JOURNAL OF CURRENT RESEARCH IN SCIENCE (ISSN 2322-5009)
CODEN (USA): JCRSDJ
2013, Vol. 1, No. 5, pp:298-309
Available at www.jcrs010.com

REVIEW ARTICLE

BONE AND ITS DISEASES: CURRENT BIOCHEMICAL MARKERS IN DIAGNOSIS

Nnodim Johnkennedy, Uzoukwu Okechukwu A, Okwara Bede U, Okereke Bruno C, Anoruo Audrey O And Okoroji Francisca
Department of medical laboratory science, faculty of health science, Imo State University owerri, Nigeria

ABSTRACT: Bone diseases are disease associated with the bone and it is becoming increasingly relevant to everyday clinical practice. Some of them include; Osteoporosis, Osteoarthritis, Paget's disease, Rhematoid arthritis, Arthritis, Rickets, Bone cancer etc. The increases in and the need for effective measure to be used in the screening diagnosis and follow-up of such pathologies has markedly grown. Some current biochemical markers are x-rayed in the evaluation of bone diseases.
KEYWORDS: Bone, Disease, Current Biomedical Parameters, Diagnosis**.**

INTRODUCTION

Bone is a metabolically active tissue that undergoes continuous modeling by the two counteracting processes, namely bone formation and bone resorption. These processes rely on the activity of osteoclasts (resorption), osteoblasts (formation) and Osteocytes (maintenance). Under normal conditions, bone resorption and formation are tightly coupled to each other so that the amount of bone removed is always equal to the amount of newly formed bone. This balance is achieved and regulated through the action of various systemic hormones (eg Parathyroid hormone (PTH) Vitamin D, other steroid hormones) and local mediators (e.g cytokines, growth factors). In contrast, somatic growth, ageing, metabolic bone diseases, states of increased or decreased mobility, therapeutic interventions and many other conditions are characterized by more or less pronounced imbalances in bone turnover (Seibel, 2005).

The results of such uncoupling in bone turnover are often changes in bone structure, strength and mass while bone structure and strength are difficult to measure. In vivo, bone mass can be assessed by densitometric techniques. In contrast to these rather static measures, however molecular makers of bone metabolism are helpful tools to detect the dynamics of the metabolic imbalance itself (Seibel, 2006).

Although the currently available markers of bone turnover include both enzymes and non-enzymatic peptides derived from cellular and non-cellular compartments of bone, they are usually classified according to the metabolic process they are considered to reflect. Most biochemical indices of bone resorption are related to collagen breakdown products such as Hydroxyproline or the various collagens cross links and telopeptides. Other markers of bone resorption include non-collagenous matrix proteins such as bone sialoprotein (BSP) or osteoclast-specific Enzymes like tartrate – Resistant Acid phosphatase or Cathopsin K (Meir, 2006).

In contrast, markers of bone formation are either by-products of collagen neosynthesis (eg propeptides of type 1 collagen) or osteoblast related proteins such as osteocalcin (OC) and Alkaline phosphatase.

For clinical purposes therefore, markers of bone formation are distinguished from indices of bone resorption but it should be borne in mind that some marker components reflect at least in past, both bone formation and bone resorption (eg. Hydroxyproline, certain Osteocalcin fragments). Furthermore, most of the molecules used as markers of bone turnover are also present in tissues other than bone and non-skeletal processes and may therefore influence their circulating or urinary levels (Calvo, 1996).

Finally, changes in Markers of bone turnover are not disease specific but Reflect, as an integral measure, alterations in the metabolism of the entire skeletal envelope independently of the underlying cause. Hence, results of bone marker measurements should always be interpreted against the background of their basic science and the clinical picture.

1.1. Bone Turnover throughout Life

In children, bone turnover can be more than ten times greater than in adults because of three

Corresponding Author: Nnodim Johnkennedy, Department of medical laboratory science, faculty of health science, Imo State University owerri, Nigeria. **E-mail**: johnkennedy23@yahoo.com

physiologic processes interacting in the skeleton: bone modeling, remodeling and growth levels of bone formation and resorption markers therefore are much higher in children than in adults.

In puberty, bone growth accelerates with an increase in bone turnover markers that reflects the effect of hormones that include the growth spurt. Postmenopausal women who do not use hormone replacement therapy have higher levels of bone resorption and formation markers than premenopausal women. Levels In postmenopausal women on hormone replacement are no different than in premenopausal women. In postmenopausal women not on estrogen, urinary levels have been reported to discriminate between normal bone mineral density (low level), osteopenia and osteoporosis (High level). Normal levels are found in a small percentage of women. This may be explained by the variable levels of serum estradiol in postmenopausal women (Ebeleng *et al.*, 1996). Elderly men in contrast have variable findings. However accelerated bone turnover has been noted in men with full blown hypogonadism caused by androgen suppression therapy (Meir, 2006).

