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SERUM OSTEOCALCIN: A SPECIFIC MARKER FOR BONE FORMATION IN POSTMENOPAUSALOSTEOPOROSIS

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ABSTRACT

Elderly patients often are unable to enjoy pain free physical activity due to various orthopedic problems. The orthopedic problems in the geriatric group comprises, bone disease due to metabolic conditions such as osteoporosis and osteomalacia, fractures peculiar to old age, degenerative disorders and metastatic bone disease. With the increase in life expectancy, osteoporosis has become a formidable public health problem in India and a multidisciplinary approach is needed for its management. Osteoporosis is one of the major problems facing women and older people of both sexes. The morbid event in osteoporosis is fracture. Various biochemical markers are now available that allow a specific and sensitive assessment of the rate of bone formation and bone resorption of the skeleton. Although these markers are not recommended for use in diagnosis of osteoporosis yet, they appear to be useful for the individual monitoring of osteoporotic patients treated with antiresorptive therapy. Biochemical markers provide valuable insight into the complexities of bone metabolism i.e. dynamic status of bone remodeling. It is also stimulating the search for new analytical methods (or markers) to unlock what has, for many years, been a relatively closed world for clinical biochemists: bone metabolism and biochemical markers of diseases.This brief review will provide detail information about current and specific bone formation marker i.e. osteocalcin.

KEYWORDS: *Bone formation marker, Serum Osteocalcin (S-OC), Urinary Osteocalcin (U-OC), Bone Gla Protein (BGP), Gamma-carboxyglutamic acid (Gla) and Enzyme Amplified Sensitivity Immunoassay (EASIA).*

INTRODUCTION

Osteoporosis is second only to cardiovascular disease as a leading health care problem, according to the World Health Organization.[1] Increased mortality rate associated with fracture may be the worst consequence, but the loss of independence and lowered quality of life of patients living with the disease for years might be the greatest burden of osteoporosis.[2] Earlier diagnosis and prevention of fractures should decrease the medical, social and economic burdens of this disease.

Osteoporosis is a disease that may have a tremendous impact on the lives of many postmenopausal women. Osteoporosis and it's potentially devastating sequelae of fracture are increasing as the population ages and assessment of skeletal health is an important component of a women's routine care.[3]

"Osteoporosis is a progressive systemic skeletal disorder characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture".[4] World Health Organization (WHO) defines osteoporosis as bone density that is 2.5 Standard Deviation (SD) or more below the young adult mean value (T score<-2.5).[5]

Bone is a specialized form of metabolically active, mineralized connective tissue. It is a "dynamic tissue" that is remodeled constantly throughout life.[6] In adults bone resorption by osteoclasts is closely coupled with bone formation by osteoblasts to maintain a state of equilibrium until around age 30, after which bone density starts to decline slowly. At the menopause bone loss is accelerated as estrogen deficiency increases the

activity of osteoclasts. Consequently, the net rate of bone resorption exceeds the rate of bone formation, resulting in too little bone, or osteoporosis.[4] The pathogenesis of postmenopausal osteoporosis involves the interplay of many factors- aging, hormonal, nutritional, environmental, genetic factors, and life style factors. [7]

The field of bone turnover markers has developed considerably in the past decade. Biochemical markers of bone turnover measured in plasma or urine are proteins or products derived from them. In general they are either enzymes derived from osteoblasts involved in bone formation, or from osteoclasts involved in bone resorption, or are constituents of the bone matrix, which escape into the circulation during the process of bone formation, or which are released as breakdown products during resorption (Table-1). Other criteria that determine the value of any biochemical marker of tissue metabolism include knowledge of the factors that control its synthesis and metabolism, and its entry into and removal from the circulation.[8, 9] With most markers there is still only limited information available about their metabolism and kinetics and the factors which influence their production and degradation. In this brief review we discuss in detail about the bone formation marker i.e. osteocalcin.

TABLE 1: BIOCHEMICAL MARKERS OF BONE METABOLISM

FORMATION (Osteoblasts)	COUPLING	RESORPTION (Osteoclasts)
Serum		Urine
Osteocalcin (BGP)		Hydroxyproline
Total alkaline phosphatase		Hydroxylysine glycoside
Bone specific alkaline Phosphatase		Pyridinium crosslinks.
Procollagen peptides		

Other potential markers: -
Tartrate resistant acid phosphatase, bone sialoprotien (BSP), Osteopontin, osteonectin, alpha 2 – HS glycoprotein.

