent



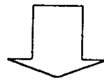
Add 200 µl of Calibrators, diluted Controls and samples to coated tubes



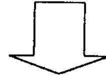
Incubate 2 hours shaking (400 rpm) at room temperature



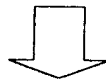
Aspirate the content of each tube
and then wash twice with 2 mL of distilled water



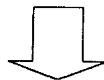
Add 200 µl of iodine labeled antibody (I^{125})



Incubate 1 hour shaking (400 rpm)at room temperature



Aspirate the content of each tube
and then wash twice with 2 mL of distilled water



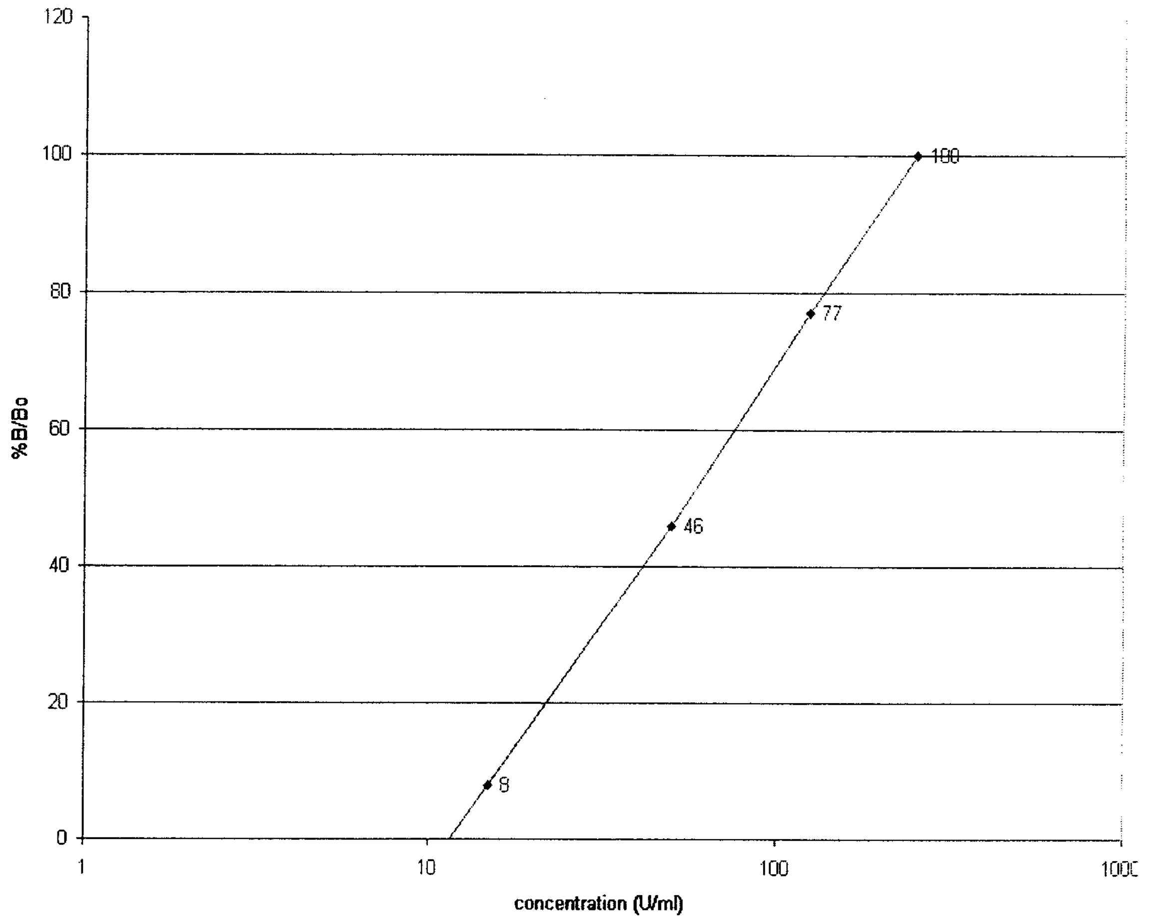
Count per minute using gamma counter

Note:

Add 200 µL of tracer in additional tube to obtain "total cpm"

Calculation of Results:

The results were calculated using a semi-logarithmic curve with B/Bo (%) on vertical axis and the CA 15-3 concentration of the standards on the horizontal axis (U/mL). Locate the B/Bo (%) for each sample on the vertical axis and read off corresponding CA15-3 concentration on the horizontal axis.



The kit was obtained from IMMUNOTECH SA, France.

Statistical Methods:

Data were analyzed using SPSS version 9.0 software. For comparison of the means of two different groups; one way analysis of variance (ANOVA) was applied. For comparison of each two individual groups separately, least significant difference (LSD) was used at 5% level of significance.

Overall survival methods were applied to death as end point. The survival curve was calculated by the Kaplan-Meier method, based on the log-rank test according to Mantel and Breslow and also was performed by Cox regression analysis. Hazard ratios (HRs) with 95% confidence intervals (CIs) were used to convey the effect of the marker.

Disease free survival methods were applied to bone metastases-free survival (defined as survival without the development of bony metastases). The survival curve was calculated by the Kaplan-Meier method, based on the log-rank test according to Mantel and Breslow and also were performed by Cox regression analysis. Hazard ratios (HRs) with 95% confidence intervals (CIs) were used to convey the effect of the marker.

RESULTS

RESULTS

Percentage of metastases:

Table (1) represents the number and percentage of different metastatic sites developed during follow-up in primary breast cancer patients. During 40 months of follow-up, 22.6% (7) were free from disease, 38.7% developed local recurrence and lung metastases, 38.7 %(12) developed bone metastases. Thus, on the bases of these results, group I (31 primary breast cancer patients) was divided into sub-groups according to the metastatic sites appeared.

Table (1): Number and percentage of different metastatic sites developed during follow-up in primary breast cancer patients.

Case	Number	%
Non-metastatic	7	22.6
Local Recurrence	7	22.6
Lung	5	16.1
Bone	12	38.7
Total	***31***	***100***

Bone TRAP 5b and Bone metastases:

Table (2) and fig. (14) represents the serum level of bone TRAP 5b (U/L) in control, non-metastatic, local recurrence, lung, and bone metastases sub-groups before surgery, 10 and 20 months after surgery. The mean ± S.E. for control group was 3.2± 0.4 U/L and for non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery were 3.5 ±0.5, 4.1±0.2, 3.9 ± 0.5 and 3.8± 0.3 U/L respectively. Also, the mean ± S.E. for non-metastatic, local recurrence, lung, bone metastases sub-groups after 10 and 20 months were 3.9 ±0.4, 4.1± 0.4, 4.1± 0.4, 6.0±0.2, 3.7±0.4,3.8±0.2, 3.6±0.5 and 6.6±0.3 U/L respectively.

There was no statistically significant difference in the serum level of bone TRAP 5b between control group and all sub-groups before surgery, p = 0.628, 0.115, 0.302 and 0.206 respectively. Also, There is no statistically significant difference in the serum level of Bone TRAP 5b between control group and non-metastatic, local recurrence, lung sub-groups after 10 and 20 months, p = 0.207, 0.104, 0.152, 0.426,0.274, and 0.484 respectively. While, in the bone metastases sub-group, there is a statistical significant difference between this sub-group and control group after 10 and 20 months, p =0.0001, 0.0001.

Table (3) and fig (15) shows the percent of elevation in the serum level of bone TRAP 5b in different sub-groups before surgery, 10 and 20 months after surgery. The percent of elevation is defined as the value that higher than (M +2 S.D) of the control group multiplied by 100. The percent of elevation in all sub-groups before surgery were 0, 0, 0 and 8.3% respectively. After 10 and 20 months, the percent of elevation in non-metastatic, local recurrence, lung and bone metastases sub-groups were 14.3, 28.6, 20.0, 75.0, 14.3, 0, 20.0 and 83.3% respectively.

Table (2): Serum level of Bone TRAP 5b (U/L) in control, non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery, 10 and 20 months after surgery.

Sub-groups	(M ±SE)		
	Before surgery	10 months after surgery	20 months after surgery
Non-metastatic	3.5±0.5	3.9±0.4	3.7±0.4
Local recurrence	4.1±0.2	4.1±0.4	3.8±0.2
Lung	3.9±0.5	4.1±0.4	3.6±0.5
Bone	3.8±0.3	6.0±0.2* p =0.0001	6.6±0.3* p =0.0001
Control	3.2±0.4		

Table (3): The percent of elevation in serum Bone TRAP 5b in different sub-groups before surgery, 10 and 20 months after surgery.

Sub-groups	Number	% of elevations (M ±2SD)		
		Before surgery	10 months after surgery	20 months after surgery
Non metastatic	7	0	14.3(1)	14.3(1)
Local recurrence	7	0	28.6(2)	0
Lung	5	0	20.0(1)	20.0(1)
bone	12	8.3(1)	75(9)	83.3(10)

Fig (14) : Serum bone TRAP 5b activity U/L in control, non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery, 10 and 20 months after surgery.

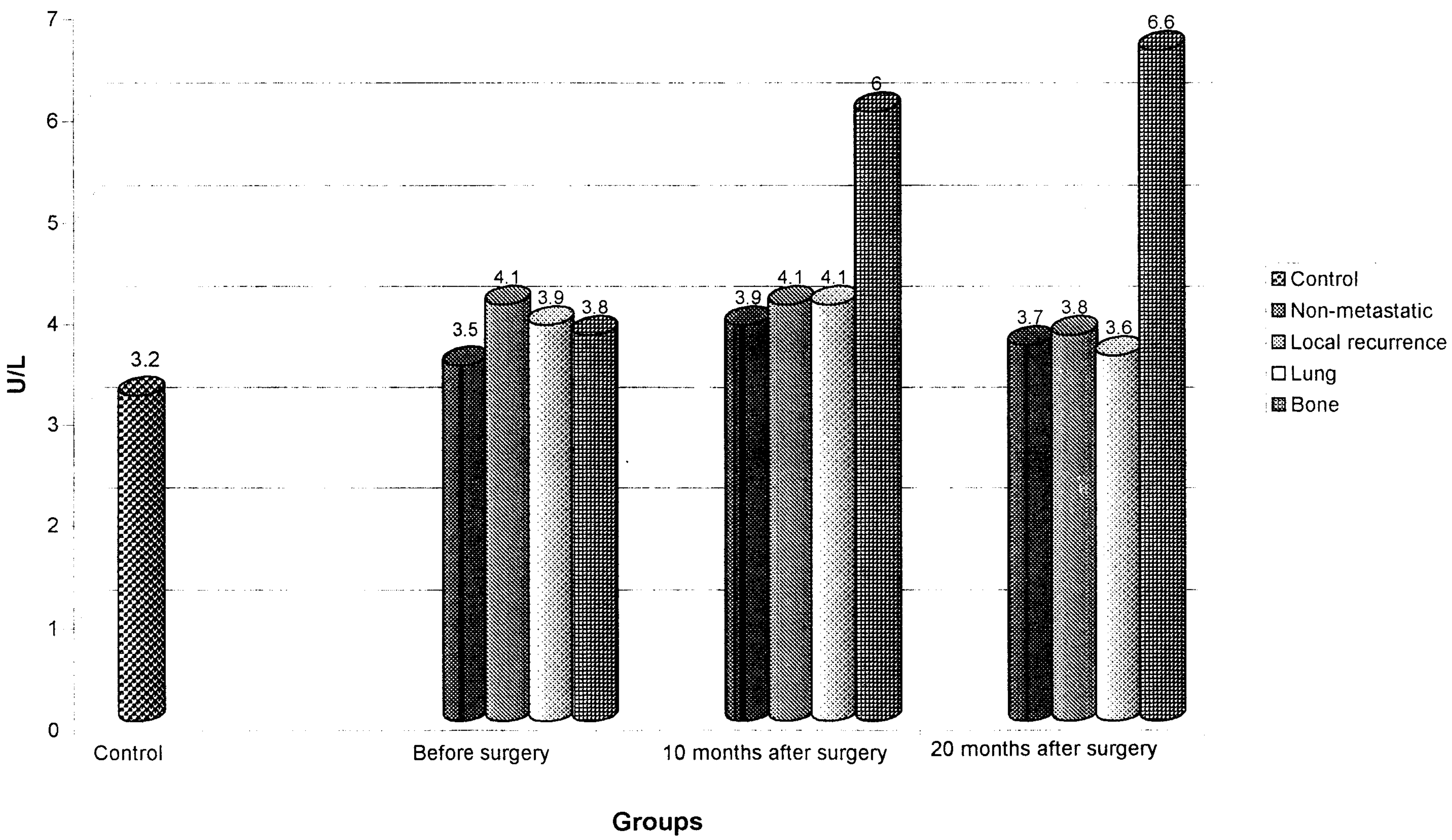
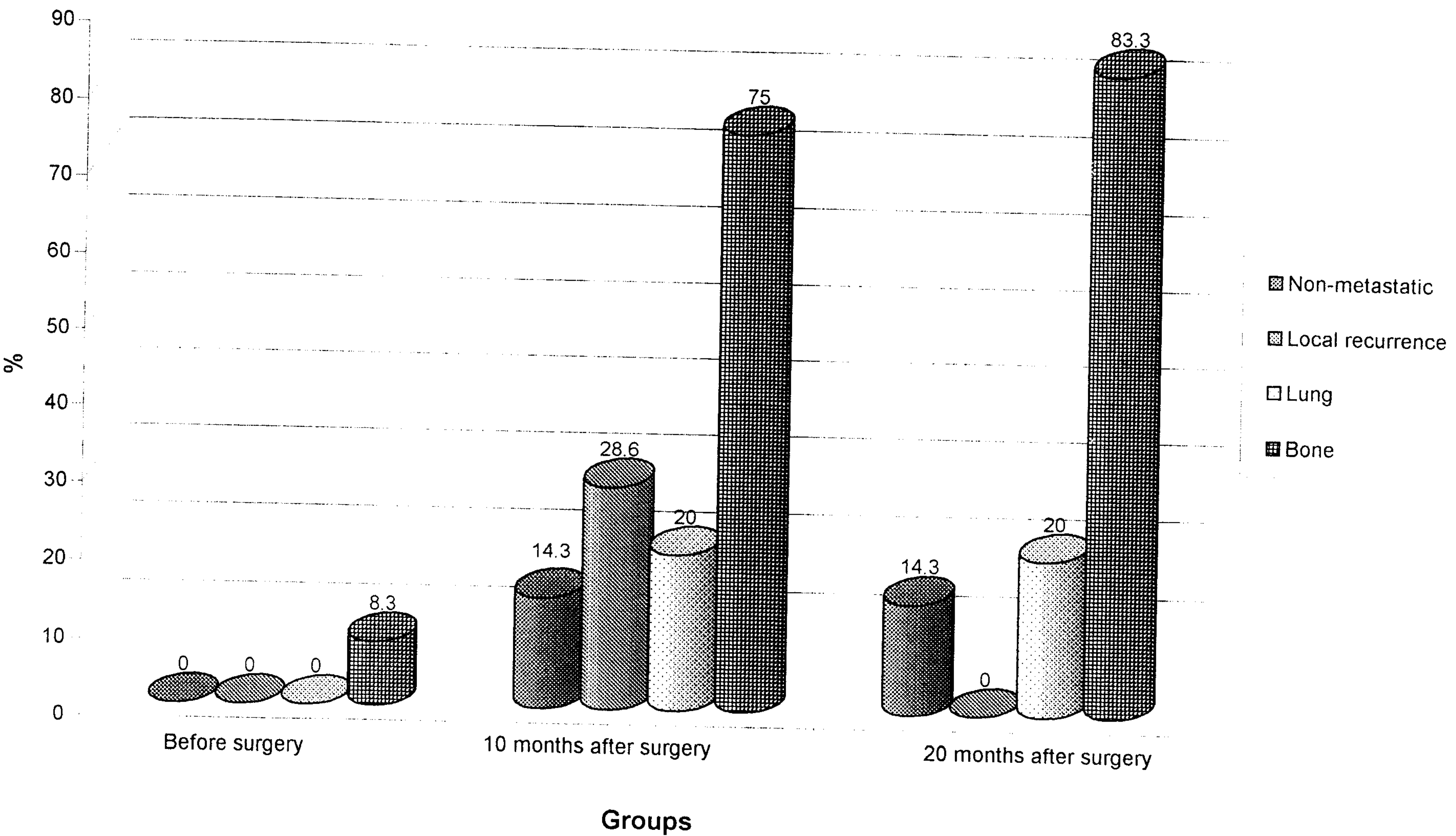


Fig (15) :The percent of elevation in serum bone TRAP 5b in different sub-groups before surgery, 10 and 20 months after surgery.