BONE

Bone is a type of connective tissue in which the intercellular matrix is highly specialized for rigidity and strength. The structure of mature bone seen in the microscope is the last of numerous cycles in which bony tissue is formed, and then resorbed, followed by formation of new bone tissue. Bone is usually formed in layers or lamellae which contain collagen fibres in a nearly parallel array and a small proportion of proteoglycans and other substances (calcium phosphate and calcium carbonate). This Organic matrix is soon mineralized by formulation of minute apatite-like crystals oriented along the collagen fibres. The collagen fibres of adjacent lamellae are laid down obliquely or perpendicularly. This plywood-like arrangement increases the overall strength (Lee *et al.*, 2007)

A specialized layer of dense connective tissue called periosteum covers the outer surface of the bone. The inner surface enclosing the narrow cavity is lined with a very thin delicate connective tissue the endosteum. The cells of the endosteum and the inner layer of the periosteum resemble fibroblasts, but can differentiate into osteoblasts under appropriate stimulation. The principal bone cells are the osteocyte, osteoblast and the osteoclast. The osteocyte (bone cell proper) is found in launae lying in or between the lamellae. Very fine channels (canaliculi) containing osteocyte cell processes connect lacunae to each other and to tissue fluid spaces providing for nutrition and metabolic exchange. The osteocyte is derived from the osteoblast as it becomes surrounded by matrix. The osteoblast is found on the surfaces of bone and is involved with bone deposition. The osteoclast is usually found in depressions (howship's lacunae) at the surfaces of bony tissue and is associated with bone resorption (Clarke *et al.*, 1996).

Bony tissue is classified as either spongy (trabecular, cancellous) or compact bone depending on the relative proportions of mineralized and soft tissue. Most bones contain both spongy and compact regions. In compact bone however, the predominant structural unit is the osteon or Haversian system. Compact bone forms the outer shell of bones; it consists of a hard virtually solid mass made up of bony tissue arranged in concentric layers surrounding a central canal which contains nerves, connective tissue and blood vessels. In spongy bone found beneath compact bone, consists of a meshwork of bony bars with many interconnecting spaces containing marrow. Individual bones may be classed as long, short, flat or irregular. Bones not only form the skeleton but also act as stores for mineral salts and play an important part in the formation of blood cells. The skeleton is the rigged framework of connected bone that gives form to the body, protects and supports its soft organs and tissues and provides attachment for muscles and a system of levers essential for locomotion. The 206 named bones of the body are organized into the axial skeleton (Head and trunk) and the appendicular skeleton (limbs). Skeletal homeostasis is dependent upon the delicate balance between bone formation and bone resorption. Bone loss often occurs as a result of their imbalance (Lee et al., 2007).

2.1. Bone Formation

The basic process in bone formation consists of the deposition of a calcifiable organic matrix by osteoblasts and its subsequent mineralization. In early development, osteoblasts differentiate from mesenchyme cells and from fibroblast like cells with mesenchymal potentialities. In later development, they differentiate from fibroblast–like osteogenic cells only. Osteoblasts remain relatively stationary while laying down the matrix and become complexly embedded in matrix at which time they become known as osteocytes. Bone formation is classified into (2) two main types according to the milieu in which it occurs.

2.1.1. Intramembranous

The bone is formed in and replaces a pre-existing membrane of embryonic connective tissue. A periosteum develops on the surface of the newly formed bone and osteoblasts which differentiate in it continue the process of osteogenesis. Bones of the skull and Jaw are examples.

2.1.2. Endochondral Bone Formation

A cartilage model is formed initially; the cartilage is a temporary structure and is eventually destroyed except for the articular surface. Short and long bones are formed this way. The long bones are formed by a combination of endochondral and intramembranous bone formation. The initial bone collar at the primary centre is made by intramembranous ossification. Half way between the ends of the cartilage model, the osteogenic capacity of cells of the perichondrium is activated leading to development of the bone collar. Subsequently, blood vessels penetrate into the cartilage model leading to endochondral ossification. Growth in width of diaphysis of large bones occurs in a manner similar to that of the membrane bones. Most of the true endochrondral bone serves merely as a temporary framework which is ultimately resorbed. Mature bone of the intramembranous or endochondral origin has essentially the same histological structure (Cloos *et al.*, 2004).

A non-lamellar form of bone is normally found in rapidly growing areas of embroynic or developing bone and in healing fractures. Most woven bone is replaced by lamellar bone during remodeling but woven bone persists at certain sites in the adults (eg tooth socket). The process of bone remodeling occurs continually in the embryo, fetus and the adult in order to maintain the proper shape of the bone during growth and for adaption to normal variations in physiological conditions and to changes in stress. Throughout life, Remodelling involves the removal of osseous tissue (resorption) in some locations and adding tissue (accretion) in others. In bone resorption, normally both the organic matrix and the mineral crystals are removed simultaneously.

2.2. Bone Turnover and Bone Loss

Bone mass, rates of bone loss and the risk of fractures are interrelated and both low bones mass and rapid down loss have been shown to be independent predictors of future fracture risk. The rate of bone less is determined by a number of factors one of which appears to be the rate of bone remodeling. Earlier observations has demonstrated that bone formation and bone resorption increase shortly after natural menopause, a phase that in most women is also associated with significantly accelerated bone loss. Similar observations have been made in ovarectomised pre-menopausal women and in castrated men indicating that the withdrawal of endogenous sex steroid induces both high bone turnover and rapid bone loss. Conversely, Markers of bone metabolism return to premenopausal levels during hormone Replacement therapy. Other biochemical studies suggest that high rates of bone turnover may be sustained well into advanced ages. However, it is unclear whether this applies to all women as most longitudinal studies support the notion that individuals with high rates of bone turnover lose bone at a faster rate than subjects with normal or low bone turnover. Other groups argue that due to the high degree of variability in urinary markers of bone turnover, predicting either bone density or changes therein for an individual patient from a single marker measurement may not be possible. Taken together, there is evidence that rates of bone remodeling are associated with bone loss. However, the strength of this association seems to depend on a number of factors such as menopausal age, skeletal site and gender. Bone remodeling markers are no substitute for individual bone mass measurement or for a careful assessment of the patients' personal and family history (Clarke *et al.*, 1996).