The main markers available for measuring the coupled process of bone formation and resorption are indicated, together with other potential markers.

Osteocalcin or Bone Gla Protein (BGP) is a bone specific protein. Osteocalcin is the major and most thoroughly characterized noncollagenous protein in mature human bone, where it constituents 1-2% of the total protein.[10-12] It is a small protein of 49 amino acids including 3 residues of gamma-carboxyglutamic acid (**FIG-2**) with a molecular weight of 5800 Da[13]. Because it is rapidly cleared by the kidney, the half life of circulating osteocalcin is short i.e. approximately 5 minutes[14].

Osteocalcin [Bone Gla Protein (BGP]):

1				5			10		
Tyr	Leu	Tyr	Gln	Trp	Leu	Gly	Ala	Pro	Val
11				15					20
Pro	Tyr	Pro	Asp	Pro	Leu	**Gla**	Pro	Arg	Arg
21				25					30
Gla	Val	Cys	**Gla**	Leu	Asn	Pro	Asp	Cys	Asp
31				35					40
Glu	Leu	Ala	Asp	His	Ile	Gly	Phe	Gln	Glu
41				45			49		
Ala	Tyr	Arg	Arg	Phe	Tyr	Gly	Pro	Val	

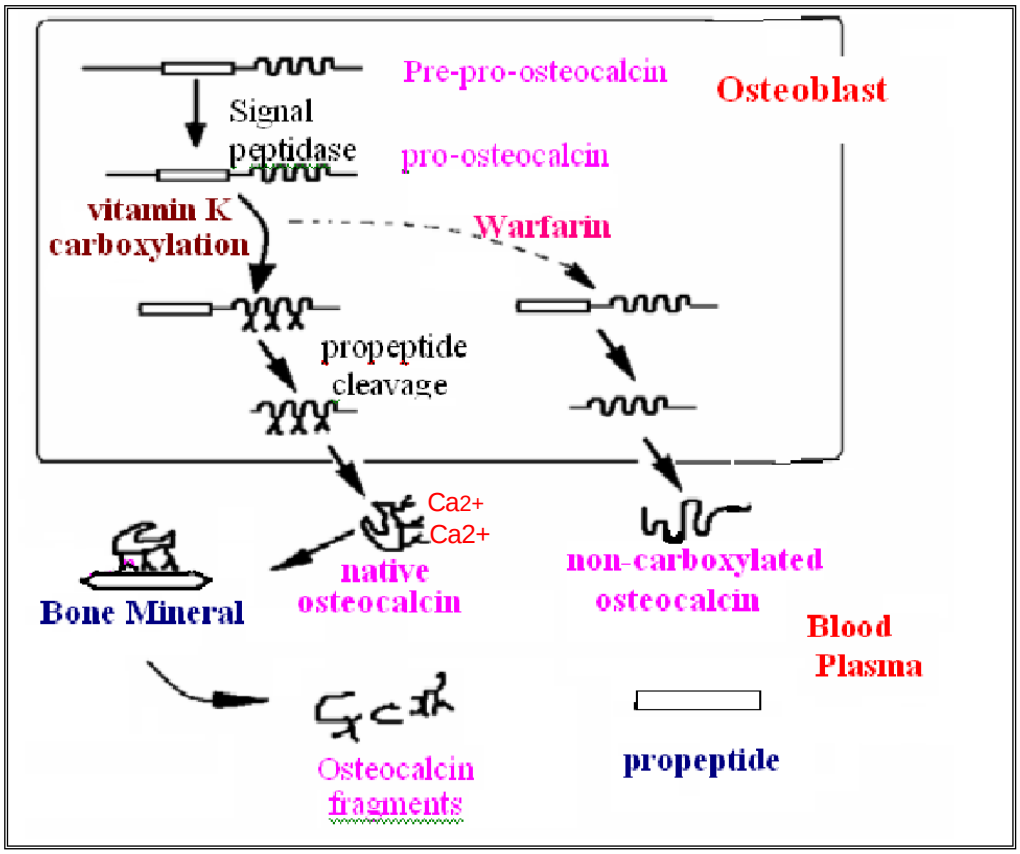
Figure 1: Amino acid sequence of osteocalcin:

Three glutamyl residues at position 17, 21, and 24 can be carboxylated in a posttranslational, vitamin K- dependent, enzymatic step producing γ-carboxyglutamyl (Gla) residues.

