Osteoprotegerin and Crosslaps, New Markers of Bone Remodeling in Postmenopausal Osteoporosis.

Thesis
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By
Asmaa Ismail Ahmed
MB., B.ch., M.Sc. Clinical & Chemical Pathology

Supervisors

Prof. Dr.
Omnia Ahmed Yousef
Professor of Chemical & Clinical Pathology
Faculty of Medicine
Cairo University

Prof. Dr.
Lamia Ali Mansour
Professor of Chemical & Clinical Pathology
Faculty of Medicine
Cairo University

Prof. Dr.
Manal Mohamed Sedky
Prof. of Rheumatology & Rehabilitation
Faculty of Medicine
Cairo University

Faculty of Medicine
Cairo University
2006

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List of Abbreviations

ALP	alkaline phosphatase
BAP	bone-specific alkaline phosphatase
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
CAD	coronary artery disease
CTx	carboxy terminal crosslinked telopeptide of type i collagen.
DDH	death domain homologous
DEXA	dual- energy x ray absorptiometry.
DM	diabetes mellitus
DPD	deoxypyridinoline
EIA	enzyme immunoassay
ELISA	enzyme linked immunosorbant assay
ERK	extracellular signal regulated kinase
HPLC	high performance liquid chromatography
HRT	hormonal replacement therapy
ITAM	immunoreceptor tyrosine based activation motif
MMP	matrix metallo proteinase
MAPK	mitogen activated protein kinases
NTx	amino terminal crosslinked telopeptide of type i collagen
OC	osteocalcin
OH Lys	hydroxylysine
OH Pr	hydroxyproline
OPG	osteoprotegerin
PINP & PICP	amino & carboxyteminal propeptide of type i collagen
PTH	parathyroid hormone
PYD	pyridinoline
RANK	receptor activator of nuclear factor kappa b
RANKL	receptor activator of nuclear factor kappa b ligand
TGF	tissue growth factor
TNF	tumour necrosing factor
TRAF	tnf receptor associated factor
TRAIL	tnf related apoptosis inducing ligand
TRAP	tartarate resistant acid phosphatase

Introduction & Aim of the Work

Bone mineral density (BMD) decreases with advancing age among both women and men. Women in particular, suffer accelerated bone loss after menopause . While changes in sex hormone levels are considered very important in the development of osteoporosis, other factors, including genetic ones, nutrition, body weight, muscle function, and calcium absorption, are thought to play a significant role as well . Moreover, recent studies suggest that peak bone mass may be a key determinator of bone mineral density in adult life . It is thought likely that all these factors influence bone turnover in an interrelated and complex way, either directly or indirectly (Olafur et al.,2005).

Osteoprotegerin (OPG) is a glycoprotein that belongs to the TNF-receptor superfamily. It is a part of a newly described cytokine system that is important in the control of osteoclast maturation. OPG acts as a decoy receptor, binding to receptor activator of nuclear factor kappa B ligand (RANKL),preventing the activation of precursor cells, thus inhibiting bone resorption (Olafur et al.,2005).

Attempts have been made to develop a bone resorption assay based on serum specimens, like serum crosslaps . Recent clinical data provide supportive evidence that serum crosslaps levels reflect specific bone resorption (Peichl et al.,2001).

Overweight is associated with both higher bone mineral density, and higher serum leptin concentrations(Ruhl et al.,2002). Leptin was initially identified as an anorexigenic hormone. It is now known to have actions on different systems of the body, including skeletal system either centrally or peripherally (Moran & Philipp,2003).

The aim of this work is to evaluate the diagnostic significance of serum crosslaps & osteoprotegerin as markers of osteoporosis, to study their correlation with obesity, leptin and BMD, & to select the best combination of serum markers for follow up of osteoporosis.

Osteoporosis

Definition & Introduction

Osteoporosis literally means porous bone .Bone is a live and constantly changing structure and osteoporosis is accelerated bone loss. It is a disease that results in weak, fragile bones that can break easily (Lindsay,1988; Bell , 2003).

