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Review article

**THE VALUE OF OSTEOPONTIN AS A DIAGNOSTIC BIOMARKER IN
POSTMENOPAUSAL OSTEOPOROSIS; A REVIEW.**

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Abstract:

Although increased awareness of morbidity and costs related to osteoporotic fractures, real progress achieved only through early detection of osteoporosis before any fractures occur. Dual Energy X-ray Absorptiometry (DEXA) is commonly used for diagnosis of osteoporosis by measuring bone mineral density (BMD), but it has its technical and biological limitations. Moreover, DEXA scanning only assesses mineral content in bones, which is only one component of bone strength, BMD cannot define other biological factors which contribute to fracture risk. The biology of bone turnover can be assessed by bone turnover markers (BTMs). They are metabolic indicators released into serum and their quantity reflects the metabolic activity of bones. Markers of bone remodeling predominantly resorption markers have been shown to predict fracture risk independent from BMD. Osteopontin (OPN) is a non-collagenous bone matrix protein marker, it is a key regulator of many metabolic, inflammatory diseases and contributing to osteoporosis pathogenesis. Published studies suggested that, high OPN level may predict the risk of osteoporotic fractures in postmenopausal women (PMW) independent of BMD. The aim of the present review is to integrate the relationship between OPN and BMD in PMW and to discuss the role of OPN as an early diagnostic factor for osteoporosis

Keywords: Osteopontin; Bone Turnover Markers; Bone remodeling; Postmenopausal Osteoporosis; DEXA Scanning.

Introduction

Osteoporosis is a treatable condition, and yet many people with this condition remain undiagnosed and untreated [1]. Prevention of fractures through identification of subjects at risk remains challenging [2]. It is recommended that postmenopausal women >50 years of age who are at risk of having osteoporotic fractures should be assessed [3].

Riggs *et al.*, 2003 [4], reported that, bone strength is dependent on four factors, which are: (1) Bone mineral density, (2) Bone structure, (3) Bone matrix, and (4) Accumulation of micro-cracks. Osteoporotic fractures, therefore, are the MACROSCOPIC result of a sequences of multiple NANO- and MICRO- structural events [5].

Until now, in clinical practice, DEXA considered standard tool for osteoporosis diagnosis [6]. The use BMD alone is less than optimal as an intervention threshold as it ignores other biological determinants of bone strength and does not cover all variations in bone fragility[3]. When BMD measurement by itself does not provide a clear answer to identify individuals at

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higher risk for developing osteoporotic fractures, combined use of measuring BMD and blood BTMs is likely to improve fracture risk assessment in those cases [7]

The current review article was done to summarize published studies concerning association between postmenopausal estrogen withdrawal, screening investigation using DEXA and serum Osteopontin levels to define the optimal tool to be used in clinical practice to evaluate bone quality to improve early prediction of fracture risk.

Osteoporosis:

Bone is a metabolically active tissue that undergoes continuous remodeling throughout life by two counteracting processes, namely bone formation and bone resorption which involves removal of old bone and replace it with new bone. These processes rely on activity of bone cells; osteoclasts (resorption), osteoblasts (formation), and osteocytes (maintenance). Under normal conditions, bone resorption and bone formation are tightly coupled to each other, so that the amount of bone removed is always equal to amount of bone newly formed. This balance is achieved and regulated through action of various systemic hormones (e.g., PTH, vitamin D, steroid hormones) and local mediators (e.g., cytokines, growth factors) [8–10].

Osteoporosis, is the most prevalent metabolic bone disorder, it is a progressive, silent asymptomatic disease characterized by decreased bone mass and strength due to increased bone turnover, which compromises bone architecture and results in increased fracture risk [3]. Loss of BMD by itself rarely affect quality of life. However, the effect of fractures associated with osteoporosis on health-related quality of life is typically profound, reflecting the following dimensions of well-being; physical, psychological, social and financial [1,11].

A great number of women aged 50 years and older will experience an osteoporosis-related fracture in their lifetime [12]. Women are more susceptible to osteoporosis than men, representing 80 % of all patients with osteoporosis. Generally, they have smaller and thinner bones than men, in addition, at menopause, there is a sharp decline in estrogen production with subsequent increased bone resorption [13]. It was stated that one in three women over age of 50 and one in five men will have osteoporotic fracture in their lifetime [14]. Osteoporotic fractures may occur spontaneously without evident trauma or often as a result of trivial trauma [15].