2.2.1. Bone Turnover and Fracture Risk

Bone turnover is an independent predictor of fracture risk. Earlier post-hoc analysis of data from clinical trials suggested that in untreated Ostesporotic women, vertebral fractures increase as a direct function of either increased bone turnover or of decreased vertebral BMD. Thus, at a given level of vertebral BMD (Bone Mineral Density), the rate of vertebral fractures increases with the rate of bone turnover. When bone turnover is normal, the main determinant of vertebral fractures is vertebral BMD. All types of non-vertebral fracture but especially fractures of the hip and the upper humerus were associated with urinary levels of DPD above the pre-menopause mean independent of bone mineral density and disability fractures risk increased dramatically when elevated rates of bone resorption were combined with low BMD. The relative fracture risks as defined by either BMD or marker measurement were similar with combined measurement of hip bone density and of bone resorption markers increased the predictive power for hip fractures (Meir, 2006).

Thus in Elderly Women, the relative risk of hip fracture seems to be highest in individuals with both low hip BMD and high rates of bone resorption. In another study, low serum levels of both the carboxy terminal propeptide and teleopetide of type 1 collagen were associated with an increased risk of hip fracture independent of age and BMD. Thus increased rate of bone resorption or decreased rates of bone formation seem to be associated with future Osteoporotic fractures. Accelerated bone resorption was associated with increased risk of Osteoporotic fracture independent of BMD. Combining measurements of BMD and bone turnover improved fracture prediction in Elderly Men (Meir, 2006). Prospective data from the Australian FREE study of 1112 frail elderly men and women indicate that high bone turnover is also an independent predictor of cause mortality. This association appeared to be mainly manifested in deaths from cardiovascular causes. In summary, data from several Independent and large prospective studies indicate that in both postmenopausal women and healthy men, increased rates of bone resorption are associated with an increased risk of vertebral fractures independent of BMD, age and disability. In the future, markers of bone turnover in combination with other risk factors for Osteoporotic fracture may be used to define fracture risks and intervention threshold (figure 1).

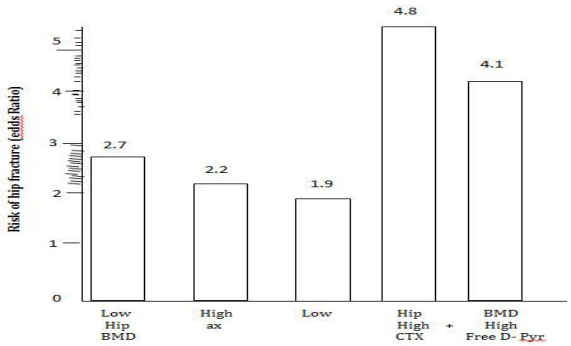

Figure 1: low Bone density plus high resorption marker levels best predict fracture.

2.2.2. Pretreatment Bone Turnover and Therapeutic Effect

From both a theoretical and clinical point of view, it is conceivable that intervention strategies may differ between patients with accelerated, normal or even abnormal low bone turnover at the time of diagnosis, hence a patient presenting with high rates of bone resorption may benefit from antiresorptive therapy, whereas in an individual with a low bone turnover, a stimulator of bone formation may yield better long- term Results. Some studies have shown that in Osteoporotic patient treated with sub cutaneous calcitonin, increases in lumbar BMD were significantly greater in individuals with high than with normal or low base line Rates of bone turnover. Similar results were later reported for short term alendronate treatment although one report suggests that changes in BMD during treatment with alendronate are independent of pre-therapeutic bone turnover rates. A similar post-hoc analysis of fracture intervention Trial examining the influence of pretreatment bone turnover on the anti-fracture efficacy of daily alendronate in postmenopausal women found that the non-spine fracture efficacy of alendronate was significantly greater among both Osteoporotic and non-Osteoporotic women with baseline levels of the bone formation marker aminoterminal propeptide of type 1 collagen. However, no such association was observed for vertebral fracture and changes in both BSAP (Serum bone Alkaline Phosphatase) and the carboxyterminal crosslinked telopeptide (CTX-1) were not associated with fracture outcomes at any site. This observation is in agreement with the hypothesis that changes in bone turnover affects the risk of vertebral fractures only if bone turnover is significantly accelerated, while more subtle changes appear to be without major effect. Taken together, it remains unclear whether there is a clinically relevant Relationship between bone turnover at baseline and the response to antiresorptive Treatment. Drugs even of the same class may differ in this respect.

BONE DISEASES

The various bone diseases include:

a. Osteoporosis
b. Osteoarthritis
c. Paget's disease
d. Rhematoid arthritis
e. Arthritis
f. Rickets
g. Bone cancer

3.1. Osteoporosis

This is a heterogeneous chronic and slowly developing condition. It is characterized by a reduction of bone mass and micro-architectural integrity making patients more susceptible to fractures. Osteoporosis generally occurs with increasing age and in post-menopausal women. It is also a feature of Cushing's syndrome and prolonged steroid therapy. It causes loss of bony tissue resulting in bones that are brittle and liable to fracture. Infection, injury and synovitis

can cause localized Osteoporosis of adjacent bone. Osteoporosis can be detected by Quantitative digital radiography and by DEXA scans. A diet with adequate calcium, exercise and hormone replacement therapy are preventive and bisphosphonates and be used to reduce or halve further bone loss.