ICTP and Bone metastases:

Table (4) and fig (16) represents the serum level of ICTP (µg/L) in control. non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery. The mean ± S.E. for control group was 2.8± 0.3 µg/L and for non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery were 3.3 ±0.4, 4.1±0.3, 4.2±0.3 and 5.4±0.6 µg/L respectively. Also, the mean ± S.E. for non-metastatic, local recurrence, lung, bone metastases sub-groups 10 and 20 monthsafter surgery were 3.4 ±0.4, 3.9± 0.4, 4.4± 0.5, 5.8±0.7, 3.5±0.4, 4.1±0.3, 4.2±0.6 and 7.0±0.9 µg/L respectively.

There is no statistically significant difference in the serum level of ICTP between control group and non-metastatic, local recurrence and lung sub-groups before surgery, 10 and 20 months after surgery, p = 0.478, 0.066, 0.058 0.450, 0.167, 0.058, 0.444, 0.188 and 0.180 respectively. While, in the bone metastases sub-group, there was a statistical significant difference between this sub-group and control group before surgery, after 10 and 20 months, p =0.0001, 0.003, 0.0001 respectively.

Table (5) and fig. (17) Shows the percent of elevation in the serum level of ICTP in different sub-groups before surgery, 10 and 20 months after surgery. The percent of elevation in all sub-groups before surgery, after 10 and 20 months were 28.6, 42.9, 60.0, 66.7, 28.6, 42.9, 60.0, 75, 28.6, 28.6, 40.0 and 75% respectively.

Table (4): Serum level of ICTP (μg/L) in control, non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery, 10 and 20 months after surgery.

Sub-groups	(M ±SE)		
	Before surgery	10 months after surgery	20 months after surgery
Non met static	3.3±0.4	3.4±0.4	3.5±0.4
Local recurrence	4.1±0.3	3.9±0.4	4.1±0.3
Lung	4.2±0.3	4.4±0.5	4.2±0.6
bone	5.4±0.6* p = 0.0001	5.8±0.7* p = 0.003	7.0±0.9* p = 0.0001
control	2.8±0.3		

Table (5): The percent of elevation in serum ICTP in different sub-groups before surgery, 10 and 20 months after surgery.

Sub-groups	Number	% of elevations (M ±2SD)		
		Before surgery	10 months after surgery	20 months after surgery
Non met static	7	28.6(2)	28.6(2)	28.6(2)
Local recurrence	7	42.9(3)	42.9(3)	28.6(2)
Lung	5	60.0(3)	60.0(3)	40.0(2)
bone	12	66.7(8)	75(9)	75(9)

Fig (16) :Serum ICTP concentration μg/L in control, non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery, 10 and 20 months after surgery.

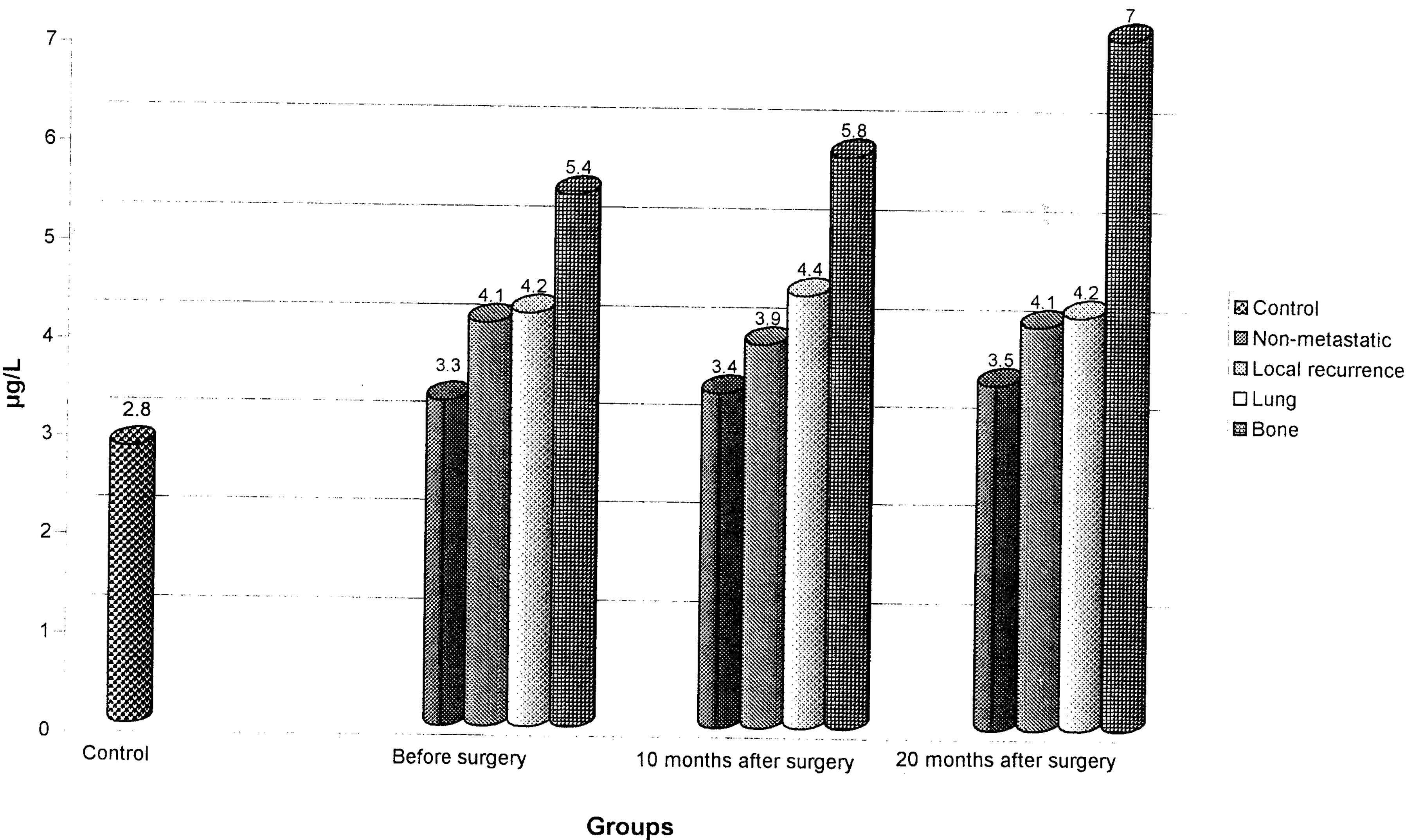
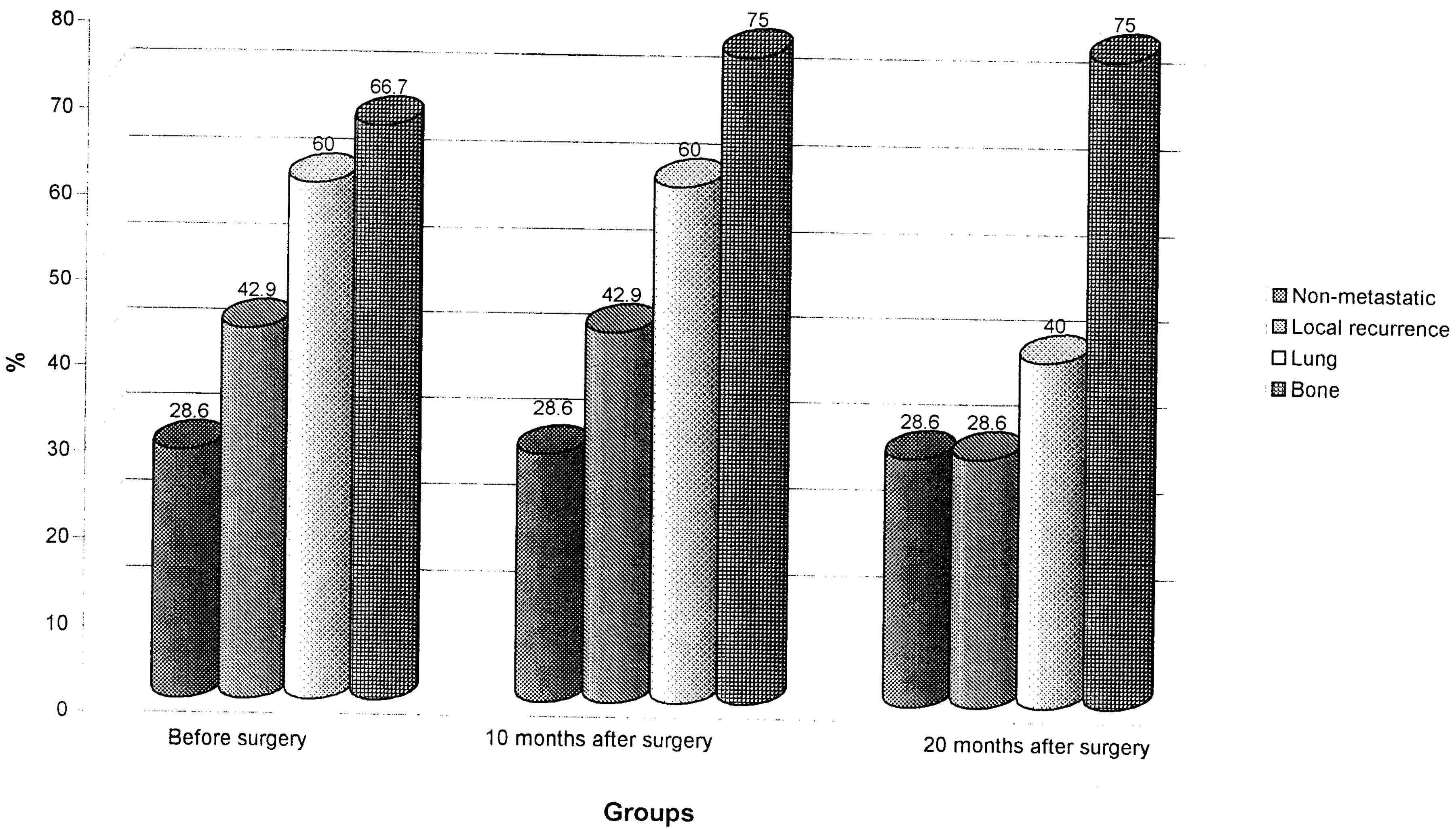


Fig (17): The percent of elevation in serum ICTP in different sub-groups before surgery, 10 and 20 months after surgery.



CA15.3 and Bone metastases:

Table (6) and fig.(18) represents the serum level of CA15.3 (U/L) in control, non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery, 10 and 20 months after surgery. The mean ± S.E. for control group was 15 ± 2.1U/L and for non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery were 16.4 ±3.0, 16.8±2.6, 17.6 ± 3.6 and 18.0±2.4 U/L respectively. Also, the mean ± S.E. for non-metastatic, local recurrence, lung, bone metastases sub-groups after 10 and 20 months were 14.5±2.8, 19.2±4.8, 20.2±3.9, 23.2±3.6, 16.5±2.8,31.0±11.4, 33.8±16.9 and 50.1±12.3 U/L respectively.

There was no statistically significant difference in the serum level of CA15.3 between control group and non-metastatic, local recurrence, lung, bone metastasers sub-groups before surgery and after 10 months, p = 0.723, 0.641, 0.541, 0.363, 0.921, 0.403, 0.354 and 0.066 respectively. Also, There was no statistically significant difference in the serum level of CA15.3 between control group and non-metastatic, local recurrence, lung metastases sub-groups after 20 months, p = 0.921, 0.283 and 0.257 respectively but, there was a statistically significant difference in the serum level of CA15.3 between control group and bone metastases sub-group after 20 months, p = 0.009.

Table (7) and fig.(19) shows the percent of elevation in the serum level of CA15.3 in different sub-groups before surgery, 10 and 20 months after surgery. The percent of elevation in all sub-groups before surgery, after 10 and 20 months were 0, 14.3, 20.0, 16.7, 0, 14.3, 20.0, 33.3, 14.3, 57.1, 40.0 and 58.3% respectively.

Table (6): Serum level of CA15-3(U/mL) in control, non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery, 10 and 20 months after surgery.

Sub-groups	(M ±SE)		
	Before surgery	10 months after surgery	20 months after surgery
Non met static	16.4±3.0	14.5±2.8	16.5±2.8
Local recurrence	16.8±2.6	19.2±4.8	31.0±11.4
Lung	17.6±3.6	20.2±3.9	33.8±16.9
bone	18.0±2.4	23.2±3.6	50.1±12.3* p = 0.009
control	15±2.1		

Table (7): The percent of elevation in serum CA15-3 in different sub-groups before surgery, 10 and 20 months after surgery.

Sub-groups	Number	% of elevations (M ±2SD)		
		Before surgery	10 months after surgery	20 months after surgery
Non met static	7	0	0	14.3(1)
Local recurrence	7	14.3(1)	14.3(1)	57.1(4)
Lung	5	20.0(1)	20.0(1)	40.0(2)
bone	12	16.7(2)	33.3(4)	58.3(7)

Fig (18) :Serum CA15-3 concentration U/ml in control, non-metastatic, local recurrence, lung, bone metastases sub-groups before surgery, 10 and 20 months after surgery.