In the literature, the pathogenic mechanism underlying postmenopausal osteoporosis (PMO) is an imbalance between bone resorption and bone formation where resorption exceeds formation [16,17], The estrogen decline occurring in women after menopause frequently leads to derangement of this homeostasis [18], this imbalance has effects on trabecular bone; loss of connectivity and cortical bone; cortical thinning and porosity [1]. The long-term imbalance of bone turnover leads to change in bone mass, bone microstructure, bone strength and ultimately may leads to osteoporosis and fragility fractures [19].

The relationship between age / body weight and osteoporosis is well known. Age and body weight are both important independent clinical risk factors for PMO that can also have a profound effect on fracture risk. Aging is associated with significant bone loss, the rates of osteoporosis increase with age [20]. Fodor *et al.*, 2013 [21], suggested that, BMD varies markedly with age. For any BMD, fracture risk is much higher in elderly than in young. Moreover, Al-Nejjar *et al.*, 2015 [22], stated that, aging means longer total exposure to chronic oxidant stress and inflammation, both of which contribute to development of

osteoporosis. Overweight [body mass index (BMI) > 30Kg/m²] has been postulated as a protective factor for osteoporosis due to increased amount of biologically endogenous estrogen in adipose cells [18,23].

Observation showed that most women achieved peak bone density by 18 to 25 years of age [24], after this, bone continues to be remodeled throughout person's lifetime to respond to new stresses and to replace older weakened bone. After attainment of peak bone density, age-related bone density loss begins owing to an imbalance of bone formation and bone resorption in remodeling process. After menopause, the rate of bone remodeling increases, which accelerates bone loss owing to this inherent imbalance [12]. It is important to allude to fact that, in menopausal women with age advancing, there is a doubled mechanism of bone loss; Rapid process is estrogen-deficiency bone loss, occurs at a rate of 2% to 3% of total bone mass per year, which begins after menopause and my last from 6 to 10 years. Subsequently, it is followed by a constant stage that is age-related bone loss [20,25,26].

Estrogens act as anti-resorptive hormones that limit bone resorption and bone loss. They regulate the (RANK)/(RANKL)/(OPG) pathway at several levels by downregulate the expression of RANKL in the osteoblastic cell lineage and induce the expression of OPG, a naturally occurring antagonist of RANKL. Moreover, estrogens interfere with osteoclast signaling downstream of RANKL/RANK, thereby preventing osteoclast differentiation and promoting osteoclast apoptosis via increased production of Transforming growth factor β (TGF β) by osteoblasts [1,27]. Under conditions of acute estrogen deficiency, osteoclast differentiation and activity are increased by a higher expression of RANKL and other pro-osteoclastic cytokines; including tumor necrosis factor alpha (TNF α), Interleukin 1 (IL-1) and Interleukin 6 (IL-6) by Mesenchymal stem cells (MSCs) and lymphocytes [1,28,29].

Screening Investigation:

Bone radiological studies are of little predictive utility, because biochemical and biological changes precede radiological changes [30]. Decreased skeletal radio-density in conventional radiography is a late and unreliable sign of bone loss; it becomes apparent only after about 30 % reduction in bone mineral or skeletal mass [31], and even then one cannot tell whether this is due to Osteoporosis (decreased bone mass) or Osteomalacia (bone insufficient mineralization) or a combination of both [15].

Screening investigation for measurement of bone-losing disorders has been greatly advanced by development of methods for measuring BMD and bone mass [32]. Bone mineral density is a key tool in identifying osteoporosis, it has been shown to correlate with bone strength, as it accounts for approximately 60-70% variation of bone fragility. In clinical practice, determining BMD via DEXA is the most widely screening form for diagnosing osteoporosis [33,34]. A working group of WHO has defined osteoporosis as a BMD that is 2.5 standard deviations below the peak bone mass for adult women, or lower [1].

Measurement is based on the principle that a beam of energy is attenuated as it passes through bone, and the degree of attenuation is related to bone mass and the mineral content of the bone [15], DEXA have advantages of being cheap compared to alternative methods as magnetic resonance imaging and expose patient to minimal amounts of radiation compared to computed tomography [35]. Other practical advantages including; it is painless, short scanning times, easy patient setup, good measurement precision and stable calibration [36].

However, DEXA scan has its own limitations being static measure, does not reflect bone remodeling with respect to bone formation and resorption, does not reflect metabolic rate of bone turnover and does not define molecular and cellular biology of bone [7, 37].

Furthermore, DEXA scanning can not define other factors contribute to fracture risk, BMD only provides information regarding mineral quantity in bones which is only one component of bone strength [5].

Moreover, in clinical practice, Observations have demonstrated that a majority of fragility fractures occur in subjects whose BMD values are not in the osteoporotic range, Some reports recorded about 50% of menopausal women who had sustained osteoporotic fracture have BMD above WHO definition of osteoporosis; T score in osteopenic range [7].