"And Athena lavished a marvelous splendor on the prince so that all the people gazed in wonder as he came forward. The elders making way as he took his father's seat. The first to speak was an old lord, Aegyptius, stooped with age, who knew the world by heart."
(Homer, the Odyssey: translation by Robert Fagles).

Loss of height (stooping), Dowager's hump, and kyphosis are some of the most visible signs of old age in humans. The primary reason for these involutional changes is a progressive loss of bone mass that affects the axial (primarily trabecular) as well as the appendicular (primarily cortical) skeleton. Loss of bone mass, along with microarchitectural deterioration of the skeleton, leads to enhanced bone fragility and increased fractures—the bone disease known as osteoporosis (Riggs & Milton , 1986).

The process of bone remodelling from resorption to matrix synthesis to mineralization normally takes about 8 monthes , a slow but constant process . Bone in older persons just is not as efficient as bone in younger persons at maintaining itself . There is decreased activity of osteoblasts and decreased production of growth factors and bone matrix. Bone loss is greater in women past menopause than in men of the same age (Deal ;1997). Both men and women start losing bone in their 40s. However, women experience a rapid phase of loss during the first 5–10 yr after menopause, due to the loss of estrogen. In men this phase is obscure, since there is only a slow and progressive decline in sex steroid production; hence, the loss of bone in men is linear and slower. In addition to losing bone faster at the early postmenopausal years, women also accumulate less skeletal mass than men during growth, particularly in puberty, resulting in smaller bones with thinner cortices and smaller diameter. Consequently, the incidence of bone fractures is 2- to 3-fold higher in women as compared with men (Riggs&Milton, 1986; Manolagas,2000).

Diagnosis of Osteoporosis

There are three available diagnostic methods to estimate the metabolic state of bone: bone density, bone biopsy, and measurement of specific biochemical markers. Bone density measurements are essential for the diagnosis of osteoporosis and biopsies are useful for assessing bone metastases (Kanakis et al.,2004).

In contrast to biochemical markers of bone resorption, bone mineral density (BMD) measurements are subject to low intraindividual variation. However, only small annual responses in BMD are seen when using antiresorptive therapy. In fact, the BMD response of individual patients over a 1 year treatment period is typically of the same magnitude as the precision error for BMD measurements: 1%–5%. Thus, follow-up periods of 1 year are necessary to monitor a statistically significant effect of antiresorptive therapy with BMD measurements. Hence, methods for rapid evaluation of the response to antiresorptive therapy, as well as assessment of prognosis, will be of considerable value for its clinical use (Qvist et al.,2002).

Dual- Energy X Ray (DEXA)

Assessment of existing bone mass, determining the fracture risk based on this clinical assessment, and making decisions regarding the appropriate therapeutic intervention are the ultimate goals when evaluating patients for osteoporosis. The WHO established diagnostic criteria for osteoporosis on the basis of BMD T-scores. The T-score describes the patient's BMD in terms of the number of standard deviations (SDs) by which it differs from the mean peak value in young, healthy persons of the same sex. The WHO uses a threshold of 2.5 SDs below the mean of young adult women as the criterion for a diagnosis of osteoporosis. The criterion for a diagnosis of osteopenia (low bone mass) is more than 1.0 SD but less than 2.5 SDs below the reference mean. However, T-scores were developed for the estimation of the prevalence of osteoporosis across populations, not for the assessment of osteoporosis in specific patients. Moreover, although T-scores originally were based on the BMD of the hip measured by dual-energy x-ray absorptiometry (DEXA), the scores are now applied to BMD at other skeletal sites and/or measured by different methods. Currently, the National Osteoporosis Foundation and the International Society for Clinical Densitometry consider central DEXA of the hip and/or spine as the preferred measurement for a diagnosis of osteoporosis (Lane et al.,2005).