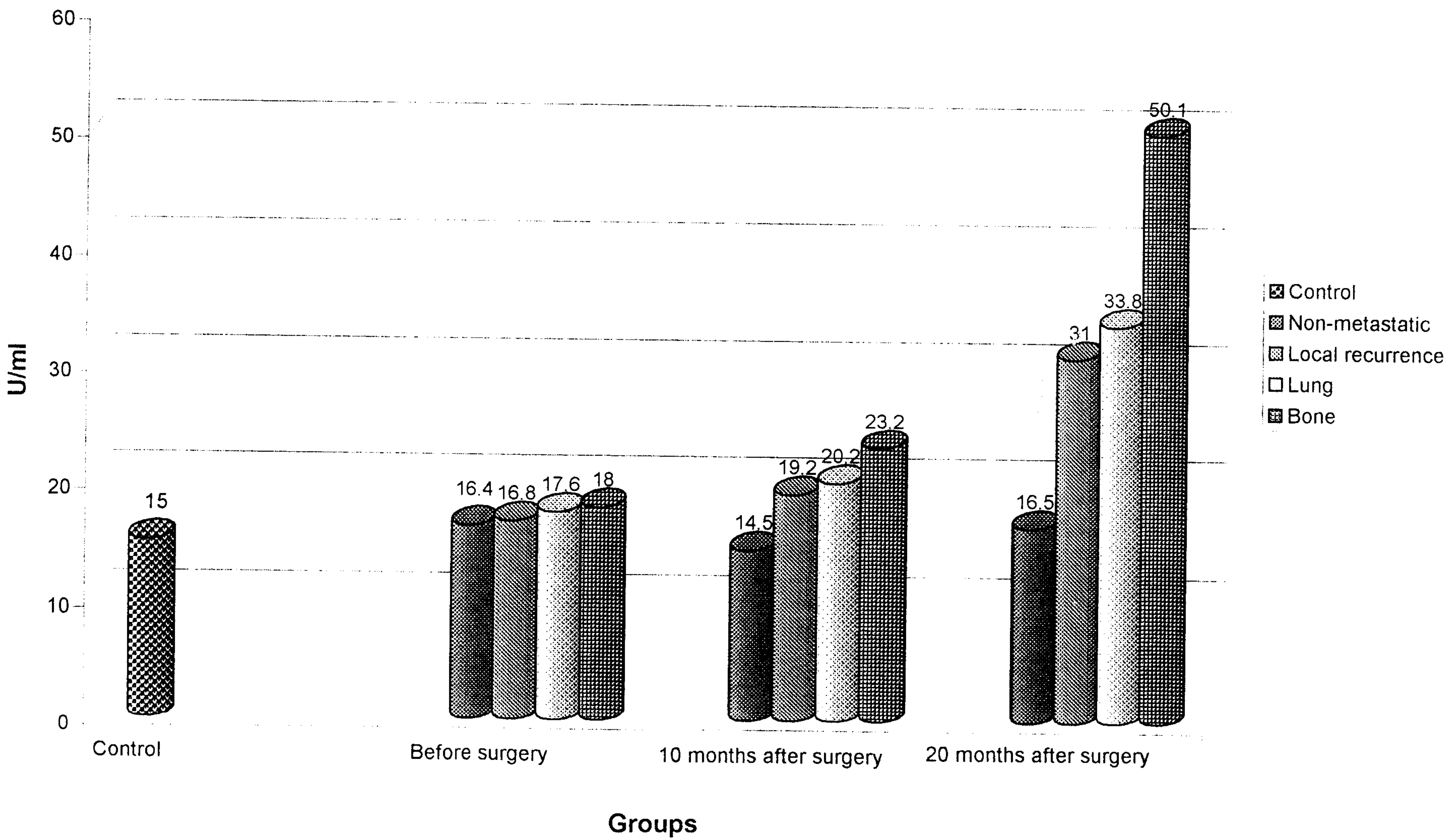
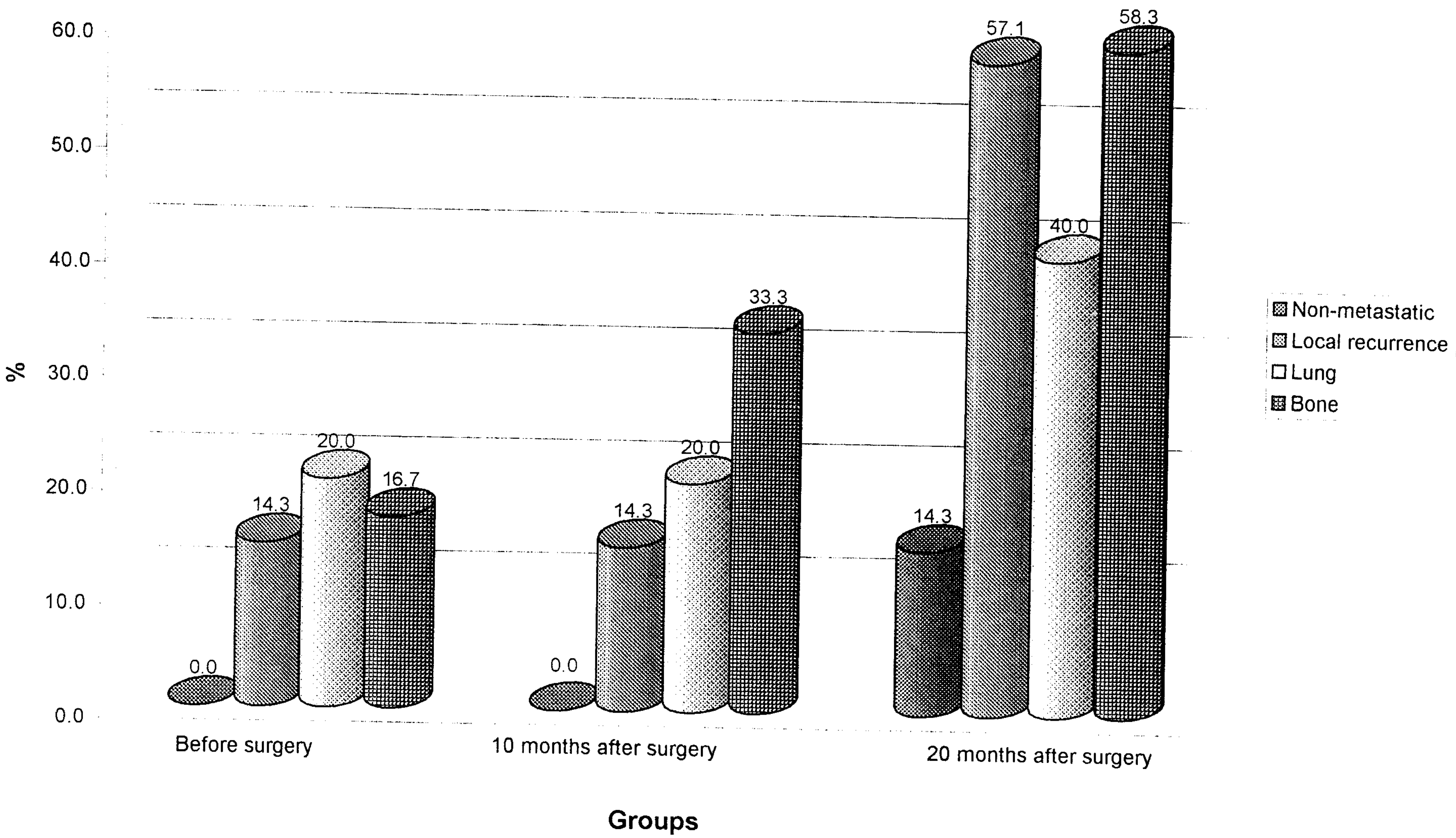


Fig (19): The percent of elevation in serum CA15-3 in different sub-groups before surgery, 10 and 20 months after surgery.



The Overall survival curves:

Fig. (20) represents Overall survival according to serum bone TRAP 5b activity in patients with primary breast cancer after 10 months. There is a statistically significant difference between mean of two groups of patients with Bone TRAP 5b values < and ≥5.4 U/L, where mean of months for patients who have < 5.4 U/L was 32.16 months, while for patients who have ≥5.4 U/L was 23.09 months, log Rank-test = 4.7, p = 0.03. Hazard ratio (HR) = 2.4, P= 0.04; this means that patients with Bone TRAP 5b ≥ 5.4 U/L were at double risk of death compared to patients with low levels. Thus, patients with serum Bone TRAP 5b ≥ 5.4 U/L had a worse overall survival pattern than those with low levels of the marker.

Fig. (21) represents Overall survival according to serum bone TRAP 5b activity in patients with primary breast cancer after 20 months. There is a statistically significant difference between mean of two groups of patients with Bone TRAP 5b values < and ≥ 5.4 U/L, where mean of months for patients who have < 5.4 U/L was 31.81 months, while for patients who have ≥ 5.4 U/L was 24.25 months, log Rank-test = 4.17, p = 0.04. Hazard ratio (HR) = 2.3, p = 0.05; this means that patients with Bone TRAP 5b ≥5.4 U/L were at double risk of death compared to patients with low levels. Thus, patients with serum Bone TRAP 5b ≥5.4 U/L had a worse overall survival pattern than those with low levels of the marker.

Fig. (22) represents Overall survival according to preoperative serum ICTP concentrations in patients with primary breast cancer. There is a statistically significant difference between mean of two groups of patients with ICTP values < and ≥4.6 µg/L, where mean of months for patients who have < 4.6 µg/L was 32.67 months, while for patients who have ≥4.6 µg/L was 25.09 months, log Rank-test = 7.34, p = 0.007. Hazard ratio (HR) = 3.2, p = 0.01; this means that patients with ICTP ≥4.6 µg/L were at trible risk of death compared to patients with low levels. Thus, patients with serum ICTP ≥4.6 µg/L had a worse overall survival pattern than those with low levels of the marker.

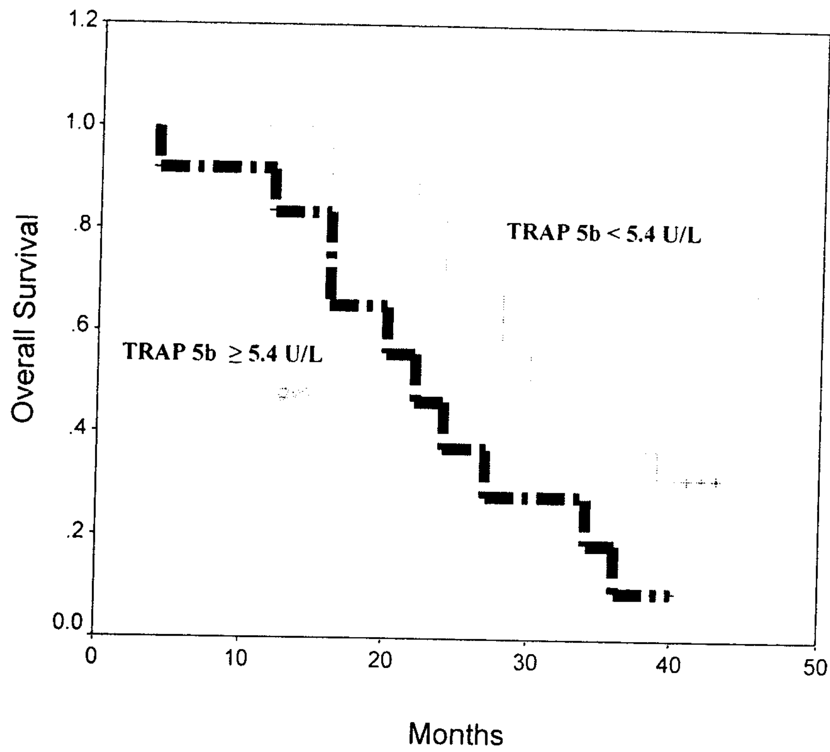


Fig.(20): Overall survival according to serum bone TRAP 5b activity in patients with primary breast cancer after 10 months. Thin line, TRAP 5b <5.4 U/L(n= 19) thick line; TRAP 5b ≥ 5.4 U/L(n= 12). HR = 2.4, p = 0.04.

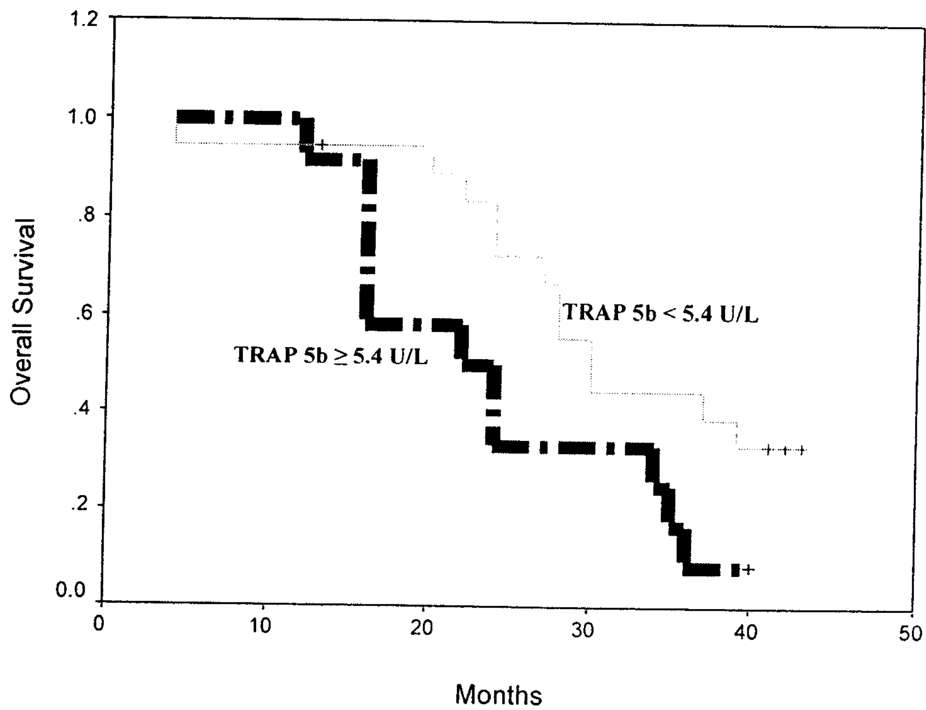


Fig.(21): Overall survival according to serum bone TRAP 5b activity in patients with primary breast cancer after 20 months. Thin line, TRAP 5b <5.4 U/L(n= 19); thick line, TRAP 5b ≥ 5.4 U/L(n= 12). HR = 2.3, p = 0.05.

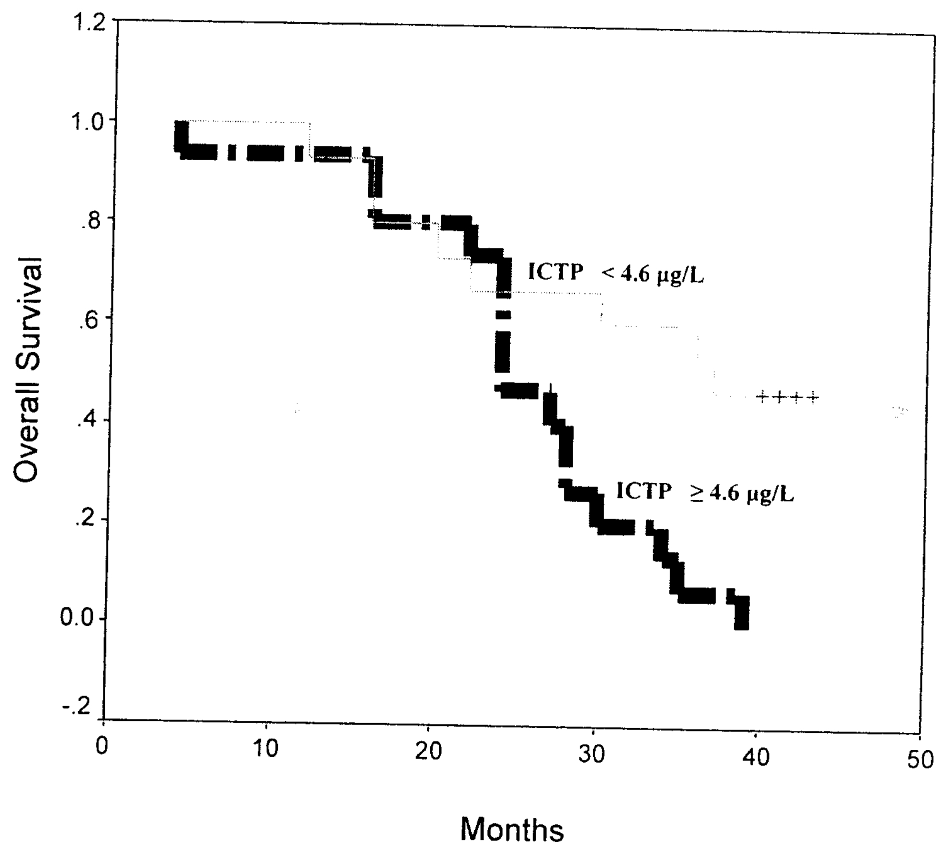


Fig.(22): Overall survival according to preoperative serum ICTP concentration in patients with primary breast cancer. Thin line, ICTP <4.6 μg/L(n= 15); thick line, ICTP ≥ 4.6 μg/L(n= 16). HR = 3.2, p =0.01.

Disease Free Survival:

Fig. (23) represents Disease Free Survival according to serum bone TRAP 5b activity in patients with primary breast cancer after 10 months. There is a statistically significant difference between mean of two groups of patients with Bone TRAP 5b values < and ≥5.4 U/L, where mean of months for patients who have < 5.4 U/L was 35.74 months, while for patients who have ≥5.4 U/L was 21.33 months, log Rank-test = 7.18, p = 0.0074. Hazard ratio (HR) = 4.3, p = 0.017; this means that patients with Bone TRAP 5b ≥5.4 U/L were at four times risk of developing bone metastases compared to patients with low levels. Thus, patients with serum Bone TRAP 5b ≥5.4 U/L had a worse Disease Free Survival pattern than those with low levels of the marker.

Fig. (24) represents Disease Free Survival according to preoperative serum ICTP concentrations in patients with primary breast cancer. There is a statistically significant difference between mean of two groups of patients with ICTP values < and ≥4.6 µg/L, where mean of months for patients who have < 4.6 µg/L was 36.3 months, for patients who have ≥4.6 µg/L was 17.0 months, log Rank-test = 5.16, p = 0.02. Hazard ratio (HR) = 3.9, p= 0.04; this means that patients with ICTP ≥4.6 µg/L were at four times risk of developing bone metastases compared to patients with low levels. Thus, patients with serum ICTP ≥4.6 µg/L had a worse Disease Free Survival pattern than those with low levels of the marker.