BIOSYNTHESIS OF OSTEOCALCIN:

The schematic representation of the biosynthesis and metabolism of osteocalcin shown in **Figure- 2** illustrates the issues to be considered in utilizing this component as a serum marker of bone formation. Osteocalcin synthesis is essentially specific to osteoblasts with only small amounts being produced by odontoblasts. In common with most secreted proteins, osteocalcin has a signal sequence that is removed in the rough endoplasmic reticulum to give pro-osteocalcin. Before secretion from the osteoblast, specific glutamic acid residues are carboxylated by a posttranslational, vitamin K- dependent enzymatic carboxylation to form gamma-carboxyglutamic acid (Gla). Human osteocalcin contains a maximum of three Gla residues per molecule[15]. After cleavage of the propeptide and secretion, a large proportion of the native osteocalcin is incorporated into the mineralizing matrix (bone matrix), assisted by the calcium binding properties of the Gla residues[15], and partly 10% to 30% of the osteocalcin synthesized by osteoblast is released into the circulation[16]. In patients treated with the anticoagulant, warfarin, inhibition of the carboxylase leads to the secretion of non carboxylated osteocalcin, and a similar situation may, to a lesser degree, occur in individuals with vitamin K deficiency[17]. The under carboxylated molecules will, of course, be less likely to be incorporated into the matrix and a higher proportion will therefore be released into the blood.

Figure 2: BIOSYNTHESIS AND METABOLISM OF OSTEOCALCIN:



METABOLISM OF OSTEOCALCIN:

After biosynthesis of osteocalcin native, calcium-binding molecule and the propeptide appear in the serum. Osteocalcin fragments are released in the plasma after degradation of the matrix- bound material. In addition, lack of adequate carboxylation can produce non-carboxylated molecules.

REGULATION OF OSTEOCALCIN SYNTHESIS:

Its synthesis is dependent upon the presence of active metabolites of vitamin D, especially 1, 25-dihydroxyvitamin D[12] and it requires vitamin K for the conversion by carboxylation of three glutamate residues to gamma-carboxyglutamate.

STRUCTURE FUNCTION RELATIONSHIP OF OSTEOCALCIN:

Osteocalcin has a high affinity for calcium and exhibits a compact- calcium dependent α-helical conformation, in which the γ -carboxyglutamic acid (Gla) residues binds and promote absorption to hydroxyapatite in the bone matrix[18, 19]. It might be associated with the process of mineralization. The exact physiological function of osteocalcin is still unclear. A large number of studies show that the circulating levels of osteocalcin are associated with changes in the rate of bone turnover in metabolic bone diseases such as osteoporosis[16, 20-22].

MEASUREMENT OF OSTEOCALCIN:

Several immunoassays of osteocalcin have been developed, including radio (IRMA) and enzyme

immunoassays [EIA- ELISA and EASIA[23, 24] (Enzyme Amplified Sensitivity Immunoassay)] involving the use of either monoclonal and polyclonal antibodies or chemiluminescent compounds (ICMA). Most (previous/ old) immunoassays (RIA) for osteocalcin recognize both the native and non-carboxylated molecule equally and this heterogeneity with respect to carboxylation can therefore complicate interpretation of the results. In recent years new developments include the development of assays utilizing antibodies against human rather than bovine osteocalcin and the use of sandwich assays with two monoclonal antibodies, so that only the intact osteocalcin molecule is measured rather than (non-carboxylated) osteocalcin fragments[23, 24]. This assay appears to provide a valid indication of osteoblastic activity without any resorptive component. Some attempts to establish the degree of osteocalcin carboxylation in serum have been made using hydroxyapatite binding in vitro, [25, 26] but the results appear to be very dependent on the precise conditions used for these estimations[27].

A further complication for the interpretation of osteocalcin determinations in blood is that fragments of osteocalcin released from the matrix can also appear in the blood, and may react with osteocalcin antibodies in some assays[28]. These considerations may to some extent explain the large variations between different centers in the determination of osteocalcin[29]. There is also interest in developing non- isotopic methods, and in assays for fragments of osteocalcin that might be released during the resorption of bone matrix[8]. Fragments of osteocalcin are also found in urine, and the measurement of urinary osteocalcin (U-OC) is another method for monitoring bone metabolism. Urinary osteocalcin was measured with U-Mid OC, U-Long OC and U-Total osteocalcin assays, which have unique specificities toward different naturally occurring U-OC fragments[9].