3.2. Clinical Application of bone markers

3.2.1. In Postmenopausal Osteoporosis

To asses fracture risk Osteoporosis is diagnosed on the basis of bone mineral density. A better marker of risk is the combination of low density in the hip and high level of marker of bone resorption (deoxypyridinoline).

3.2.2. In Glucocorticoid Induced Osteoporosis

Glucocorticoid therapy causes bone loss and an increased incidence of fractures when given in high does or for prolonged periods by the oral, parental or inhaled routes. Also levels of bone formation markers are generally low and those of bone resorption markers are either normal or low. Presumably, the reduction in bone resportion is not enough to overcome the reduction in bone formation and bone loss ensues which are particular profound in children as liner growth may be retarded (Clowes *et al*., 2000).

3.2.3. In Immobilization Induced Osteoporosis

In a long term cross-sectional study of paraplegic men with spinal cord injuries, bone turnover patterns changed over time. In the first years after injury, urinary Deoxypyridinoline excretion was markedly elevated whereas blood total alkaline phosphatase and Osteocalcin levels were normal to slightly elevated. Over a 30 years period after injury, the bone resorption marker returned to normal level in most patients and the markers of bone formation were normal. Fracture incidence rose but leveled off after 20 years (Seibel, 2006).

3.3. Osteoarthritis

Osteoarthritis is a condition that causes changes in cartilage, the elastic tissue that cushions the joints. Healthy cartilage allows bone to glide over one another, while absorbing energy from the shock of physical movement. In Osteoarthritis, the surface layer of cartilage breaks down and wears away allowing the bones under it to rub together causing pain, swelling and loss of motion of the joint. This degenerative disease resulting from the wear of the articular cartilage leading to secondary changes in the underlying bone can be primary or secondary occurring as a result of abnormal load to the joint or damage to the cartilage from inflammation or trauma. The joints are painful and stiff with restricted movement. Osteoarthritis can also arise from Paget's disease of the bone. Osteoarthritis is recognized on x-ray by narrowing of the joint space (due to cartilage loss) and the presence of Osteophyte, Osteosclerosis and cysts in the bone.

3.3.1. Treatment

The goal of osteoarthritis therapy is to improve joint function and control pain and swelling. Treatment approaches include exercise weight control (use of walking stick), rest, joint care, prescription and over the counter medicines (Analgesics), pain relief techniques and Alternative therapies such as Acupuncture and Nutritional supplements. In certain cases surgery on the affected joint may be needed (Osteomy, arthrodesis, or arthroplasty).

3.3.2. Diagnosis

No single test can diagnose osteoarthritis especially in a person with Paget's disease. Diagnosis of osteoarthritis in a patient with Paget's diseases may involve blood tests, x-ray images or the examination of fluid drawn from the joint. Blood and urine tests may also be used to help find out if something other than Paget's disease is causing the arthritis.

3.4. Paget's Disease

Paget's disease is a chronic disorder that can result in Enlarged and misshapen bones. The excessive breakdown and formation of bone tissue causes affected bone to weaken resulting in pain, misshapen bones, fractures and other bone and joints problems including osteoarthritis. Paget's disease a localized disorder marked by a rapid increases in bone turnover resulting in woven bone which is susceptible to fracture. It typically affects just one or a few bones mostly in the elderly and most frequently affecting the skull, backbone, pelvis and long bones.

Affected bones become thickened and their structure disorganized with x-ray revealing patchy sclerosis.

3.4.1. Symptoms

There are after no symptoms but pain, deformity and fracture can occur when the skull is affected, blindness and deafness can occur due to nerve compression. There is a very small (1%) risk of malignant change (osteosarcoma).

3.4.2. Treatments

This is done with bisphoshonates or calcitonin. The goal of Paget's disease therapy is to relieve pain and control the progress of the disorder therefore treatment strategies should include the use of appropriate forms of exercise and over the counter pain medications and in some cases, surgery on the affected bone or joint. Scientists do not know for sure what causes Paget's diseases.

3.4.3. Clinical Application of Bone Markers

Paget's disease is characterized by an early Osteolytic phase to secondary osteoblastic activity dominance. Patients with Extensive polyostotic disease, bone resorption and formation marker levels may be higher than in almost any other skeletal disoder with an exception of Osteocalcin (Itannon *et al.*, 2004).

3.5. Rhematoid Arthritis

Rheumatoid Arthritics is a disease of the synovial lining of joint, the joints are initially painful, swollen and stiff and are usually affected symmetrically. It typically involves the joints of the fingers, wrists, feet and ankles with later involvement of the hips, knees, shoulders and Neck. As the disease progress, the ligaments supporting the joints are damaged and there are erosions of the bone leading to deformity of the joints. Tendon sheath can be affected, leading to tendon rupture. Onset can be at any age and there is a considerable range of severity. Women are at a greater risk. Rheumatoid Arthritics is an Auto- immune disease and most patients show the presence of rheumatoid factor in their serum. There are characteristic changes on X-ray. In the early stage there is soft tissue swelling and periarticular Osteoporosis; late stages are characterized by marginal bony erosions, narrowing of the auricular space, articular destruction, and joint deformity.

3.5.1. Symptom

It is usually characterized by deformity of the joint ligament.