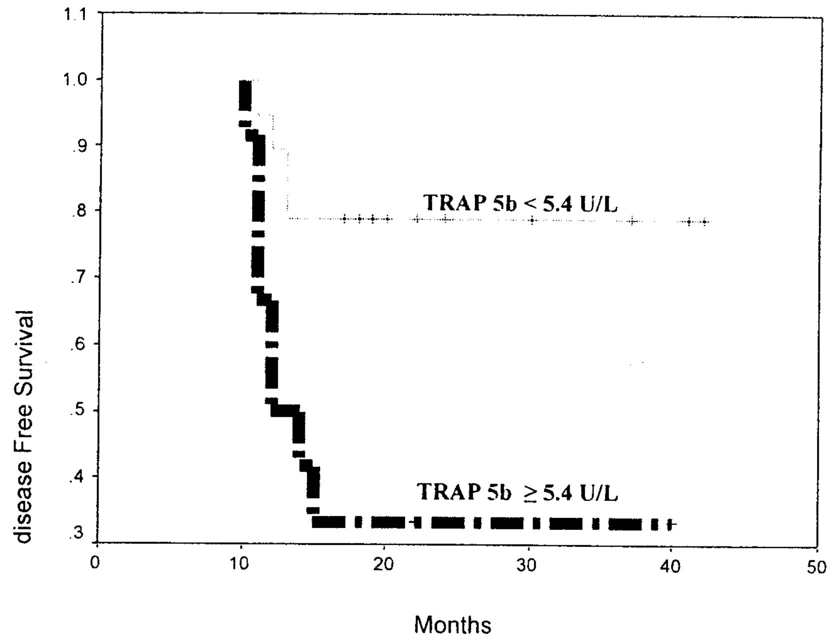


Fig.(23): Disease free survival according to serum bone TRAP 5b activity in patients with primary breast cancer after 10 months. Thin line, TRAP 5b <5.4 U/L(n= 19); thick line, TRAP 5b ≥ 5.4 U/L(n= 12). HR = 4.3, p = 0.017.

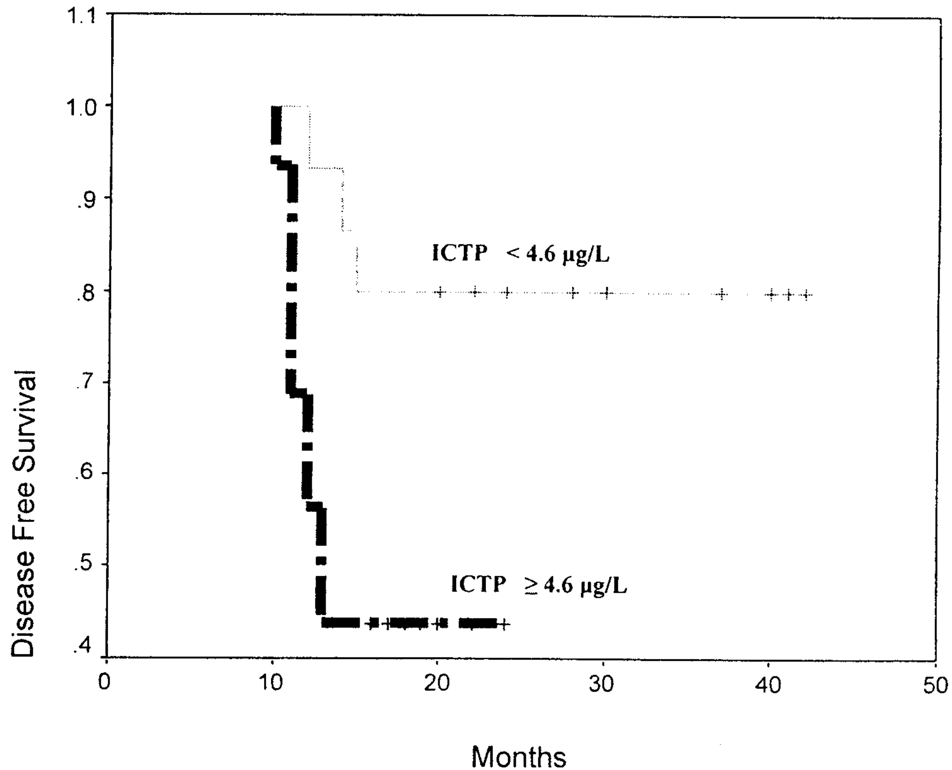


Fig.(24): Disease free survival according to serum ICTP concentration in patients with primary breast cancer before surgery. Thin line, ICTP < 4.6 μg/L(n= 15); thick line, ICTP ≥ 4.6 μg/L(n= 16). HR = 3.9; p = 0.04.

Discussion

DISCUSSION

DISCUSSION

Breast cancer is the most frequent cancer in women and represents the second leading cause of cancer death among women (after lung cancer). [136] Metastatic spread of tumors continues to be a major obstacle to successful treatment of malignant tumors. Approximately 30% of those patients diagnosed with a solid tumor have a clinically detectable metastasis and for the remaining 70%, metastases are continually being formed throughout the life of the tumor. Even after the tumor is excised, the threat of death is attributable to the metastasis that may occur through the remaining tumor cells. [137]

Coleman had reported that nearly 70% of patients dying with breast cancer have bone metastases. [139]

In the current study, 31 patients with primary breast cancer were followed up from preoperative until developing different types of metastases. During the follow up 22.6% (7) was free from disease, 22.6% (7), 16.1% (5), 38.7% (12) developed local recurrence, lung and bone metastases respectively.

Metastatic bone disease develops as a result of the many interactions between tumor cells and bone cells. This leads to disruption of normal bone metabolism, with the increased osteoclast activity by cancer cells that have migrated to the bone microenvironment. [139, 140] Indeed, results of immunohistochemistry using antibody against tartrate-resistant acid phosphatase (TRAP) show a layer of osteoclasts between the bone matrices and tumor cells in tissue samples taken from both nude mice and humans. Therefore, the measurement of bone resorption markers, both collagen degradation products as ICTP and osteoclast-secreted proteins such as TRAP, is thought to reflect the bone resorption process produced by bone metastases. [141]

TRAP and bone metastases:

Tartrate-resistant acid phosphatase is described as a marker enzyme of bone resorbing osteoclasts, where the increase in osteoclast activity is accompanied by an increase in the synthesis and secretion of type TRAP 5b. [43, 72] This enzyme is an iron-containing protein, exists as isoforms 5a and 5b, whereas exclusively the active isoform 5b of TRAP is specifically associated with osteoclasts, while inactive isoform 5b of the enzyme is released by macrophages, whereas the origin of 5a is unknown. [63, 72]

TRAP 5b is secreted by Osteoclasts into the blood circulation as an enzymatically active form that is rapidly inactivated before removal. Thus, TRAP 5b activity does not accumulate into the circulation in renal or hepatic failure. Diurnal variability of serum TRAP 5b activity is low and the levels are not affected by feeding, allowing sample collection at any time of day. [40, 72]

In this study, there was no statistically significant difference in the serum activity of bone TRAP 5b between the control group and preoperative patients group. So, it could not be used as a preoperative prognostic marker of bone metastases from breast cancer.

In addition, there was no statistically significant difference in the serum activity of bone TRAP 5b between the control group and 10 and 20 months after surgery in non-metastatic, local recurrence, lung metastases sub-groups. While, after 10 and 20 months from surgery, there was a statistically significant difference in the serum activity of bone TRAP 5b between the control group and the patients' sub-group who developed bone metastases during follow up.

The M ± SE level of bone TRAP 5b among the control group was 3.2±0.4U/L and in bone metastases sub-group after 10 and 20 months were 6.0±0.2 and 6.6±0.3U/L ($P<0.05$). For bone sub-group, the percent of supranormal values (>M+2SD of the age matched control) were about 75 and 83.3% after 10 and 20 months from surgery, respectively. So, bone TRAP 5b may be used as a highly predictive marker of bone metastases from breast cancer.

TRAP 5b had a good prognostic role in bone metastases and might be useful in identify patients who need antiresorptive treatment from the start before being metastatic.. In addition, it may be a good diagnostic marker of bone metastases.

Moreover, we were able to show that , after 10 months from surgery, patients with serum bone TRAP 5b values in excess of two SD of the normal value for control group (≥ 5.4U/L) had a four fold higher risk of developing skeletal metastases than those who had serum activity <5.4U/l. Furthermore, after 10 and 20 months from surgery, patients with serum Bone TRAP 5b values ≥ 5.4U/L had shorter overall survival by two times of risk than those who had serum bone TRAP 5b values <5.4U/l. Thus, it is a worthwhile bone resorption marker. This agrees with many previously reported results.[71,143-148]

Chao and his colleages reported that the study included 208 breast cancer patients, who were sub grouped into breast cancer with bone metastases (BCBM), nonosseous metastatic breast cancer (NoMBC), and early breast cancer without any metastasis (EBC). They observed that the mean serum TRAP 5b activity of BCBM, NoMBC, EBC patients, and normal women were 6.78, 3.22, 3.05, and 2.12, respectively. Serum TRAP 5b activity of BCBM patients is significantly higher than that of BC patients without BM and normal volunteer. Therefore, TRAP 5b activity can be considered a surrogate indicator of BM in BC patients. Prospective longitudinal studies are warranted to gauge the value of TRAP 5b as a means for early detection of bone involvement and to monitor anti-resorptive therapy in such patients. [143]

Capeller et al. reported that TRAP 5b serum levels were measured in 192 samples from patients with breast cancer with and without bone metastases and in 53 healthy women using the enzyme immunoassay Bone-TRAP. They found that serum TRAP 5b levels are elevated in patients with bone metastases and breast cancer compared to healthy women. Therefore, active TRAP 5b seems to be a useful serum marker for bone metastases in breast cancer patients. [146]

Lyubimova et al. observed that serum activity of TRAP 5b in patients with breast cancer having bone metastases was much higher than in healthy donors and patients without skeletal injuries. TRAP 5b activity in patients with breast cancer and multiple bone metastases surpassed than in patients with single bone metastases. Diagnostic sensitivity and specificity of TRAP 5b as a marker of skeletal metastases in patients with breast cancer were 82 and 87%, respectively. Therefore, detection of this marker in tumor patients holds much promise for early diagnostics of bone metastases, estimation of the severity of skeletal metastases. [147]

Chao et al. reported in a study consisted of 30 early breast cancer patients without bone metastasis and 30 age-matched breast cancer patients with bone metastases together with 60 normal control volunteers. This result showed that the overall mean TRAP 5b activity in normal women (M±SD; 2.83±1.1 U/l) did not differ from that of the mean TRAP 5b activity in early breast cancer patients (2.93±0.64), $p = 0.66$. While, mean TRAP 5b activity in breast cancer patients with bone metastases was significantly higher than in

normal group (5.42±2.5 vs. 2.83±1.1 U/l), $p < 0.0001$. Therefore, TRAP 5b activity can be considered a surrogate indicator of bone metastases in breast cancer patients. [144]

On the other hand, the present results were in a disagreement with those of Ebert et al., 2004 who reported that the study consisted of 49 patients with bone metastasis confirmed by plain radiography and/or computed tomography, 89 patients without bone metastasis, 12 patients with benign lung diseases and 18 healthy persons. All patients were of male gender. There was no significant difference in the TRAP 5b levels between the two groups. [149]

Ek-Rylander et al. reported that TRAP can remove phosphate groups from osteopontin, an event that consequently disrupts adhesion of osteoclasts to the bone. This suggests that the enzyme might regulate osteoclast adhesion to the bone and also enable migration of osteoclasts to adjacent sites of resorption. Of further interest, the bone matrix is rich in pyrophosphate, a known inhibitor of bone resorption. TRAP can hydrolyse and therefore liberate pyrophosphate from the bone matrix. This hydrolysis event would enable bone resorption activity by osteoclasts to begin. [75] These events help in explaining how the TRAP 5b increase bone resorption. Halleen et al concluded that bone matrix proteins are destroyed within transcytotic vesicles by TRAP facilitated formation of ROS and the subsequent degradation of bone proteins by the ROS generated. [50] Therefore, these events may help to explain why the concentration of TRAP increases in the circulation during bone resorption. [43, 46]

ICTP and bone metastases:

The carboxy terminal cross linked telopeptide of type I collagen (ICTP) seems to represent a new marker of type I collagen degradation. It is released into the circulation during degradation of mature type I collagen primarily in bone and therefore reflects the degree of bone resorption and the severity of metabolic bone disease. [150, 151]

A significant relation between ICTP and the degree of bone resorption, as assessed by histomorphometry, has been described in patients with different types of metabolic bone diseases. The sites of degradation of ICTP are unknown. ICTP is a small molecule with a molecular weight of 9–12 kDa and may therefore be eliminated by the kidneys. [150]

Results of the present study revealed that there was no statistically significant difference in the serum concentration of ICTP between the control group and different sub-groups of patients before surgery, 10 and 20 months after surgery, except patients who developed bone metastases during follow up.

The M ± SE level of ICTP in the control group was 2.8±0.3 µg/L and among bone metastases sub-group was 5.4±0.6, 5.8±0.7 and 7.0 ±0.9 µg/L before surgery, after 10 and 20 months, respectively ($P < 0.05$). For bone sub-group, the percent of supranormal values were about 66.7, 75 and 75% before surgery, after 10 and 20 months, respectively. Suggesting that ICTP might be an excellent bone resorption marker in prognosis, diagnosis and monitoring.

In the present study, we found that an augmentation of the time from marker increase to the appearance of clinical or radiological signs of bone metastasis (preoperative, 10 and 20 months). Therefore, ICTP could be used in prediction of bone metastases from primary breast cancer and might be useful in identify patients who need proper antiresorptive treatment before being metastatic. Furthermore, it may be a good diagnostic marker of bone metastases.

In our study of the prognostic value of serum ICTP in patients with primary breast cancer, we were able to show that preoperative patients with ICTP values in excess of two SD of the normal value for control group (≥4.6 μg/L) had a bad prognosis and were at four times risk of death relative to those who had ICTP values <4.6 μg/L . In addition, patients with serum ICTP concentrations ≥4.6 μg/L had a three time risk for developing skeletal metastases than those with ICTP <4.6 μg/L. Therefore, it is thought that ICTP would be used as a prognostic and diagnostic marker of bone metastases from breast cancer.