In addition, there are a number of technical limitations in general application of DEXA for diagnosis of PMO. A disease which can mimic osteoporosis, for example, osteomalacia [3]. By definition, osteoporosis is a debilitating disease of abnormally low bone mass, but, the existing bone is fully mineralized. It is characterized by bone micro-architecture degradation and defects in bone structure manifested by more porous in the cancellous regions and cortices are thinner than normal contributing to fragility fractures. Osteoporosis is therefore a quantitative, not a qualitative defect in bone. The presence of osteomalacia, a common complication of poor nutrition in elderly. It is a disorder of mineralization, inadequate bone mineralization. Osteoid throughout the skeleton is incompletely calcified resulted in a large amount of un-mineralized osteoid (qualitative defect) and bone is therefore, "softened" [15], DEXA will underestimate total bone matrix because of decreased bone mineralization [3].

Another example for technical limitations of DEXA, osteoarthritis at spine or hip are common in elderly [3]. Published clinical trials noted that, the presence of artifacts such as hypertrophic bony changes, vertebral spurs or osteophytes and intervertebral bone bridges associated with degenerative joint diseases can often be detected on scan and can lead to falsely elevated BMD making density measurements less reliable. Elderly people with osteoarthritis show an artefactual increase in spine BMD by 10 percent [15].

Bone structures are difficult to measure in vivo, bone mass can be assessed by densitometric techniques. In contrast to these rather static measures, however, molecular markers of bone metabolism (BTMs) are helpful tools to detect the dynamics of metabolic imbalance [8]. Bone mass measurements for prognosis depends upon accuracy, accuracy in this context is the ability of measurement to predict fracture. In general, all "densitometric" techniques have high specificity but low sensitivity. [3]. In clinical experience, at the age of 50 years, for example, the proportion of women with osteoporosis who will fracture their hip, spine, forearm or proximal humerus in the next 10 years (i.e. positive predictive value) is approximately 45 %. Despite this, overall detection rate for these fractures (sensitivity) is low, and 96 % of vertebral and non-vertebral fractures will occur in women without osteoporosis [3,38]. The low sensitivity is one of the reasons why DEXA screening for BMD alone is not an optimal tool in women at the time of menopause [3,39].

The limitation of screening approach is that it increases a risk factor to the status of a diagnostic criterion. Thus, it is a matter of arguing regarding the sensitivity and specificity of DEXA as an optimal tool to predict fracture risk. Hence new tools are needed to consider structural measures of bone rather than focusing on BMD alone and to evaluate bone quality to improve prediction of fracture risk. Therefore, it is recommended to use BTMs as a complementary tool for assessing bone quality [7].

Bone Markers - Osteopontin:

Biochemical markers of bone turnover are increased after the menopause, and in several studies, the rate of bone loss varies according to the markers values, predominantly resorption markers [40]. Thus, a potential clinical application of biochemical indices of skeletal metabolism is in assessing fracture risk independently of BMD in postmenopausal women [41]. A consistent feature of PMW is the concurrent increase of bone resorption and bone formation at the tissue level, termed high bone turnover. However, at cellular level, multicellular bone-remodeling unit, there is a negative imbalance in bone remodeling, with bone resorption outpacing bone formation. After menopause and beyond the age of 50, bone resorption on the basis of biochemical markers is increased by 90%, but bone formation is only increased by 45%, thus resulting in net bone loss with each remodeling cycle [1,42].

Several prospective studies have shown that serum levels and urinary excretion of BTMs correlate with subsequent risk of fractures in PMW [43,44]. Thus, women that have marker values of bone turnover above the premenopausal range (25–40 % of PMW) have been shown in several but not all studies to have approximately a 2-fold increased risk of vertebral and non-vertebral fractures, including those at hip, independently of age and of BMD [3,40].

Molecular markers of bone metabolism are novel tools which detect bone dynamics with respect to bone formation and bone resorption, also play a golden role in management of osteoporosis, especially in the follow up of the patients who had been on anti-resorptive or bone formation therapies. Furthermore, it carry the advantages of sensitive and specific assays, in addition to wider availability and reliability [7].

The biochemical markers currently available for bone turnover assessment include enzymes and non-enzymatic peptides derived from the cellular and non-cellular compartments of bone [45]. Bone turnover markers are grouped into two categories based on the metabolic phase during which they are produced: Bone Formation Markers and Bone Resorption Markers [40]. Markers of bone resorption are related to collagenous and none collagenous matrix breakdown, in contrast to, markers of bone formation are either by-products of collagen or osteoblast-related protein [8].