Specimen requirements:

Serum is the most widely used specimen for the measurement of osteocalcin; heparinised plasma can be used with some methods. The stability of osteocalcin in samples is method dependent. Decreases in osteocalcin immunoreactivity of 50% to 70% after 6 to 24 hours at room temperature and 40 % to 80% after 2 weeks at 4^0C have been reported[30-32].Trasylol[30], mixed protease inhibitors[32], and collection on ice[31,32] improve the stability of osteocalcin with some but not all methods. Serum osteocalcin concentrations are more stable with methods measuring both intact osteocalcin and the N- terminal/midregion fragement (1-43). Concentrations were unchanged after 3 hours at room temperature and after 24 hours at 4^0C[31, 32].

REFERENCE INTERVALS:

Methods for osteocalcin are not harmonized as illustrated by the reference intervals listed below.

	ηg/mL (µg/L)
IRMA	
Children	
6-9.9 yrs	40.2-108
10-13.9y	35.8-166
14-17.9y	27.8-194
Adult male	11.3-35.4
Adult female	7.2-27.9
ICMA	
Adult male	1.1-7.2

Adult female (premenopause)	0.5-7.0
EASIA (Immunoenzymatic assay)	
Adult male	5-25
Adult female (premenopause)	0.4-8.2
(postmenopause)	3-13

Osteocalcin concentrations are influenced by age, gender, and diurnal variation[13,23,33&34]. Osteocalcin exhibits a diurnal variation with a nocturnal peak, dropping by as much as 50% to a morning nadir. Concentrations are higher in children. With the highest concentrations observed during periods of rapid growth. Males have somewhat higher concentrations of osteocalcin. Osteocalcin concentrations have been reported to increase, decrease, or remain unchanged with advancing age, a probable consequence of the heterogeneity of circulating osteocalcin and differences in immunoassay specificity.

Elevated levels of serum osteocalcin may be associated with increased activity of osteoblast. Osteocalcin levels are generally increased during menopause. Increased levels of osteocalcin have been reported in patients with high bone turnover osteoporosis and fractures[35]. Verit et al.[36] studied and found that serum osteocalcin levels in postmenopausal osteoporotic women were significantly higher than in premenopausal non-osteoporotic women. Osteocalcin has a high affinity for calcium and exhibits a compact-calcium dependent α-helical conformation, in which the Gla residues binds and promote absorption to hydroxyapatite in the bone matrix, in this way mineralization of bone takes place. In osteoporotic women, deficiency of calcium may lead to lowering of formation of hydroxyapatite crystals. Thus, in the state of decreased rate of bone mineralization, free osteocalcin may be available for circulation in the blood. This may explain the increased concentration of osteocalcin in the serum of osteoporotic postmenopausal women. Pino et al.[37] found that osteocalcin is a promising marker of bone turnover useful in the diagnosis and follow-up of high turnover osteoporosis. Similar observations were reported by a number of other studies Ones et al.[38], Yasumura et al. [39], Rosenquist et al.[40]. In osteoporosis, Brown et al.[41] found that serum osteocalcin level correlated well with histological markers of bone formation rate. Serum osteocalcin has also been reported as being predictive of the rate of bone loss after menopause[42]. As a tool for selecting the appropriate treatment [43] and as a measure of the response to estrogen replacement therapy [42]. Measurement of osteocalcin is very useful in the study or management of osteoporosis. Although osteocalcin is accepted as being a marker of bone formation, its serum concentration is also increased in predominantly bone resorbing states so long as the two processes are coupled[29]. Gundberg et al.[44] studied and found that osteocalcin may play a role in the local control of calcium deposition or removal in mineralized tissue. Measurement of urinary and serum osteocalcin may provide important insights into the metabolic derangements in osteoporosis and other bone disorders [44.]

CONCLUSION

In developed countries a number of research projects are undertaken for the study of bone biomarkers, because of the current interest in osteoporosis. They also focused much interest on measurement of serum osteocalcin, because its concentration in serum appears to be a specific index of bone formation. Similarly, in Western Maharashtra, there is a current need for establishment of more rapid assays and for improvement in technical methods for

measurement of these markers with a high priority in the management of osteoporosis. In Western Maharashtra, assessment of BMD is the standard criteria for diagnosis and evaluation of osteoporosis. But BMD provide a static picture of skeleton whereas, the biochemical markers (osteocalcin) of bone turnover can provide dynamic status of bone remodeling and rapid measurement of skeletal metabolism. The major advantage of using osteocalcin as a clinical index of bone turnover is its tissue specificity and it's relatively low within person's variation. Thus osteocalcin is a specific, sensitive, promising and currently used marker for better prognosis of osteoporosis and for monitoring responses to antiresorptive therapy.

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