This is in agreement with the result previously reported. [98,118,120,151-156]

Yamamoto et al. who reported that the 43 patients under investigation (41 of them are breast cancer), serial serum samples were obtained during 0.5 to 2.4 years (mean 1.4 years). In all of nine patients, who developed bone metastasis, serum ICTP level increased, and by successful treatment to the metastatic lesions, ICTP level decreased, while in some cases, tumor markers remained to be elevated. Thus, serial measurement of ICTP is suggested to be useful for detection and evaluation of therapeutic responses in patients with bone metastasis.[151]

In the study of Maemura M et al. ICTP was examined in 83 patients with metastatic breast cancer. ICTP levels were significantly higher in patients with bone metastases than in those without bone metastasis. In patients with bone metastasis, significantly higher ICTP levels were observed in those with multiple lesions than in those with a solitary lesion. Sequential monitoring of ICTP revealed that this elevation was correlated with disease progression. Combined with imaging studies, monitoring of ICTP appears to offer additional information for detection of bone metastasis. [98]

The study of Ulrich et al. was consisted of 106 patients with breast cancer. Based on scintigraphic and radiological findings, patients were divided into 3 groups: 19 patients with bone metastases, 65 patients without bone metastases and normal bone scintigrams, and 22 patients with pathological, non-malignant findings on scintigraphy without proof of bone metastases. ICTP was significantly higher in patients with bone metastases compared to both patients without skeletal recurrence and those with pathological, non-malignant scintigraphic findings. There were no statistically significant differences between the latter two groups. The clinical sensitivity for diagnosing bone metastases was 65% for ICTP. The clinical specificitiy for discriminating patients with bone disease from those without were 91%. [119]

Chinchilli V et al. who reported that the serum samples from 254 metastatic breast cancer patients (182 with bone metastases BM+, 72 without bone metastases BM-) were obtained to measure ICTP concentrations. The mean follow–up was 868 days (264-1466). The normal level was 3.0+1.6 μg/l (M+ SD), the cutoff value was 6.2 μg/l (M+2SD), by using this cutoff 62/182 had elevated ICTP concentrations in BM+, While 6/72 in BM- .The mean serum ICTP levels were significantly higher in BM+ compared to BM- (P= 0.0002). Overall survival was significantly worse in patients who had elevated level of ICTP in BM+ and BM-, P=0.0001 and 0.02, respectively. [156]

One hundred and eighty-four breast cancer patients without advanced disease were enrolled in the study of Keskikuru et al. They reported that sixty-five out of the 184 patients developed metastatic disease during the follow-up. The median follow-up time was 62 months (range 6-111 months). Preoperatively elevated serum ICTP is a prognostic factor for Overall and Disease free survival in breast cancer and the measurement of which should improve the accuracy of predictions of the clinical outcome. [157]

On the other hand, the results disagrees with many previous reports. [118,158,159]

Kiuchi et al. who reported that ICTP is not sensitive enough to detect an early stage of bone metastasis from breast cancer. [158]

Schoenberger et al were. prospectively studied 88 patients aged 21 - 82 years with malignant tumors. The serum concentrations of ICTP was measured and compared to the results of bone scintigraphy, radiological bone series, CT, MRI and clinical follow-up. Osseous metastases were found in 21 patients, 19 of them were correctly identified by bone scintigraphy (sensitivity: 90%). ICTP sensitivity: 71%, specificity: 42%. So, ICTP showed low sensitivity and/or specificity for the detection of osseous metastases and did not seem to be sufficient enough to identify patients with bone metastases or to replace established screening methods. [118]

CA15-3 and bone metastases:

CA 15-3 is the most widely used serum marker in breast cancer. Currently, its main uses are in the surveillance of patients with diagnosed disease and monitoring the treatment of patients with advanced disease. [129]

CA15-3 is a double-determinant radioimmunometric assay that detects a breast cancer-associated mucin of 300 to 450 kD that bears epitopes recognized by two murine monoclonal antibodies. [160]

Results of the present study revealed that there was no statistically significant difference in the serum concentration of CA15-3 between the control group and patients before surgery, after 10 and 20 months but after 20 months, there was a statistically significant difference between controls and patients whom developed bone metastases during follow up.

The M ± SE level of CA15-3 in the control group was 15±2.1 U/mL and in bone metastases sub-group after 20 months was 50.1±42.7 U/mL ($P < 0.05$). For bone sub-group, the percent of supranormal value was about 58.3% after 20 months from surgery. Suggesting that CA15-3 cannot be sensitive enough to be used for screening and early diagnosis of primary breast cancer but may be used as a monitoring parameter to the response of therapy in bone metastases.This finding agrees with the other results reported. [161-170]

Sanchez GRE et al. [165] repoted that they reviewed the files of 100 female patients in the Nuclear Medicine Department, which was done bone scan and CA 15-3 from January to December 2000. All patients were in stage III and IV. The mean patient's age was 59.39 years. The mean value of CA 15-3 for the patients with the absence of metastatic disease is 16.18 U/mL and 164.02 U/mL in the presence of metastatic disease ($p < 0.00001$). The high level was 35 U/mL. The sensitivity founded was 82% and the specificity was 87%. In patients with metastatic disease and normal CA 15-3, the tumor marker will increase gradually. Therefore, CA 15-3 can be use as a simple method that reflects the presence of bone metastases in association with bone scan.

Yildiz et al. [167] studied 98 female breast cancer patients when underwent bone scintigraphy during follow-up. In these patients, values of CA15-3 was compared with the results of bone scintigraphy. resulting in 49 patients with bone metastasis and 74 patients without bone metastasis being included in the study. In patients with bone metastasis, serum CA 15-3 concentrations was elevated above the cut-off in 33/49 cases. Among

patients without bone metastasis, CA 15-3 serum concentrations were normal in 55/74 cases. Therefore, CA15-3 measurements do not allow prediction of the bone scan results and it is not justifiable to reject a bone scintigraphy on the basis of this marker.

Seregni E et al. (168) showed that CA15-3 was not sensitive enough to be used for screening and early diagnosis of primary breast cancer.

The study of Begic A et al. (170) included 25 patients with breast cancer, previously surgically treated. All patients underwent total body scintigraphy. Presence, number and location of bone metastases were correlated with CA15-3 levels. Bone scintigraphy revealed bone metastases in 16 (64%) patients. 11 (44%) patients with metastases had elevated CA15-3 levels. Since no significant correlation was found between level of CA15-3 and number of metastases, they consider scintigraphy an appropriate method for assessment of bone metastases in breast cancer.

On the other hand, the results disagree with many previously authors (129.171-173)

Nishimura R et al. (173) who reported that Two hundred-twenty deceased patients who had had recurrent or advanced breast cancer were enrolled in a retrospective study. Serum CA 15-3 was measured regularly during the clinical course until death. The rate of positivity was 50.9% at the time of recurrence, and rose to 76.8% after recurrence. Serum CA15-3 status correlated significantly with survival after recurrence. Multivariate analysis revealed that CA15-3 status was one of the significant factors for survival after recurrence.

Duffy et al. (129) who reported that the Preoperative CA 15-3 concentrations were measured prospectively in 600 patients with histologically confirmed breast cancer. After a median follow-up of 6.27 years, patients with high preoperative concentrations of CA 15-3 (>30 units/L) had a significantly shorter overall survival pattern than those with low concentrations. Therefore, Assay of CA 15-3 is a relatively inexpensive, convenient, and noninvasive method for evaluating prognosis in newly diagnosed breast cancer patients.

Summary

SUMMARY

SUMMARY

Breast cancer is the most frequently diagnosed cancer in women. Worldwide, it is a major public health problem. One-third of cancer patients will have metastases of their primary tumor to bone. The incidence of bone metastases from primary breast cancer is approximately 73%. Patients with breast cancer, for example, have a four- to five fold higher rate of vertebral fracture than those with age-matched women.

Bone consists of two physically and biologically distinctive Structures: cortical (compact) and trabecular (spongious). The outer cortical bone is hard mineralized matrix in which cellular and metabolic activities are relatively low. Cortical bone makes up 85% of the total bone in the body and Cancellous or trabecular bone constitutes the remaining 15% of the skeleton. Cortical bone functions as a mechanical support, while trabecular bone is involved in metabolic processes.

The cellular part of bone is composed of osteoblasts and osteoclasts. Osteoblasts are responsible for production and subsequent mineralisation of bone matrix. Osteoclasts are responsible for bone resorption. Bone growth and remodelling are normal physiological events that occur at a high rate throughout childhood and adolescence, and to a much lesser extent during adult years. It is the net result of the activity of two types of bone cell, which have opposing actions: those that synthesise new bone material, mainly, osteoblasts, and cells called osteoclasts, which are responsible for resorbing or breaking down existing bone material. Two processes are balanced to maintain the homeostasis of bone tissue throughout life. The normal balance between resorption and formation is disturbed by cancer. Because when the tumor has metastasized to bone, it can directly alter the bone and cause lesions that may be lytic (due to increased resorption), blastic (due to increased bone formation) or a combination of both, causing a mixed lesion.

Currently, imaging techniques are the leading methods used to diagnose of metastasis to bone. However, these techniques are expensive, expose patients to toxic and radioactive compounds, and monitor response to treatment poorly; these drawbacks have prompted the search for alternative screening methods. Therefore, bone metabolic markers have been evaluated as possible methods to diagnose and monitor the development and progression of metastatic bone disease. Although bone metabolic markers are often grouped as either resorption or formation markers. Bone marker measurements are noninvasive, inexpensive, and can be repeated often. Major changes occur in a short time. Markers are derived from both cortical and trabecular bone and reflect the metabolic activity of the entire skeleton.

Tartrate-Resistant Acid Phosphatase (TRAP) 5b is a marker of osteoclastic activity and bone resorption. Osteoclasts secrete TRAP 5b into the blood circulation as an active enzyme that is inactivated and degraded to fragments before it is removed from the circulation. Thus, TRAP 5b activity does not accumulate into the circulation in renal or hepatic failure. All serum TRAP 5b activity is derived from osteoclasts. Diurnal variability of serum TRAP 5b activity is low and the levels are not affected by feeding, allowing sample collection at any time of day. TRAP-5b might be a suitable marker to evaluate status of bony metastases in patients with breast cancer and might provide a reference to therapeutic response in this patient group. Active TRAP 5b seems to be a useful serum marker for bone metastases in breast cancer patients, especially to detect progressive disease.

ICTP is the carboxyterminal telopeptide region of type I collagen. It seems to be the leading marker in bone resorption and the promising indicator in detecting bone metastases from primary breast cancer. It is released into the circulation during degradation of mature type I collagen primarily in bone. Therefore, it reflects the degree of bone resorption and the severity of metabolic bone disease. It is produced under the actions of matrix metalloproteinases mainly MMp 2 and MMP 13.

Serum CA15.3 is elevated in 54–80% of patients with metastatic breast cancer. The CA 15-3 assay measures the protein product of the MUC1 gene. The preoperative CA15-3 concentrations are rarely elevated in patients with primary breast cancers. It might appear reasonable to use this marker to monitor response to treatments.

The goal of this study is to determine serum Tartrate Resistant Acid Phosphatase 5b(TRAP 5b), Carboxy-terminal telopeptide of type I collagen (ICTP) and CA15.3 preoperatively in patients with primary breast cancer to asses their values as prognostic factors for the subsequent metastatic disease.

This study included 41 persons divided into two groups; Group I: included 31 primary breast cancer patients and Group II: included 10 control of matched age and sex. Three serum samples were obtained from each patient in the group I; before surgery, after 10 and 20 months from surgery. One serum sample was collected from each person in the group II. Bone TRAP 5b, ICTP and CA15-3 assays were measured in the serum samples using Enzyme Immunoassay techniques.

Results of our study revealed that 22.6% (7) was free from disease, 22.6 (7), 16.1(5), 38.7(12) developed Local Recurrence, Lung and Bone metastases. Thus, on the bases of these results, group II was divided into sub-groups according to the metastatic sites appeared.

There is no statistically significant difference in the serum level of Bone TRAP 5b between control group and all sub-groups before surgery. There is no statistically significant difference in the serum level of Bone TRAP 5b between control group and Non-metastatic, Local Recurrence, Lung sub-groups after 10 and 20 months. While, in the bone metastases sub-group, there is a statistical significant difference between this sub-group and control group after 10 and 20 months. Patients with Bone TRAP 5b $\geq$5.4 U/L were at double risk of death and at four times risk of developing bone metastases compared to patients with low levels of the marker. Therefore, Bone TRAP 5b could be used as a prognostic and diagnostic marker of bone metastases from primary breast cancer.

There is no statistically significant difference in the serum level of ICTP between control group and Non-metastatic, Local Recurrence and Lung sub-groups before surgery, after 10 and 20 months. While, in the bone metastases sub-group, there is a statistical significant difference between this sub-group and control group before surgery, after 10 and 20 months. Patients with preoperative ICTP $\geq$4.6 µg/L were at trible risk of death and at four times risk of developing bone metastases compared to patients with low levels of the marker. Therefore, preoperative serum ICTP concentrations would be highly predictive of subsequent skeletal metastases and might identify patients whom need antiresorptive treatments with chemotherapy. In addition, it could be useful in diagnosis of bone metastases from breast cancer.

There is no statistically significant difference in the serum level of CA15.3 between control group and Non-metastatic, Local Recurrence, Lung, Bone metastasers sub-groups

before surgery and after 10 months. There is no statistically significant difference in the serum level of CA15.3 between control group and Non-metastatic. Local Recurrence. Lung metastases sub-groups after 20 months but, there is a statistically significant difference in the serum level of CA15.3 between control group and Bone metastases sub-group. Thus, it may be used in the follow up of patients.

CONCLUSION

CONCLUSION

- The percentage of bone metastases from primary breast cancer was 38.7%.
- Bone TRAP 5b could be used as a specific prognostic marker of subsequent bone metastases from primary breast cancer especially serum samples collected after 10 months from surgery.
- Bone TRAP 5b would be used as a highly specific diagnostic marker of bone metastases from primary breast cancer.
- Patients with Bone TRAP 5b ≥ 5.4 U/L were at double risk of death and four times risk of subsequent bone metastases.
- preoperative serum ICTP concentrations might be highly predictive for subsequent skeletal metastases and might identify patients who need antiresorptive treatments.
- ICTP might be used as a prognostic marker of bone metastases from primary breast cancer especially serum samples collected after 10 months from surgery.
- ICTP could be useful in diagnosis of bone metastases from primary breast cancer
- Patients with ICTP ≥ 4.6 µg/L were at trible risk of death and four times risk of subsequent bone metastases.
- CA15-3 may be used in the follow-up of patients.

REFERENCES

REFERENCES

1. Boring CC, Squires TS, Tong T and Monegomery S: Cancer statistics, 1993.Cancer J Clin 1994; 44:7-26.
2. Fidler IJ: Invasion and Metastasis. In: Clinical Oncology. Abeloff MD, Armitage JO, Lichter AS and Niederhuber JE (eds.) Churchill Livingstone, New York, 1995.pp55-69.
3. Ilic D, Genbacev O, Jin F, Caceres E, Almeida EA, Dubouchaud BV, Schaefer EM, Damsky CH and Fisher SJ: Plasma membrane-associated pY397FAK is a marker of cytotrophoblast invasion in vivo and in vitro. Am J Pathol 2001; 159: 93-108.
4. Reddi AH, Roodman D, Freeman C and Mohla S: Mechanisms of Tumor Metastasis to the Bone: Challenges and Opportunities. J Bone Miner Res 2003; 18: 190-4.
5. Thersea GA and Mundy GR: Cancer and Bone. Endocrine Review 1998; 19: 18-54.
6. Casey JJ, Stempel BG and Scanlon EF: The Solitary pulmonary nodule in the patient with breast cancer. Surgery 1984; 96: 801-5.
7. McDonald ML, Deschamps C, Ilstrup DM, Allen MS, Trastek VF and Pairolero PC : Pulmonary resection for metastatic breast cancer. Ann Thorac Surg. 1994; 58: 1599-602.
8. Singletary SE, Walsh G, Vauthey JN, Curley S, Sawaya R, Weber KL, Meric F and Hortobagyi GN: A Role for Curative Surgery in the Treatment of Selected Patients with Metastatic Breast Cancer. The Oncologist 2003; 8: 241-51.
9. Jardines L, Callans LS and Torosian MH: Recurrent breast cancer: Presentation, Diagnosis and treatment. Semin Oncol 1993; 20: 538-47.
10. Hoe AL, Royal GT and Taylor I: Breast liver metastases- incidence, diagnosis and outcome.J R Soc Med 1991; 84: 714-16.
11. Patanaphan V, Salazar OM and Risco R : Breast cancer: Metastatic patterns and their prognosis. South Med J 1988; 81: 1109-12.
12. Atalay G, Biganzoli L and Renard F Paridaens R, Cufer T, Coleman R, Calvert AH, Gamucci T, Minisini A, Therasse P and Piccart MJ : Clinical outcome of breast cancer patients with liver metastases in the anthracyclin-taxane era. Eur J Cancer. 2003; 39: 2439-49.
13. Boogerd W, Hart AA and Tjahja IS: Treatment and outcome of brain metastasis as first site of distant metastasis from breast cancer. J Neurooncol 1997; 35: 161-7
14. Coia LR: The role of radiotherapy in the treatment of brain metastases. Int J Radiat Oncol Biol Phys 1992; 23: 229-38
15. Pladdet I, Boven E and Nauta J: palliative care for brain metastases of solid tumour types. Neth J Med 1989; 34: 10-21.
16. Therialut RL and Hortobagyi GN: Bone metastases in breast cancer. Anticancer Drugs 1992; 3: 455-62

17. Demers LM: Biochemical Markers in the Management of Patients with Metastatic Bone Disease. Clin Chem 1999; 45: 1131-2

18. Coleman RE and Rubens RD: The clinical course of bone metastases from breast cancer. Br J Cancer 1987; 55: 61-6

19. Choong PFM: The molecular basis of skeletal metastases. Clin Orthop 2003; 415: S19-S31

20. Luc AM, Vakaet L and Boterberg T: Pain control by ionizing radiation of bone metastases. Int J Dev Biol 2004; 48: 599-606

21. Dogan E and Posaci C: Monitoring hormone replacement therapy by biochemical markers of bone metabolism in menopausal women. Postgrad Med J 2002; 78: 727-31

22. Kurland ES, Cosman F, McMahon DJ, Rosen CJ, Lindsay R and Bilezikian JP: Parathyroid hormone as a therapy for idiopathic osteoporosis in men: effects on bone mineral density and bone markers. J Clin Endocrinol Metab 2000 ; 85: 3069-76

23. Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P (eds.): Cells in Their Social Context. In: Molecular Biology of the Cell. Garland science in New York (pub.) 2002,pp.3404-618.