3.5.2. Treatment

This is done using a variety of drugs including anti-inflammatory Analgesics, steroids, immunosuppressant, and gold salts. Surgical treatment is by excision of the synovium in early cases or by fusion or joint replacement once bony changes have occurred. The condition may resolve spontaneously but is usually relapsing and remitting with steady progression. It may finally burn itself out leaving severely deformed joints.

3.5.3. Clinical Application of Bone Markers

Fracture incidence is increased in patients with Rheumatoid arthritis generally; studies have reported increased bone resorption based on type 1 collagen markers although serum total TRAP protein is elevated in Rheumatoid Arthritis patients. This is probably due to the 5a Isoform the Origin of which may be macrophages and dendritic cells. The influence of Abnormalities in bone formation on bone loss is less clear. Levels of bone formation markers have been reported to be normally elevated or reduced.

3.6. Arthritis

This is the inflammation of one or more joint characterized by swelling, warmth, redness of the overlying skin, pain and Restriction of motion. Over 200 diseases may cause arthritis inducing rheumatoid Arthritis, Osteoarthritis, gout, tuberculosis and other infections. Diagnosis is assisted by examination of the pattern of distribution of affected joints, X-rays, blood tests and examination of synovial fluid obtained by aspiration of a swollen joint. Mono- or oligo Arthritis is inflammation of one joint, pauciarthritis of a few (four or less), and polyarthitis of many joints either simultaneously or in sequence. Any disease involving the synovial membranes or causing degeneration of cartilage may cause arthritis.

3.6.1. Symptoms

Swelling, pain and redness of the overlying skin are associated with the condition.

3.6.2. Treatment

This depends on the cause but aspirin and similar analgesics are often used to suppress inflammation and hence reduce pain and swelling.

3.6.3. Clinical Application of Bone Markers

Treatment of arthritis with high dose glucocorticoid pulse therapy is effective in controlling symptoms and some manifestations of the immune system in patients with the disorder. The latter effect would be expected to have a beneficial effect on bone metabolism. This is the case as there are only Transient decrease in bone formation markers and no significant reduction in bone density. Similarly, there is only a transient decrease in serum Osteocalcin after an intra- articular injection of a glucocorticoid and no effect on urinary pyridinoline.

3.7. Rickets

This is a disease of childhood in which the bones do not harden due to a deficiency of vitamin D which is responsible for calcium salts deposition in bones to make them rigid. Consequently they become soft and malformed. This is particular noticeable in the long bones which become bowed, and in the front of the ribcage where a characteristic rickety "rosary' may become apparent. Vitamin D decency may be dietary or due to lack of exposure to sunlight which is important in the conversion of Vitamin D to its active form.

Renal Rickets to due to impaired kidney function causing bone forming minerals to be excreted in the urine resulting in softening of the bones.

3.7.1. Clinical Application of Bone Markets

Rickets of any cause is characterized by increased Osteoblstaic activity. If the underlying causes are vitamin D deficiency, genetic or acquired defects in calcitriol synthesis or vitamin D resistance, then hyper parathyrodism with increased bone resoption is a secondary feature. Serum total alkaline phosphatase activity has been a useful marker of disease activity as bone resoprtion markers are elevated in Vitamin D deficiency but not widely used in chemical practice as serum parathyroid hormone is an excellent indirect means of assessing the presence of increased bone resorption and the Response to therapy (Miller *et al.*, 1999).

3.8. Bone Cancer

Bone metastases are common complication in cancer patients. It is very common in breast and prostate cancer but less common in other tumors. They are classified as Osteolytic, Osteoblastic or mixed on the basis of radiographic features. Bone cancer is a highly malignant tumour arising from within a bone usually in the metaphysis of the long bones especially that of the knees and the proximal end of the humerus. It is usually seen in children and adolescent but can occur in adults of all ages. The usual site of the tumor in children is the leg particularly the femur associated with secondary growth (e.g. in the lungs, liver)

3.8.1. Symptoms

Pain and swelling at the site of the tumor.

3.8.2. Treatment

Traditionally, treatment of disease localized to the primary site was by amputation of the limb; limb-sparing surgery is now possible after neoadjuvant chemotherapy with replacement of the diseased bone by a metal prosthesis.

3.8.3. Clinical Application of Bone Markers

Biochemical markers of bone turnover have proven useful in assessing the magnitude of the metastases, the response to therapy and even the prognosis for survival. Osteolytic metastases which are common in breast cancer are associated with increases in bone resorption markers and after treatment with intravenous bisphosphonates the levels decrease nearly 70%. Patients with high urinary N-telopeptides have higher risk of skeletal complications and disease progression those patients with low level across multiple tumor groups including multiple myeloma. In Osteoblastic metastases, prostate cancer patients typically have predominant osteoblastic lesions with high serum total alkaline phosphatase activity and other markers of bone formation and bone resorption. Decrease is associated with treatment, urinary N-telopeptides markedly but serum bone specific alkaline phosphatase decrease only slightly upon treatment with Zometa (Zoledronic Acid) Intravenously. In hormone-suppression therapy; two of the most successful cancer Therapies accelerates bone loss through marked suppression of gonadal steroids. Bone markers increase and bone loss ensues as resorption exceeds formation. Estrogen suppression appears mainly responsible in both sexes.