Biochemical Bone Markers :

Bone remodeling is often considered ‘‘coupled’’. Coupling involves a link between bone formation and bone resorption. This term, however, should not be confused with ‘‘balance’’, which implies the complete replacement of removed bone. By means of coupling, biochemical bone markers reflect the general process of bone turnover when the bone is in a steady state. However, markers are classified on the basis of the remodeling process that they mainly reflect in acute situations. The process of resorption takes place in less time (7–10 days) than the process of formation (2–3 months). Resorption markers therefore allow a faster response (2–12 weeks) to changes in remodeling than formation markers (3–6 months). A number of biochemical assays readily detect the above molecules released from the bone matrix and bone collagen degradation in both serum and urine (Bernardi et al.,2004).

Table 1 Biochemical Markers of bone metabolism

Marker and its abbreviation	specimen	Biochemical features
Bone formation markers		
Alkaline phosphatase(AlP)	serum	Exists in several forms ,not specific for osteoblastss
Bone specific alkaline phosphatase(BAP)	serum	Bone specific isoform of AP, osteoblastic specific
Osteocalcin(OC)	serum	Non collagenous bone matrix protein,osteoblastic specific
Amino & Carboxyteminal propeptide of type I collagen(PINP & PICP)	serum	Extention domain of type I collagen ,serum levels reflect formation rate of type I collagen molecules
Bone resorption markers		
Hydroxyproline(OHPr)	urine	Degradation product of all collagens ,unspecific marker of bone resorption
Hydroxylysine(OHLys)	urine	Degradation product of all collagens ,unspecific marker of bone resorption
Pyridinoline & deoxypyridinoline(PYD &DPD)	urine	Degradation product of maturel collagens ,originates from bone & dentin,urine levels reflect bone resorption
Amino terminal crosslinked telopeptide of type I collagen(NTx)	Urine,serum	Degradation product of mature collagen ,originates from all tissues containing type I collagen, fluid levels reflect bone resorption
Carboxy terminal crosslinked telopeptide of type I collagen(CTx)	Urine,serum	Degradation product of mature collagen ,originates from all tissues containing type I collagen, fluid levels reflect bone resorption
Tartarate resistant acid phosphatase(TRAP)	serum	Exists in 2 isoforms ,5a and 5b,5b is osteoclast specific.

Quoted from Zittermann et al.,(2006)

Others Markers:

Osteoprotegerin & Receptor Activator of Nuclear factor K Ligand(OPG & RANKL)

Bone remodeling is a complex process characterized by coordinated resorption and formation of new bone and is regulated by systemic and local factors that affect osteoclast and osteoblast cells. In pathological conditions, the process may be up- or down-regulated. Recently, a cytokine system regulating osteoclastogenesis with a new role on osteoblasts have been elucidated: Osteoclastogenesis inhibitory factor, or osteoprotegerin (OPG), is a natural decoy receptor for osteoclast differentiation factor, produced by osteoblasts. OPG and its ligand receptor activator of nuclear factor K ligand (RANKL), represent complex mediators of regulation of bone resorption, probably playing an important role in homeostasis of bone turnover (Malyszko et al.,2003).

Markers of bone formation

Bone-specific alkaline phosphatase

The bone specific ALP isoenzyme(BAP) , a tetrameric glycoprotein, predominates during childhood and adolescence and represents less than 40% of the total serum concentration. It has been demonstrated in matrix vesicles deposited as ‘‘buds’’ derived from the cells membrane. These deposits seem to play an important role in bone formation(Moss et al.,1992; Terpos et al.,2005). BAP is produced by osteoblasts during the bone formation phase by clipping off the membrane and released into the circulation. It is produced in extremely high amounts during bone formation phase of bone turnover and is, therefore, an excellent indicator of bone formation activity(Harris ,1989 ; Loffman et al.,2005).

Variations in bone ALP

Bone ALP activity predominates during childhood and adolescence. It also increases during the menopause but returns towards premenopausal levels after 60–65 years of age. Approximately 15% higher activities of ALP were observed in menopausal women compared with premenopausal women and higher inter-individual variations were seen in the older age groups. Even though total ALP is a less specific marker than BAP, bone ALP comprise approximately 50% of the total