24. Yin JJ, Pollock CB and Kelly K: Mechanisms of cancer metastasis to the bone. Cell Research 2005; 15: 57-62.

25. Miyamoto T and Suda T: Differentiation and function of osteoclasts. Keio J Med 2003; 52 : 1–7.

26. Janssens K and Van Hu W: Molecular genetics of too much bone. Human Molecular Genetics 2002; 11: 2385–93.

27. Sly WS, Hewett E, Whyte MP, Yu YS and Tashian RE: Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. Proc Natl Acad Sci 1983; 80: 2752–6.

28. Watts NB: Clinical Utility of Biochemical Markers of Bone Remodeling. Clin Chem 1999; 45: 1359–68.

29. Ross JR, Saunders Y, Edmonds PM, Patel S, Wonderling D, Normand C and Broadly K: A systemic review of the role of biphosophonates in metastatic disease. Health Technology Assessment 2004; 8: 1-192.

30. Van Poznak C H: The Use of Bisphosphonates in Patients with Breast Cancer. Cancer Control 2002; 9: 480-9.

31. Yin JJ, Selander K, Chirgwin JM, Dallas M, Grubbs BG, Wieser R, Massague J, Mundy GR and Guise TA: TGF-beta signaling blockade inhibits PTHrP secretion by breast cancer cells and bone metastases development. J Clin Invest 1999; 103:197-206.

32. Paget S: The distribution of secondary growths in cancer of the breast. Lancet 1889; i: 571-3.

33. Guise TA: Molecular mechanisms of osteolytic bone metastases. Cancer 2000: 88: 2892-8.

34. Kanis JA: Bone and cancer: Pathophysiology and treatment of metastases. Bone 1995; 17: 101S-105S.

35. Orr FW, Lee J, Duivenvoonder WCM and Singh G: Pathophysiologic interactions in skeletal metastasis. Cancer 2000; 88: 2912-18.

36. Lemieux P, Harvey J, Guies TA, Dallas A, Oesterriech S and Yin JJ: Low cell motility induced by hsp27 overexpression decreases osteolytic bone metastases of human breast cancer cells in-vivo. J Bone Miner Res 1999; 14: 1570-5.

37. Roodman GD: Mechanisms of bone metastasis. N Engl J Med 2004; 350: 1655-64.

38. Rubens RD and Coleman RE: Bone metatases. In; Clinical Oncology. Abeloff MD, Armitage JO, Lichter AS and Niederhuber JE (eds.) Churchill Livingstone. New York, 1995.pp55-69.

39. Fohr B, Colin R. Dunstan, and Seibel MJ: Markers of Bone Remodeling in Metastatic Bone Disease. J Clin Endocrinol Metab 2003; 88: 5059–75.

40. Alatalo S, Zhiqipeng, Janckila AJ, Kaija H, Vihko P, Vaananen HK and Halleen JM: A Novel Immunoassay for The Determination of Tartrate-Resistant Acid Phosphatase 5b From Rat Serum. J Bone Miner Res 2003; 18: 134-9.

41. Vincent JB, Crowder MW and Averill BA: Hydrolysis of phosphate monoesters: a biological problem with multiple chemical solutions. Trends Biochem Sci 1992: 17: 105-10.

42. Kaija H: Tarate-Resitant Acid Phosphatase: Three-dimentional structure and structure-based functional studies. Oulo University Press 2002; http://herkules.oulu.fi/isbn9514267761/.

43. Bull H, Murray PG, Thomas D, Fraser AM and Nelson PN: Acid phosphatases. J Clin Pathol: Mol Pathol 2002; 55: 65–72.

44. Halleen JM, Hentunen TA, Mattikarp, Käkönen SM, Pettersson K,3 and Väänänen HK :Characterization of Serum Tartrate-Resistant Acid Phosphatase and Development of a Direct Two-Site Immunoassay. J Bone Miner Res 1998; 13: 683-7.

45. Bevilacqua MA, Lord DK, Cross NC, Whitaker KB, Moss DW and Cox TM: Regulation and expression of type V (tartrate-resistant) acid phosphatase in human mononuclear phagocytes. Mol Biol Med 1991; 8:135-140.

46. Räisänen SR, Halleen J, Parikka V and Väänänen HK: Tartrate-Resistant Acid Phosphatase Facilitates Hydroxyl Radical Formation and Colocalizes with Phagocytosed Staphylococcus aureus in Alveolar Macrophages. Biochem Biophys Res Commun 2001; 288: 142-50.

47. Reinholt FP, Widholm SM, Ek-Rylander B and Andersson G: Ultrastructural localization of a tartrate-resistant acid ATPase in bone. J Bone Miner Res 1990; 5: 1055-61.

48. Schindelmeiser J, Munstermann D and Witzel H: Histochemical investigations on the localization of the purple acid phosphatase in the bovine spleen. Histochemistry 1987; 87: 13-9.

49. Hayman AR, Bune AJ, Bradley JR, Rashbass J and Cox TM: Osteoclastic tartrate-resistant acid phosphatase (Acp 5): its localization to dendritic cells and diverse murine tissues. J Histochem Cytochem 2000; 48: 219-28.

50. Halleen JM, Räisanen S, Salo JJ, Reddy SV, Roodman GD, Hentunen TA, Lehenkari PP, Kaija H, Vihko P and Väänänen HK: Intracellular fragmentation of bone resorption products by reactive oxygen species generated by osteoclastic tartrate-resistant acid phosphatase. J Biol Chem 1999; 274: 22907-10.

51. Clark SA, Ambrose WW, Anderson TR, Terrell RS and Toverud SU: Ultrastructural localization of tartrate-resistant, purple acid phosphatase in rat osteoclasts by histochemistry and immunocytochemistry. J Bone Miner Res 1989; 4: 399-405.

52. Andersson GN, Ek-Rylander B, Hammarström LE, Lindskog S and Toverud SU: Immunocytochemical localization of a tartrate-resistant and vanadate-sensitive acid nucleotide tri- and diphosphatase J Histochem Cytochem 1986; 34: 293-8.

53. Igarashi Y, Lee M and Matsuzaki S: Acid phosphatases as markers of bone metabolism. J Chromatogr B. 2002; 781: 345-58.

54. Ketcham CM, Baumbach GA, Bazer FW and Roberts RM: The type 5, acid phosphatase from spleen of humans with hairy cell leukemia. Purification, properties, immunological characterization, and comparison with porcine uteroferrin. J Biol Chem 1985; 260: 5768-76.

55. Schindelmeiser J, Gullotta F and Munstermann D: Purple acid phosphatase of human brain macrophages in AIDS encephalopathy. Pathol Res Pract 1989; 185: 184-6.

56. Halleen J, Hentunen TA, Hellman J and Väänänen HK: Tartrate-resistant acid phosphatase from human bone: purification and development of an immunoassay. J Bone Miner Res 1996; 11: 1444-52.

57. Delmas PD: Clinical use of biochemical markers of bone remodeling in osteoporosis. Bone 1992; 13: S17-S21.

58. Cheung CK, Panesar NS, Haines C, Masarei J and Swaminathan R: Immunoassay of a tartrate-resistant acid phosphatase in serum. Clin Chem 1995; 41: 679-86.

59. Ljusberg J, Ek-Rylander B and Andersson G: Tartrate-resistant purple acid phosphatase is synthesized as a latent proenzyme and activated by cysteine proteinases. Biochem J 1999; 343: 63-9.

60. Orlando JL, Zirino T, Quirk BJ and Averill BA: Purification and properties of the native form of the purple acid phosphatase from bovine spleen. Biochemistry 1993: 32: 8120-9.

61. Lam Kw, Lee P, Li CY and Lung T. Yam LT: Immunological and Biochemical Evidence for Identity of Tartrate-Resistant Isoenzymes of Acid Phosphatases from Human Serum and Tissues. Clin Chem 1980; 26: 420-2.

62. Janckila AJ, Nakasato Y, Neustdt DH and Yam LT: Disease-Specific Expression of Tartrate-Resistant Acid Phosphatase Isoforms. J Bone Miner Res 2003; 18: 1916-9.

63. Janckila AJ, Takahashi K, Sun SZ and Yam LT: Tartrate-resistant Acid Phosphatase Isoform 5b as Serum Marker for Osteoclastic Activity. Clin Chem 2001; 47: 74–80.

64. Minkin C: Bone acid phosphatase: tartrate-resistant acid phosphatase as a marker of osteoclast function. Calcif Tissue Int 1982; 34: 285–90.

65. Kaye M: When is it an osteoclast? J Clin Pathol 1984; 37: 398–400.

66. Zaidi M, Moonga B, Moss DW and MacIntyre I: Inhibition of osteoclastic acid phosphatase abolishes bone resorption. Biochem Biophys Res Commun 1989: 159: 68–71.

67. Angel NZ, Walsh N, Forwood MR, Ostrowski MC, Cassady AI and Hume DA: Transgenic mice overexpressing tartrate-resistant acid phosphatase exhibit an increased rate of bone turnover. J Bone Miner Res 2000; 15: 103-10.

68. Halleen JM, Karp M, Viloma S, Laaksonen P, Hellman J, Kakonen SM, Stepan JJ, Holmes S, Vaananen H and Pettersson K: Two-site immunoassays for osteoclastic tartrate-resistant acid phosphatase based on characterization of six monoclonal antibodies. J Bone Miner Res 1999; 14: 464–9.

69. Chamberlain P, Compston J, Cox TM, Hayman AR, Imrie RC, Reynolds K and Holmes SD: Generation and characterisation of monoclonal antibodies to human type-5 tartrate-resistant acid phosphatase: development of a specific immunoassay of the isoenzyme in serum. Clin Chem 1995; 41: 1495–9.

70. Lau KHW, Onishi T, Wergedal JE, Singer FR, Baylink DJ, Chamberlain P, Compston J, Cox TM, Hayman AR, Imrie RC, Reynolds K and Holmes SD.: Characterisation and assay of tartrate-resistant acid phosphatase activity in serum: potential use to assess bone resorption. Clin Chem 1987; 33: 458–62.

71. Rico H and Villa LF: Serum tartrate resistant acid phosphatase (TRAP) as a biochemical marker of bone remodelling. Calcif Tissue Int 1993; 52: 149–50.

72. Halleen JM, Alatalo SL, Janckila AJ, Woitge HW, Seibel MJ and Väänänen HK: Serum tartrate-resistant acid phosphatase 5b is a specific and sensitive marker of bone resorption. Clin Chem 2001; 47: 597-9.

73. Halleen JM, Alatalo SL, Suominen H, Cheng S, Janckila AJ and Väänänen HK: Tartrate-resistant acid phosphatase 5b: A novel serum marker of bone resorption. J Bone Miner Res 2000; 15: 1337-45.

74. Ek-Rylander B, Bergman T and Andersson G: Characterization of a tartrate-resistant acid phosphatase (ATPase) from rat bone: hydrodynamic properties and N-terminal amino acid sequence. J Bone Miner Res 1991; 6: 365-73.

75. Vincent JB and Averill BA: An enzyme with a double identity: purple acid phosphatase and tartrate-resistant acid phosphatase. FASEB J 1990; 4: 3009-14.

76. Halleen JM, Kaija H, Stepan JJ, Vihko P and Vaananen HK: Studies on the protein tyrosine phosphatase activity of tartrate-resistant acid phosphatase. Arch Biochem Biophys 1998; 352: 97-102.

77. Andersson G, Ek-Rylander B, Hollberg K, Sjölander JL, Lång P, Norgård M, Wang Y and Zhang S-J: TRACP as an Osteopontin Phosphatase. J Bone Miner Res 2003;18: 1912-5.

78. Antanaitis BC and Aisen P: Effects of perturbants on the pink (reduced) active form of uteroferrin. Phosphate-induced anaerobic oxidation. J Biol Chem 1985; 260: 751-6.

79. Hayman AR, Warburton MJ, Pringle JA, Coles B and Chambers TJ: Purification and characterization of a tartrate-resistant acid phosphatase from human osteoclastomas. Biochem J 1989; 261: 601-9.

80. Allen SH, Nuttleman PR, Ketcham CM and Roberts RM: Purification and characterization of human bone tartrate-resistant acid phosphatase. J Bone Miner Res 1989; 4: 47-55.

81. Crans DC, Simone CM, Holz RC and Que L Jr: Interaction of porcine uterine fluid purple acid phosphatase with vanadate and vanadyl cation. Biochemistry 1992; 31: 11731-9.

82. Janckila AJ, Woodford TA, Lam KW, Li CY and Yam LT: Protein-tyrosine phosphatase activity of hairy cell tartrate-resistant acid phosphatase. Leukemia 1992; 6: 199-203.

83. Andersson G and Ek-Rylander B: The tartrate-resistant purple acid phosphatase of bone osteoclasts--a protein phosphatase with multivalent substrate specificity and regulation. Acta Orthop Scand Suppl 1995; 266: 189-94.

84. Beck JL, Durack MC, Hamilton SE and De Jersey J: Irreversible inactivation of purple acid phosphatase by hydrogen peroxide and ascorbate. J Inorg Biochem 1999; 73: 245-52.

85. Ek-Rylander B, Barkhem T, Ljusberg J, Öhman L, Andersson KK and Andersson G: Comparative studies of rat recombinant purple acid phosphatase and bone tartrate-resistant acid phosphatase. Biochem J 1997; 321: 305-11.

86. Hayman AR and Cox TM: Purple acid phosphatase of the human macrophage and osteoclast. Characterization, molecular properties, and crystallization of the recombinant di-iron-oxo protein secreted by baculovirus-infected insect cells. J Biol Chem 1994; 269:1294-1300.

87. Ek-Rylander B, Flores M, Wendel M, Heinegard D and Andersson G: Dephosphorylation of osteopontin and bone sialoprotein by osteoclastic tartrate-resistant acid phosphatase. Modulation of osteoclast adhesion in vitro. J Biol Chem 1994; 269: 14853-6.

88. Halleen J, Raisanen SR, Alatalo S, and Vaananen HK: Potential Function for the ROS-Generating Activity of TRACP. J Bone Miner Res 2003;18: 1908-11.

89. Salo J, Lehenkari P, Mulari M, Metsikkö Kand Väänänen HK: Removal of osteoclast bone resorption products by transcytosis. Science 1997; 276:270-273.

90. Nesbitt SA and Horton M: Trafficking of matrix collagens through bone-resorbing osteoclasts. Science 1997; 276: 266-69.

91. Kaija H, Alatalo SL, Halleen JM, Lindquist Y, Schneider G, Väänänen HK and Vihko P: Phosphatase and oxygen radical generating activities of mammalian purple acid phosphatase are functionally independent. Biochem Biophys Res Commun 2002; 292:128-132

92. Brehme CS, Roman S and Shaffer J: Tartrate-resistant acid phosphatase forms complexes with a-macroglobulin in serum. J Bone Miner Res 1999; 14: 311–8.

93. Chu CT and Pizzo SC: Alpha2-macroglobulin, complement and biologic defence: antigens, growth factors microbial proteases and receptor ligation. Lab Invest 1994: 71:792–812.