CURRENT DIAGNOSTIC MARKERS

A variety of biochemical assays that Reflect the activity of Ostexblasts (The bone-forming cells) and ostexlasts (The bone-resorting cells) have been developed for durual use based on these markers. They have helped increase our raderslanding of the bone remodeling cycle, the pathagemesis of skeletal disorders and the response of these disorders to therapy. These markers are measured in serum or plasma and sometimes in urine.

4.1. Alkaline Phosphates (Ap)

Alkaline phosphatase is a ubiquitous membrane-bound tetrameric enzyme attached to glycosyl-phosphatidylinositiol moieties located on the quter cell surface it was introduced into clinical use in 1929. The total tlkaline phosphatase post consists of several demeric Isoforms found in the plasma membrane of osteoblasts and also originates from various tissues liver, bone, intestine, spleen, kidney and placenta. In addition, certain tumors may express macromoleiular forms of tlkaline phosphatase. The differences between these isoforms are due

to post-translation modification in the carbohydrate meuty content.

The precise function of the Enzyme is yet unknown but it obviously plays an important role in Osteoil formation and mineralization. In normal adults with normal liver function, approximately 50% of total AP activity in serum is derived from the liver, whereas 50% arises from bone (Macier *et al.,* 2006). In children and adolescents the bone-specific isoenzyme predominates (up to 90%) because of skeletal growth.

Many techniques have been developed to differentiate between the two main isopesme of circulating AP including heat denomination, electrophoresis, precipitation, selective inlubation and more recently, immunoassays. In healthy adults, most methods show a good correlation between bones specific and total AP. The never immunoassays allow simple and rapid quantization of either Enzyme activity of enzyme mass however, like all the other techniques even these assays show a certain degree of cross reactivity between the bone and liver alkaline phosphatase (15-20%) therefore, in subjects with High liver AP, results of bone AP measurement may be artificially high leading to false positive Results.

Alkaline phosphatase was the first biochemical marker of bone turnover and is the most widely used marker of born metabolum) in chemical practice due to the wide availability of inexpensive and simple methods. Once liver disease is ruled out, semen levels of total alkaline phosphatase provides a good impression of the extent of new bone formation and osteoplastic activity.

4.2. Osteocaccin (Oc)

Ostencatan is a large peptide that is synthesized by osteoblasts, Osteotablasts and hypertrophic choadrocytes. It binds to hydrozyapatite and much of it is deposited in the bone matrix because Osteocalan payments are released from the bone matrix during resonption assays for circulating Osteocatun and its fragments reflect both bone formation and resorption (Cloos *et al.,* 2004). The exact function of osteocatein in bone is still unclear but recent studies raise the surprising possibility that it is a hormen that influences energy metalodum by modulating the production and action of incline osteocalane is the most abundant non-collagenous protein in bone which comprises 1-2% of the total bone protein. It is an excellent master of bone formation (osteoblast function). Serum levels of immunoreactive osteocalane have been shown in correlate well with the bone formation Rate. However, the peptide is readily subject to degradation in serum so that both intact peptides and OC fragments of various sizes coexist in the circulation. This helerogeneity of OC fragments in serum results in considerable limitations. In the chemical application of this a priors highly specific master, thus the various assays used to measure OC in serum detect fragments of various sizes and usually epitspe specificity and antibody cross-reactivity of the assays are ill defined. However, only one third of the total OC serum past represents intact OC and due to its instability in serum rapid loss of immunoreativity is seen with these assays when samples are left for more than 1 hour at room temperature. Therefore quick processing of the blood sample after drawing is essential for most assays sine a loss of reactivity is noted within a few hours at Room temperature. The same applies to sera subjected to multiple having or prolonged storage at temperature above 25^0C. Serum OC is elevated in patients at increased fracture risk due to coleomalace and Paget disease (Lee *et al.,* 2000).

4.3. Procollagen Type I Properties

Procollagen type I properties are cleared from the ends of the procellagen include and can be detected in the circulation. These from ammo-terminal end are called PINB, these from the carboxy-terminal end are called PICP, although these propeplide are also syathesized in the skin teadons ligaments, connect blood vessels fib-ocarlilage and many other tissues their main source is bone. The level of each of the propeplides in blood is thought to reflect the amount of newly synthesized collagen. After secretion into the extracellular space, the gladder trimoric propeplides are enzymatically cleared and liberated into circulation. PICP is stabilized by disulphide bones, direct by liver endothelial cells via the measure, receptor and therefore has a short serum half-life of 6-8mine PINP is much in produce and hydroxyproline and is uliminated from the circulation by liver andothhal cells by the searenger receptor. The propeptidas are considered quantitate measure meant of newly formed type 1 collagen. Although type 1 collagen propepltides may also arise from other sources, most of the non-skeletal tissues exhibit a slower turnover than bone and contribute little to the circulating propeptide pool studies has shown good correlations between serum PICP levels and the rate of bone formation. While the clinical relevance of PICP in the evaluation of metabolic bone diseases is still viewed with skepticism, serum PINP appears to be of greater diagnostic

validity. The thermostability of the propeptide is an advantage in that extended transport and stonage times are well tolerated without significant loss of signal (Boyce, 2007).