94. Harringtone D J: Bacterial Collagenases and Collagen-Degrading Enzymes and Their Potential Role in Human Disease. Inefct Immun 1996; 64: 1885–91.

95. Ramachandran GN: Stereochemistry of collagen. Int J Pept Protein Res1988: 31:1–16.

96. Geoffrey CM (ed.): Cell Structure and Function. In: The Cell - A Molecular Approach. Sinauer Associates, Inc. Sunderland (MA), 2000.

97. Maemura M : Serum concentration of pyridinoline cross-linked carboxyterminal telopeptide of type I collagen in patients with metastatic breast cancer. Oncol Rep 2000; 7: 1333-8.

98. Kanakis I, Nikolaou M, Pectasides D, Kiamouris C and Karamanos NK: Determination and biological relevance of serum cross-linked type I collagen N-telopeptide and bone-specific alkaline phosphatase in breast metastatic cancer. J Pharmaceut Biomed Anal 2004; 34: 827–32.

99. Villa I, Saccon B and Rubinacci A: Response to Acute Osteoclast Activity Inhibition Assessed by the Determination of C-Telopeptide of Type I Collagen in Serum. Clinl Chem 2000; 46: 567-9.

100. Garnero P, Ferreras M, Karsdal M, Nicamhalaoibah N, Ristel J, Borel O, Qvist P, Delmas PD, Foged NT and Delaissé JM: The Type I Collagen Fragments ICTP and CTX Reveal Distinct Enzymatic Pathways of Bone Collagen Degradation. J Bone Miner Res 2003; 18: 859-67.

101. Everts V, Delaissé JM, Korper W, Niehof A, Vaes G and Beertsen W: Degradation of collagen in the bone resorbing compartment underlying the osteoclasts involves both the cysteine-proteinases and matrix metalloproteinases. J Cell Physiol 1992; 150:221-31.

102. Everts V, Delaissé JM, Korper W and Beertsen W: Cysteine proteinases and matrix metalloproteinases play distinct roles in the subosteoclastic resorption zone. J Bone Miner Res 1998; 9: 1420-30.

103. Delaissé JM, Engsig MT, Everts V, del Carmen Ovejero M, Ferrera M, Lund L, Vu TH, Werb Z, Winding B, Lochter A, Karsdal MA, Troen T, Kirkegaard T, Lenhard T, Heegaard AM, Neff L, Baron R and Foged NT: Proteinases in bone resorption: Obvious and less obvious roles. Clin Chim Acta 2000; 291: 223-34.

104. Garnero P, Sornay-Rendu E, Chapuy MC and Delmas PD: Increased bone turnover in late postmenopausal women is a major determinant of osteoporosis. J Bone Miner Res 1996; 11: 337-49.

105. Delmas PD, Eastell R, Garnero P, Seibel MJ and Stepan J: The use of biochemical markers of bone turnover in osteoporosis. Osteoporos Int 2000; 11:S2-S17.

106. Garnero P, Shih WJ, Gineyts E, Karpf D and Delmas PD: Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate. J Clin Endocrinol Metab 1994; 79: 1693-700.

107. Blomqvist C, Risteli L, Risteli J, Virkkunen P, Sarna S and Elomaa I: Markers of type I collage degradation and synthesis in the monitoring of treatment responses in bone metastases from breast cancer. Br J Cancer 1996; 73:1074-9.

108. Kylmälä T, Tammela TLJ, Risteli L, Risteli J, Konttri M and Elomaa I: Type I collagen degradation product (ICTP) gives information about the nature of bone metastases and has prognostic value in prostate cancer. Br J Cancer 1995; 71: 1061-4.

109. Aruga A, Koizumi M, Hotta R, Takahashi S and Ogata E: Usefulness of bone metabolic markers in the diagnosis and follow-up of bone metastasis from lung cancer. Br J Cancer 1997; 76: 760-4.

110. Elomaa I, Virkkunen P, Risteli L and Risteli J: Serum concentration of the cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is a useful marker prognostic marker in multiple myeloma. Br J Cancer 1992; 66: 337-41.

111. Garnero P: Markers of bone turnover in prostate cancer. Cancer Treat Rev 2001: 27: 187-92.

112. Lars FH, Theyse, Mol JA, Voorhout G, Terlou M and Hazewinkel HAW: The efficacy of the bone markers osteocalcin and the carboxyterminal cross-linked telopeptide of type-I collagen in evaluating osteogenesis in a canine crural lengthening model. Vet J 2005 ;Under press.

113. Loviselli, A, Mastinu R, Rizzolo E, Massa GM, Velluzzi F, Sammartano L, Mela Q and MariottiS: Circulating telopeptide type I is a peripheral marker of thyroid hormone action in hyperthyroidism and during levothyroxine suppressive therapy. Thyroid 1997; 7: 561–6.

114. Guillemant JA, Accarie CM, Gueronniere V and Guillemant SE: Different acute responses of serum type I collagen telopeptides, CTX, NTX and ICTP, after repeated ingestion of calcium. Clinica Chimica Acta 2003; 337: 35–41

115. Sassi ML, Eriksen H and Risteli L: Immunochemical characterization of assay for carboxyterminal telopeptide of human type I collagen: loss of antigenicity with cathepsin K. Bone 2000; 26: 367– 73.

116. Nishi Y, Atley L and Eyre DE: Determination of bone markers in pycnodysostosis: effects of cathepsin K deficiency on bone matrix degradation. J Bone Miner Res 1999; 14: 902–8.

117. Schoenberger J, Rozeboom S, Beyer EW and Eilles C: Evaluation of the clinical value of bone metabolic parameters for the screening of osseous metastases compared to bone scintigraphy. BMC Nuclear Medicine 2004; 4: 3-7.

118. Ulrich U, Rhiem K, Schmolling J, Flaskamp C, Paffenholz I, Salzer H, Bauknecht T and Schlebusch H: Cross-linked type I collagen C- and N- telopeptides in women with bone metastases from breast cancer. Arch Gynecol Obstet 2001, 264: 186-90.

119. Wada N, Fujisaki M, Ishii S, Ikeda T and Kitajima M: Evaluation of bone metabolic markers in breast cancer with bone metastases. Breast Cancer 2001, 8: 131-7.

120. Duffy MJ: C15-3 and related mucins as circulating in breast cancer.Ann Clin Biochem 1999; 36: 579-86.

121. Cheung KL, Graves CRL and Robertson JFR: Tumour marker measurements in the diagnosis and monitoring of breast cancer. Cancer Treat Rev 2000; 26: 91–102.

122. Hayes DF, Zurawski VR and Kufe DW: Comparison of circulating CA15.3 and carcinoembryonic antigen levels in patients with breast cancer. J Clin Oncol 1986: 10: 1542–50.

123. Robertson JFR, Pearson D, Price MR, Selby C, Badley RA, Pearson J, Blamey RW and Howell A: Assessment of four monoclonal antibodies as serum markers in breast cancer. Eur J Cancer 1990; 26: 1127–32.

124. Colomer R, Ruibal A and Salvador L: Circulating tumour marker levels in advanced breast carcinoma correlate with the extent of metastatic disease. Cancer 1989; 64: 1674–81.

125. Colomer R, Ruibal A, Genolla J, Rubio D, Del Campo JM, Bodi R and Salvador L: Circulating CA15.3 levels in the post surgical follow-up of breast cancer patients and in non-malignant diseases. Breast Cancer Res Treat 1989; 13: 123–33.

126. Molina R, Zanon G, Filella X, Moreno F, Jo J, Daniels M, Latre ML, Gimenez N, Pahisa J, Velasco M and Ballesta AM: Use of serial carcinoembryonic antigen and CA15.3 assays in detecting relapses in breast cancer patients. Breast Cancer Res Treat 1995; 36: 41–8.

127. Van Dalen A: New markers for breast carcinoma-associated antigen in comparison with CA15.3. Anticancer Res 1996; 16: 2339–43.

128. Duffy MJ, Duggan C, Keane R, Hill ADK, McDermott E, Crown J and Higgins NO: High Preoperative CA 15-3 Concentrations Predict Adverse Outcome in Node-Negative and Node-Positive Breast Cancer: Study of 600 Patients with Histologically Confirmed Breast Cancer. Clin Chem 2004; 50: 559–63.

129. Brayman M, Thathiah A and Carson DD: MUC1: A multifunctional cell surface component of reproductive tissue epithelia. Reprod Biol Endoc 2004; 2

130. Levi E, Klimstra DS, Adsay NV, Andea A and Basturk O: MUC1 and MUC2 in pancreatic neoplasia. J Clin Pathol 2004; 57: 456-62.

131. Duffy MJ and Sherry F: CA15-3 a new marker in breast cancer. Ann Clin Biochem 1998; 25: 53s-4s.

132. Shering s, Sherry F, McDermott E, Higgins NO and Duffy MJ: Preoperative CA15-3 concentrations predict outcome in breast cancer. Cancer 1998; 83: 2521-7.

133. Lande B: The role of CA 15.3 assays in breast cancer. Immuno-analyse Biologie spécialisée 2004; 19: 274–8.

134. Dumitrescu RG and Cotarla I: Understanding breast cancer risk - where do we stand in 2005 J. Cell. Mol. Med. 2005; 9: 208-21.

135. Johan A and Tuszynski G: The Role of Matrix Metalloproteinases in Tumor Angiogenesis and Tumor Metastasis. Path Onco Res 2001; 7: 14-23.

136. Coleman R E: Management of Bone Metastases. The Oncologist 2000; 5: 463-70.

137. Roodman GD: biology of Neoplasia: Biology of Osteoclast Activation in Cancer. J Clin Oncol 2001; 19: 3562-71.

138. Vogel CL, Yanagihara RH, Wood AJ, Svhnell FM, Henderson C, Kaplan BH, Purdy MH, Orlowski R, Decker JL, Lacerna L and Hohneker JA: Safety and Pain Palliation of Zoledronic Acid in Patients with Breast Cancer, Prostate Cancer, or Multiple Myeloma Who Previously Received Bisphosphonate Therapy. The Oncologist 2004; 9: 687-95.

139. Koizum M and Ogata E: Bone metabolic markers as gauges of metastasis to bone: a review. Annals of Nuclear Medicine 2002; 16: 161–8.

140. Wada N, Ishii S, Ikeda T, Enomoto K and Kitajima M: Serum tartrate resistant acid phosphatase as a potential marker of bone metastasis from breast cancer. Anticancer Res 1999; 19: 4515-21.

141. Chao TY, Chang HD, Huang WS, Jancklia A and Yam LT: Correlations between activity of Tartrate-Resistant Acid Phosphatase and whole body bone scan in patients of breast cancer with bony metastases. Nuclear Medicine Alasbimn Journal 2002a; 17: World Congress of Nuclear Medicine.

142. Chao TY, Lee SH, Chen MM, Janckila A and Yam LT: Tartrate-resistant acid phosphatase 5b (TRAP 5b) as a serum marker of bone metastasis in breast cancer patients. ASCO Annual Meeting 2002b; Tumor and Cell Biology.

143. Koizumi M, Takahashi S and Ogata E: Comparison of serum bone resorption markers in the diagnosis of skeletal metastasis. Anticancer Res. 2003; 23: 4095-9.

144. Capeller B, Caffier H, Sutterlin MW and Dietl J: Evaluation of tartrate-resistant acid phosphatase (TRAP) 5b as serum marker of bone metastases in human breast cancer. Anticancer Res. 2003; 23: 1011-5.

145. Lyubimova NV, Pashkov MV, Tyulyandin SA, Gol'dberg VE and Kushlinskii NE: Tartrate-resistant acid phosphatase as a marker of bone metastases in patients with breast cancer and prostate cancer. Bull Exp Biol Med. 2004; 138: 77-9.

146. Chao TY, Ho CL, Lee SH, Chen MM, Janckila A and Yam LT: Tartrate-resistant acid phosphatase 5b as a serum marker of bone metastasis in breast cancer patients. J Biomed Sci. 2004; 11: 511-6.

147. Ebert W, Muley T, Herb KP and Schmidt GH: Comparison of bone scintigraphy with bone markers in the diagnosis of bone metastasis in lung carcinoma patients. Anticancer Res. 2004; 24: 3193-201.

148. Møller S, Hansen M, Hillingsø J, Jensen JE B and Henriksen J H: Elevated carboxy terminal cross linked telopeptide of type I collagen in alcoholic cirrhosis: relation to liver and kidney function and bone metabolism. Gut 1999; 44: 417–23.

149. Yamamoto I, Miura H, Kigami Y, Yamamura Y, Ohta T, Yuu I, Yamamoto Y and Morita R: Detection of bone metastasis by serial measurement of C-terminal telopeptide of type I collagen in patients with malignancy. Kaku Igaku. 1996; 4: 423-9.

150. Plebani M, Bernardi D, Zaninotto M, De Paoli M, Secchiero S and Sciacovelli L: New and traditional serum markers of bone metabolism in the detection of skeletal metastases. Clin Biochem. 1996; 1: 67-72.

151. Tahtela R and Tholix E: Serum concentrations of type I collagen carboxyterminal telopeptide (ICTP) and type I procollagen carboxy-and aminoterminal propeptides (PICP, PINP) as markers of metastatic bone disease in breast cancer. Anticancer Res. 1996; 16: 2289-93.

152. Koizumi M, Matsumoto S, Takahashi S. Yamashita T and Ogata E: Bone metabolic markers in the evaluation of bone scan flare phenomenon in bone metastases of breast cancer. Clin Nucl Med 1999; 24: 15-20.

153. Shimozuma K, Sonoo H, Fukunaga M, Ichihara K, Aoyama T and Tanaka K: Biochemical markers of bone turnover in breast cancer patients with bone metastases: a preliminary report. Jpn J Clin Oncol 1999; 29: 16-22.

154. Chinchilli V, Demers L, Leitzel K Ali SM, . Engle L, Costa L, Risteli J and Lipton A: Effect of elevated serum carboxy terminal telopeptide (ICTP) on survival in breast cancer patients with and without bone metastases. http://herkules.oulu fi

155. Keskikuru R, Bloigu R, Risteli J, Kataja V and Jukkola A: Elevated preoperative serum ICTP is a prognostic factor for overall and disease-free survival in breast cancer. Oncol Rep. 2002; 9: 1323-7.

156. Kiuchi K, Ishikawa T, Hamaguchi Y, Momiyama N, Hasegawa S, Ishiyama A and Kono: Cross-linked collagen C- and N-telopeptides for an early diagnosis of bone metastasis from breast cancer. Oncol Rep. 2002; 9: 595-8.

157. Koizumi M, Takahashi S and Ogata E: Comparison of serum bone resorption markers in the diagnosis of skeletal metastasis. Anticancer Res. 2003; 23: 4095-9.

158. Daly L, Ferguson J, Cram GP, Hars V, George SL, McCarty KS, and Bast RC: Comparison of a Novel Assay for Breast Cancer Mucin to CA15-3 and Carcinoembryonic Antigen. J Clin Oncol 1992; 10: 1057-65

159. O'Brien DP, Horgan PG, Gough DB, Skehill R, Grimes H and Given HF: CA15-3: a reliable indicator of metastatic bone disease in breast cancer patients. Ann R Coll Surg Engl. 1992; 74: 9-11.

160. Massidda B, Ionta MT, Foddi MR, Mascia L, Bruder F, Aloi MB, Meleddu C and Giannoni MN: Usefulness of pyridinium crosslinks and CA 15-3 as markers in metastatic bone breast carcinoma. Anticancer Res. 1996; 16: 2221-3.

161. Lauro S, Trasatti L, Bordin F, Lanzetta G, Bria E, Gelibter A, Reale MG and Vecchione A: Comparison of CEA, MCA, CA 15-3 and CA 27-29 in follow-up and monitoring therapeutic response in breast cancer patients. Anticancer Res. 1999; 19: 3511-5.

162. Brown JE and Coleman RE: Assessment of the effects of breast cancer on bone and the response to therapy. Breast. 2002; 11:375-85.

163. Sanchez REG, Enriquez MM and Castaneda VN: Reliability of CA 15-3 in the follow up of female patients with breast carcinoma and bone metastases. Rev Invest Clin. 2003; 55: 412-8.

164. Oremek GM, Weis A, Sapoutzis N and Eppel SH: Diagnostic value of bone and tumour markers in patients with malignant diseases. Anticancer Res. 2003; 23: 987-90.

165. Yildiz M, Oral B, Bozkurt M and Cobaner A: Relationship between bone scintigraphy and tumor markers in patients with breast cancer. Ann Nucl Med. 2004; 18: 501-5.

166. Seregni E, Coli A and Mazzucca N: Circulating tumour markers in breast cancer. Eur J Nucl Med Mol Imaging. 2004; Suppl 1: S15-22.

167. Kurebayashi J: Biomarkers in breast cancer. Gan To Kagaku Ryoho.2004; 31:1021-6.

168. Begic A, Selimovic KE, Obralic N, Duric O, Lacevic N, Begovic S and Aganovic DM: Role of bone scintigraphy and tumor marker-Ca 15-3 in detection of bone metastases in patients with breast cancer. Bosn J Basic Med Sci. 2005; 5: 23-6.

169. Montravers F, Younsi N, Rousseau C, Philippe C, Seddiki M, Uzan S, Izrael V and Talbot JN: Comparison of bone scanning and CA 15-3 serum concentration in the follow-up of breast cancer. Anticancer Res. 1997; 17: 1683-6.

170. Chen Z, Fan Z and Yang J: Immunoradiometric assay of tumor marker CA15-3 and its clinical application. Zhonghua Zhong Liu Za Zhi 1998; 20: 125-8.

171. Nishimura R, Nagao K, Miyayama H, Matsuda M, Baba K, Matsuoka Y and Yamashita H: Elevated serum CA15-3 levels correlate with positive estrogen receptor and initial favorable outcome in patients who died from recurrent breast cancer. Breast Cancer 2003; 10: 220-7.

Arabic Summary

ARABIC SUMMARY

الملخص العربي

الملخص العربى

سرطان الثدي من اكثر السرطانات انتشارا في السيدات . علي مدار العالم ، فأنها من اكبر المشاكل الاجتماعية والصحية فثلث مرضي السرطان يعانون من انتشار السرطان الأولى إلى العظام . حيث يصل معدل الانتشار إلى العظام حوالي ٧٣ % . علي سبيل المثل ، تعاني مريضات سرطان الثدي من كسر العمود الفقري بمعدل أربع إلى خمس أضعاف اعلي من مثيلاتهم في نفس العمر .

تتكون العظام من مركبين مختلفين من حيث الطبيعة والوظائف الحيوية وهما الجزء القشري (المكتنز) والجزء التربيقي (الإسفنجي) . الجزء القشري هو عبارة عن قالب صلب من الأملاح حيث يكون به النشاط الخلوي ونشاط الايض اقل نسبيا . يمثل هذا الجزء القشري حوالي ٨٥% من كتله العظام بينما يمثل الجزء التربيقي حوالي ١٥ % من الهيكل العظمي .و يعمل الجزء القشري كدعامة للحركة بينما يشترك الجزء التربيقي في عمليات الايض .

يتكون الجزء الخلوي في العظام من خلايا بنائية وخلايا ناقضة . الخلايا البنائية هي المسئولة عن إنتاج وترسيب الأملاح في القالب العظمي ، أما الخلايا الناقضة فهي مسئولة عن الارتشاف العظمي و تعبر نمو العظام واعادة بناؤه أحداث طبيعية تتم بمعدل كبير خلال مرحلتي الطفولة والمراهقة وبمعدل اقل خلال مرحلة البلوغ

عمليتي تكوين العظام وارتشافه يكونان متوازنين حتى يحافظوا علي التوازن العام للخلايا خلال الحياة . والسرطان يدمر هذا التوزان لأنه عندما ينتشر الورم إلى العظام فانه يستطيع أن يغيرها ويسبب تقرحات تكون هادمة (نتيجة لزيادة الارتشاف العظمي) أو بنائية (نتيجة لزيادة تكوين العظام) أو تجمع بينهما لتكوين تقرحات مختلطة .

التقنيات التصويرية هي الطرق الرائدة حاليا التي تستخدم في تشخيص ثانويات بالعظام ، لكن هذه التقنيات باهظة الثمن ، تعرض المرضي إلى مركبات سامة ومركبات إشعاعية ، وسيئة في مراقبة الاستجابة إلى العلاج .

فهذه العوائق هي التي تدفع في البحث عن طرق فحص بديلة ، ولذا فان دلالات العظام الايضيه قد قيمت كطرق محتملة لتشخيص ومراقبة تطور وتعاقب انتشار أمراض العظام . أن دلالات العظام الايضية قد قسمت إلى دلالات ناقضة ودلالات بنائية وقياسهما يعتبرامن ورخيص الثمن ، ويمكن تكرارقياسهما مرات عديدة. هذه الدلالات مشتقة من الجزئى القشري والتربيقي للعظام ، لذلك فهي تعكس النشاط الايضي للهيكل العظمي بأكمله .

يعتبر الفوسفاتيز الحامضي المقاوم للتارتارات٥ ب دلالة عن نشاط الخلايا القابضة والارتشاف العظمي تفرز الخلايا القابضة الفوسفاتيز الحامضي المقاوم للتارتارات٥ ب فى صورة نشطة في الدم،و يثبط نشاطه ويكسر إلى أجزاء قبل أن يزال من الدم . وهكذا فان نشاط الفوسفاتيز الحامضي المقاوم للتارتارات٥ ب لا يتراكم في الدم فى حالات الفشل الكلوي والفشل الكبدي .

التغير اليومى لنشاط مصل الدم فى الفوسفاتيز الحامضي المقاوم للتارتارات٥ ب قليل ومستوياته لا تتأثر بالطعام فتسمح بتجميع العينات في أي وقت من اليوم ويمكن للإنزيم آن يكون دلاله مناسبة لتقييم حالة انتشار سرطان العظام من مريضات سرطان الثدي الأولى ، يمكن آن يكون دليل جيد علي الاستجابة إلى العلاج في تلك المريضات .

يبدو أن الفوسفاتيز الحامضي المقاوم للتارتارات٥ ب النشط دلالة مصل جيدة لانتشار سرطان العظام في مريضات سرطان الثدي الأولى وخاصة لتحديد تعاقب المرض .

ويبدو ان التيلوببتيد الطرفي لمجموعة الكربوكيسل من الكولاجين I دلاله رائدة في الارتشاف العظمي ومؤشر واعد في اكتشاف انتشار سرطان العظام من سرطان الثدي الأولى . وهو يخرج إلى الدم أثناء تكسير الكولاجين I خاصة في العظام بواسطة الانزيم الفلزى المحلل للبروتين وخاصة ٢، ١٣ .

يرتفع مستوى مصل الانتجين السرطانى ١٥،٣ فى حوالي ٥٤ –٨٠ % من مريضات سرطان الثدي وهو يقيس البروتين الذي ينتج من MUC-١ gene. وترتفع تركيزات مصل الانتجين السرطانى ١٥،٣ قليلا قبل الجراحة فى مريضات سرطان الثدى.و انه يمكن ان يستخدم كدلالة لمراقبة الاستجابة الى العلاج.

الهدف من هذه الرسالة هو تحديد مستويا ت الفوسفايتر الحامضي المقاوم للتارتارات٥ ب و التيلوببتيد الطرفي لمجموعة الكربوكيسل من الكولاجين I و الانتجين السرطانى ١٥،٣ في مصل دم مريضات سرطان الثدي الأولي قبل الجراحة لتقييم صلاحيتهم كمؤشرات مستقبلية لإحتمال ظهور ثانويات.

تشتمل هذه الدراسة على مجموعتين، الأولى تتكون من ٣١ مريضة بسرطان الثدي الأولي و الثانية من ١٠ أشخاص من نفس العمر و الجنس. تم أخذ ثلاث عينات من مصل دم كل مريضة في المجموعة الأولى قبل الجراحة و بعد ١٠ أشهر و بعد ٢٠ شهر من الجراحة و عينة واحدة من مصل دم كل شخص في المجموعة الثانية.ويتم قياس

التيلوببتيد الطرفي لمجموعة الكربوكيسل من الكولاجين I و الانتجين السرطانى ١٥،٣ و الفوسفاتيز الحامضي المقاوم للتارتارات٥ ب باستخدام القياس المناعي الانزيمي في كل عينة.

و من متابعة المريضات وجد أن ٢٢,٦% منهم لم تتكون لديهن ثانويات بينما عاد المرض للظهور في ٢٢,٦ % من الحالات و انتشر المرض للرئة في ١٦,١% و للعظم في ٣٨,٧% منهن. و على ذلك تم تقسيم المجموعة الأولى الي مجموعات فرعية تبعا لمكان ظهور الثانويات.

لم يوجد فارق ذو دلاله احصائية فى مستوى مصل الفوسفاتيز الحامضي المقاوم للتارتارات٥ ب بين المجموعة الضابطة و أي من المجموعات قبل الجراحة و كذا بعد ١٠ و ٢٠ شهر فيما عدا مجموعة المريضات اللاتي انتشر المرض لديهن للعظام حيث ظهر بهذه المجموعة فارق ذو دلاله احصائية، و المريضات اللاتي تخطى الفوسفاتيز الحامضي المقاوم للتارتارات٥ ب لديهن ٥,٤ وحدة/لتر كان معدل التعرض للوفاة لديهن الضعف و معدل التعرض لظهور ثانويات بالعظام أربعة أضعـــــاف مقارنة بالمريضات اللاتى لم يتخطى لديهن الانزيم هذا المستوى،ولذا فان الفوسفاتيز الحامضي المقاوم للتارتارات٥ ب يمكن ان يستخدم للتشخيص وكدلاله لاحتمال ظهور ثانويات بمريضات سرطان الثدى الاولى.

لم يوجد فارق ذو دلاله احصائية فى مستوى مصل التيلوببتيد الطرفي لمجموعة الكربوكيسل من الكولاجين I بين المجموعة الضابطة و المجموعة التى لم يتكون لديهن ثانويات والمجموعة التى عاد المرض للظهور بها والمجموعة التى تكون لديهن ثانويات بالرئه قبل الجراحة و بعد عشرة اشهروبعد عشرين شهرا،بينما يوجد فارق ذو دلاله احصائية بالمريضات اللاتي انتشر المرض لديهن للعظام. المريضات اللاتي تخطى التيلوببتيد الطرفي لمجموعة الكربوكيسل من الكولاجين I لديهن ٤,٦ ميكروجرام/لتر كان معدل التعرض للوفاة لديهن ثلاث اضعاف و معدل التعرض لظهور ثانويات بالعظم أربعة أضعـــــاف مقارنة بالمريضات اللاتى لم يتخطى لديهن التيلوببتيد الطرفي لمجموعة الكربوكيسل من الكولاجين I هذا المستوى .و لذا فان التيلوببتيد الطرفي لمجموعة الكربوكيسل من الكولاجين I يمكن أن يتنبأ باحتمال ظهور ثانويات بالهيكل العظمى بدرجة عالية فى عينة ما قبل الجراحة و يمكنه تمييز المريضات اللاتى يحتجن الى علاج مضاد للارتشاف العظمى مع العلاج الكيماوى. بالاضافة الى ذلك فانه يمكن أن يستخدم فى تشخيص انتشار سرطان الثدى الى العظام.

لم يوجد فارق ذو دلاله احصائية فى مستوى مصل الانتجين السرطانى ١٥،٣ بين المجموعة الضابطة و أي من المجموعات قبل الجراحة و كذا بعد ١٠ و ٢٠ شهر فيما عدا مجموعة المريضات اللاتي انتشر المرض لديهن للعظام حيث ظهر بهذه المجموعة فارق ذو دلاله احصائية بعد عشرين شهرا ولذا فانه يمكن ان يستحدم فى متابعة المريضات.

ولذا فإننا نستنتج أن الفوسفاتيز الحامضي المقاوم للتارتارات ٥ ب و التيلوببتيد الطرفي لمجموعة الكربوكيسل من الكولاجين I يمكن أن يستخدموا كدلالات أورام في التشخيص و في الإنذار لاحتمال ظهور ثانويات بالعظام بمريضات سرطان الثدي الأولى و بذلك فانهم يمكن أن يميزوا المريضات اللاتى يحتجن إلى العلاج المضاد للارتشاف العظمى،بينما الانتجين السرطاني ١٥،٣ فانه يمكن استخدامه لمتابعة المريضات اللاتي ظهر لديهن ثانويات بالعظام.

الفوسفاتيز الحامضى ٥ب المقاوم للتارتارات والتيلوبتيد الطرفى لمجموعه الكربوكسيل من الكولاجين I والانتيجين السرطانى ١٥,٣ كمؤشرات مستقبلية لاحتمال ظهور ثانويات بمريضات سرطان الثدى الاولى

مقدمة من

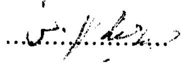
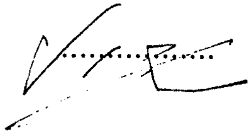
مروة سامح إبراهيم ابو العنين

بكالوريوس علوم- كيمياء/ كيمياء حيوية (٢٠٠٠)

جامعة الإسكندرية

للحصول على درجة

الماجستير في كيمياء الإشعاع

المشرفون على الرسالة	موافقون
أ.د. صافيناز محمود الزغبي أستاذ متفرغ بقسم الكيمياء الطبية التطبيقية معهد البحوث الطبية- جامعة الإسكندرية	
أم.د. محمد ابراهيم مرسى أستاذ مساعد بقسم علوم الإشعاع معهد البحوث الطبية- جامعة الإسكندرية	
أم.د. وجدى ابراهيم فايد استاذ مساعد بقسم الجراحة معهد البحوث الطبية- جامعة الإسكندرية	
أم.د. سامح الابراهيم ذكى استاذ مساعد بوحدة علاج الاورام والطب النووى معهد البحوث الطبية - جامعة الإسكندرية	

التاريخ ٢٠٠٦/٣/٤

الفوسفاتيز الحامضى ٥ب المقاوم للتارتارات والتيلوببتيد الطرفى لمجموعه الكربوكسيل من الكولاجين I والانتيجين السرطانى ١٥,٣ كمؤشرات مستقبلية لاحتمال ظهور ثانويات بمريضات سرطان الثدى الاولى

مقدمة من

مروة سامح إبراهيم ابو العنين

بكالوريوس علوم- كيمياء/ كيمياء حيوية (٢٠٠٠)

جامعة الإسكندرية

للحصول على درجة

الماجستير في كيمياء الإشعاع

لجنة المناقشة و الحكم على الرسالة	موافقون
أ.د. صافيناز محمود الزغبي أستاذ متفرغ بقسم الكيمياء الطبية التطبيقية معهد البحوث الطبية- جامعة الإسكندرية (مشرف و رئيس لجنة الحكم)	....
أم.د. محمد ابراهيم مرسى أستاذ مساعد بقسم علوم الإشعاع معهد البحوث الطبية- جامعة الإسكندرية (مشرف و عضو)	....
أ.د. سوسن مصطفى موسى استاذمتفرغ بقسم علوم الإشعاع معهد البحوث الطبية- جامعة الإسكندرية (عضو)	....
أ.د. صلاح الدين عبدالمنعم ابراهيم استاذ علاج الاورام كلية الطب- جامعة الإسكندرية (عضو)	....

التاريخ ٢٠٠٦/٣/٤

الفوسفاتيز الحامضى ٥ب المقاوم للتارتارات والتيلوببتيد الطرفى لمجموعه الكربوكسيل من الكولاجين I والانتيجين السرطانى ١٥,٣ كمؤشرات مستقبلية لاحتمال ظهور ثانويات بمريضات سرطان الثدى الاولى

رسالة

مقدمه لمعهد البحوث الطبية

للحصول على درجة

"الماجستير"

في

كيمياء الإشعاع

من

مروة سامح إبراهيم ابو العنين

بكالوريوس علوم- كيمياء/ كيمياء حيوية (٢٠٠٠)

جامعة الإسكندرية

مارس ٢٠٠٦