4.4. Hydroxyproline (Ohp)

Hydroxyproline is an amino and common 60 and character of all forms of collages and urinary hydroxyproline excretion is the oldest test of bone resorption. It is formed intracellularly from the post translation with hydroxylation of proline and constitute (12-14%) of the total amino and content of mature collagen. Ninety percent of the OHP liberated during bone degradation is primarily metabolized in the liver. It is subsequently excreted in urine where it may be detected either as free or peptide thomnd hydroxyptoline. Urinary OHP is usually considered to reflect bone resorting. However, it should be noted that significant amount of urinary OHP are derived from the degradalion of newly synthesized collagen. In addition, hydroxyproline can be found in other tissues such as the skin and moreover liberated from the metabolism of elastin and C1q. Urinary hydroxyproline is therefore considered an unspecific index of collagen turnover and consequently has been largely replaced by more specific techniques (Lee *et al*., 2007).

4.5. Collagen Cross-Links

(3-hydroxypyridinum crosslings of collagen uriary pyridinline (PYD) and deoxypyridinline (DPD) pyridindines are cross-linking amino acids that strengthen collagen fibrils in the extracellular matrix. They are found in the main fibril forming collagens (Types 1, II & III) of many tissues pyridinoline is the major chemical form but teoxypyridinoline is also unusually aboundant in bone collagen and hence is a relatively selective bone marker indicating active bone formation (Robins, 1995). PYD and DPD are formed during the extracellular mature of fibrillar collagens. As trifunctional crosslinks, they bridge several collagen peptides and mechanically stabilize the collagen moleaile. During bone resorption, croslinked collagens are preteslytically broken down and the crosslink components are released into the arculation and the urine. The measurement of hydroxypyridium crosslinks is not influenced by the depradation of newly synthesized collagens and their levels strictly reflect the degradation of mature crosslinked collagen in addition, the urinary excretion of pyridinuim crosslinks is independent of dietary sources since neither PYD nor DPD are taken up from food. Finally, PYD and DPD show high specificity for skeletal tissues while PYD is found in cartilage, bone, and vessils, DPD is almost exclusively found in bone and dantin thus they are currently viewed as the best Indies for assessing bone resorption (Clemens *et al.*, 1997). Currently, automated techniques for the measurement in urines and serum are available although the amount of free and peptide bound crosslinks seems to vary with the bone pathology, direct immunoassays for free and peptide–bound crosslinks are widely used. The first was an assay that recognized an N-telspeptide of collagen type 1 (NTx) in urine and serum several other assays target structural variants of a peptide sequence that originates from the carboraj-terminal crosslinking region of collagen type 1 (CTx) (Hanson *et al.*, 1992).

4.6. Cathepsin K

Cathepsin K is a member of the cysteine protease family that unlike other cathepsins has the unique ability to clear bothe helical and telopeptide of collagen 1. Its clonical relevance was appreciated with the discovery that pycnodysostosis, an antosomal reciesive disease characterized by ostesporoses was the result of mutations in the cathepenk gene. Immunocytochemical studies have shown that cathepsink is located intraculularly in vesicles granuls and vacuoles throughout the cytoplasm of osteoclasts and that it is secreted into bone resorption cacunae for extra-cellubr collagen degradation (Meir *et al.*, 2006).

Recently a new enzyme–linked immunoassay for measurement of cathepsin k in serum has been developed. Due to the fact that cathepein k is expressed and secreted by osteoblasts during active bone resorption cathepsin k and specifically its circulating form may be a useful and specific biochemical marker of osteodastic activity (bone resorption) as the primary proteolytic enzyme used by osteoclasts to degrade bone type 1 collagen during resorption.

4.7. Serum Tertrate- Resistant and Phosphatase (Trap)

This enzyme belongs to the family of the ubiquitous acid phosphatases of wheich at least five different isoforms are known. These isoforms are expressed by different tissues and cells such as prostate, bone, spleen, platelits, erythrocytes and macrophages all acid phosphatase are inhibited by L(+) tartrate except band 5 which was therefore termed fartrate-Resistant acid phosphatase. Of the latter, 2 subforms 50 and 56 are known and recent research has shown that TRAP – 56 is characteristic of Osteoclasts (Uebelhart *et al.*, 1991). The origin of TRAP – 5a is unknown but

may be expressed by macrophages. The two isoforms 5a and 5b are different in that 5a contains salic acid whereas 5b does not. Most assays for measuring TRAP in blood have used colorimetry and detected both isoforms without differentiating between bands 5a and 5b. More recently, specific immuwoassys for TRAP 5b have been described and clinical results indicate that the marker may be useful to assess osteoclast activity. For the conventional TRAP assays, care should be taken after phlebotomy to stabilize the enzyme since TRAP loses more than 20% of its activity per hour at Room Temperature although this can be prevented by the addition of citrate buffer to the sample.

4.8. Cross-linked Telopeptised Receptor Activator of Nuclear Factor Rappa Of Type 1 Collagen (Rank)

This is a pirotal regulator of ostesolast recruitment and activity but is limited in specificity by the broad role it plays in signaling in the immune system. The first collagen talopeptide assay was a RIA for the carboxyterminal type 1 collagen telopeptide (ICIP) in serum with respective antibodies raised against a crosslink-containg peptide isolated from human bone. Divalently and non-crosslinked pepltides do not react with the antibody nor do peptides isolated do not react with the antibody nor do peptides isolated from skin. The ICTP assay appears to be sensitive for pathological bone resorption as seen in multiple myeloma, metastatic bone disease and other degradation processes carolvig hasty breakdown of skeletal and non-skeletal type 1collagen (Boyce *et al.,* 2007). Only one peptide strand is necessary for immunoreactivity. Further to isomerisation many proteins are also subjected to racemisation of certain residues. Both processes are considered an effect of agency, as the extent of raamisation and isomerisation increases with the time elapsed since the synthesis of the protein. This immunoassays new exist (though not commercially available) for all four possible isomers of the CTx molecule (the native a-L form, the B-isomerised isoaspartyl peptide and the respective racemized forms of a-D and B-D. the differential use of these assays may possibly provide information on the age-dependent changes of collagen in health and disease. The assay formal is identical for the urine and the serum based assays and the analyle seems to be stable at Room temperature and during up to three freeze-throw cycles (Christgau *et al.,* 1998).

4.9. Variability

To avoid being misled, dimanns who use biochemical markers of bone formation should be familiar with factors that influence assay result. Most bone turnover markers exhibit significant within subject variability possing a major problem in the practical use of bone markers. Whenever a change in a bone marker level is observed in an individual patient, this change must be interpreted against a background of the respective marker's variability (Seibel, 1993).

4.10. Technical Sources of Variability

In addition to perameters of assay performance, factors such as the choice of sample (ie: serum on urine), made of sample collection (eg. 24 hours collection or second morning void) the appropriate preparation of the patient (eg. Pre-sampling diet in fasting or exercise before phlebotoring), the correct handling processing and rage of specimen should always be considered. This is important as these technical sources of variability are controllable and hence modifiable. For practical use, some technical variability is discussed.

4.10.1. Thormedgradation

Some bone markers are sensitive to conditions such as temperature as there is rapid enzymatic cleavage of the peptide into smaller fragments leading to loss of significant signal of the serum sample is kept at room temperature for more than 1-2hours. Adding protcase inhibdors will delay but by no means prevent this process some bone markers activity decrease as temperature vary (eg TRAP) while some remain stable for a longer time (eg. PYD and DPD).

4.10.2. Photolysis

Pyridinium crosslinks in agueous solutions are unstable when subjected to intensive UV irradiation. The effect increases with rising pH and has been shown to be greater for free than for total pyridinoline. Urinary NTx and CTx are not affected by UV light exposure.

4.10.3. Timing

Handling and mode of sample collection: Improper collection and handling of specimens can seriously affect assay precisean. The optimal time to collect samples is in the morning (first or second morning raid) careful sample collection and storage are particularly important in measuring serum osteocalan and TRAP.

4.10.4. Diurnal and Say to Day Variability

This is most important as levels of bone markers are highest early in the morning and lowest in the afternoon and evening varying in urine 20% to 30% from the highest to lowest value of the day. Serum markers change to a smaller degree except for serum CTx which can vary by more than 60% during the day (Wichers el al, 1999)

4.10.5. Diet

Blood for measurement of serum CTx should be taken in the marking after overnight fasting to avoid the large decrease that occurs after eating. Ingestion of hyhoxyprolina-rich ford such as meat affect 01+p measurement in urine while urinary or serum DPD is unaffected by collagen ingestion.

4.10.6. Variation between Laboratories

It is also important to use the same laboratory for serial measurement since assay results can vary considered among laboratories even if they use identical methods. Immunoassays of bone turnover markers should be include into routine proficiency testing programs.

4.10.7. Biological Sources of Variability

Intra-individual sources of variability are much larder to control than technical aspects of variability. Many biological factors cannot be modified at all (eg. Age, gender, ethnicity, etc.) while others are hard to control in clinical practice, nevertheless, every effort should be made to account for these factors when interpreting the result of bone marker measurement (Seibel, 2005).

4.10.8. Age Is Changes in Sex Hormone Level

Serum and urinary concentrations of most bone markers return to a level somewhat lower than that seen during normal puberty and growth. Once somatic growth subsides it stabilizes during the 3rd decade and in healthy man practically all markers remain stable until 70years of age. After that, a slight increase is usually seen in formation markers such as serum osteocatan and most resorption markers. Meaopause mirror 50%-100% increase in bone markers but increase in bone turnover can be attenuated by calcium supplementation. In girls, peak bone marker levels are observed approximately two years earlier than in boys. In men between 20 and 30years of age, bone markers are usually higher than in women of same age bracket (Rosen *et al.*, 2004).

4.10.9. Other Sources of Variability

A number of non-skeletal diseases have been shown to strongly affect bone turnover markers. These conditions mostly relate to improvements in the charance or metabolism of the components measured. Thus even moderate impairment of renal function has been shown to have significant effects on the serum levels of osteocalane and of the collagen type 1 telopeptides (Hassager *et al.*, 1994).

CONCLUSION

With the ageing population in most countries, disorders of bone and mineral metabolism are becoming increasingly relevant to everyday clinical practice consequently, the increases in and the need for effective measure to be used in the screening diagnosis and follow-up of such pathologies has markedly grown. Together with clinical and imaging techniques, biochemical task play an important role in the assessment and differential diagnosis of metabolic diseases. Although these bone turnover markers are not specific enough to be used alone nor to replace bone mass measurement they are however so sensitive that the reflect almost all disease processes or therapies that affect bone metabolism and they serve as excellent adjunct to bone mass measurement for improving management of fragility fractures and metabolic bone disease. These biochemical indices are non-invasive, comparatively inexpensive and when applied and treated correctly, helpful tool in the diagnostic and therapeutic assessment of metabolic bone disease and aid in managing patients with a variety of skeletal disorders (Seibel, 2005). In clinical practice, it appears adequate to use the least expensive test serum alkaline phosphatase activity to assess disease activity and the response to therapy.

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