In 1985, Osteopontin was first identified by Heingard *et al.*, as sialoprotein derived from bovine bone matrix, it is a ubiquitous matricellular protein. Also, it known as a secreted phosphoprotein 1 (SPP1), Early T-lymphocyte Activation 1 (ETA1) protein, and Bone Sialoprotein 1 (BSP1) [46]. The term “Osteopontin” was chosen by Heingard’s group, OPN is produced by cells located in the osteoid matrix, and its name “Osteopontin” denotes the property of acting as a bridge between cells and hydroxyapatite crystals (HA) of bone [47], OPN is a combination of the two words ‘Osteo’ Greek word, meaning ‘bone’ and ‘Pontin’ is derived from a Latin word ‘pons’, meaning ‘bridge’. The term osteopontin reflects its function as a linking protein and essential factor in bone homeostasis [48].

Osteopontin is known as a member of small integrin-binding ligand N-linked glycoproteins family, (SIBLINGs), is a group of five secreted non collagenous proteins which includes Bone Sialoprotein (BSP), Osteopontin (OPN), Dentin Matrix Protein-1 (DMP1), Dentin Sialophosphoprotein (DSPP), and Matrix Extracellular Phosphoglycoprotein (MEPE) [47].

It is encoded by a single copy gene *SPP1* that are located along human chromosome 4 (4q13) [49], but exists in three splice variants as a result of alternative posttranslational modifications (PTMs) including [OPN a] the full-length isoform, [OPN b] which missing exon

5 and [OPN c] which missing exon 4 [50]. Thus, alternative splicing and posttranslational modifications of OPN mRNA alter the properties of the molecule and have biological and functional significance, for example, the degree of phosphorylation regulates the adhesive properties of OPN and necessary for bone resorption by osteoclasts [51].

It is composed of approximately 314 amino acid residues with molecular weight ranging between 44 kd to 75 kd in different mammalian cells [52]. OPN consists of a number of highly conserved functional domains such as a polyaspartic acid motif, tripeptide sequence Arg-Gly-Asp (RGD), thrombin cleavage motif and calcium binding sites [50]. Additionally, it contains two terminal zones including C-terminal binds two heparin molecules as well as CD44 variants and N-terminal includes integrin receptor binding zones ($\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_4\beta_1$) [53].

Osteopontin originally described as a secreted protein (sOPN), is a soluble protein being distributed in many tissues and body fluids [21]. Although, the intracellular OPN (iOPN) form has been reported as a regulator of cytoskeleton dynamics and gene expression, most studies have focused on the secreted form (sOPN) [49].

Furthermore, it is known as a multifunctional protein [54]. It has been expressed in a broad range of cell types, including bone marrow cells, brain cells, fibroblasts, dendritic cells, immune cells like macrophages, neutrophils, B and T-cells and dendritic cells, smooth muscle cells, skeletal muscle, endothelial cells, inner ear, hepatocytes, kidney, placenta, mammary glands and tumor cells [51]. Osteopontin has been found to be implicated in important physiological and pathological processes as a result of its interactions with multiple cell surface receptors; including tissue repair, inflammation, fibrosis, tumor biology and immune responses [55,56].

At bone level, OPN has multifaceted effects on bone morphogenesis and remodeling, being associated with bone turnover and bone mineral density. It is produced by cells involved in bone metabolism such as preosteoblasts, osteoblasts, osteocytes and osteoclasts [54]. Also, it was identified as a major non-collagenous protein obtained from demineralization of bone tissue, it is one of the bone resorption biomarkers [21,57]. Osteopontin is particularly concentrated in bone surfaces; cement lines and lamina limitans, it has been shown to bind calcium ions, calcium oxalate crystal in urine and hydroxyapatite crystal [20,21], as well as facilitates osteoclast attachment and guides mineral deposition by influencing crystal shape and size [58]. These functional properties are important for the hypothesized roles of OPN in bone homeostasis and bone strength [59].

Despite the exact roles of OPN in bone are not fully understood, many animal studies have been suggested the role of OPN in the regulation of bone metabolism as an inhibitor of bone calcification [21]. Bones from OPN knockout mice have a greater mineral content and contain larger hydroxyapatite crystals (HA) than wild type animals. It is probable that osteocytes expressed OPN to inhibit the growth of hydroxyapatite [22].

During normal bone mineralization, osteoclast derived OPN inhibits the formation of HA crystals. Osteopontin acts as limiter limiting crystal formation in bones and teeth that limit the size of calcium-containing crystals by binding to existing crystals and preventing further growth which indicate that OPN is associated with bone destruction and may be considered as the risk for bone fractures [53]. Highly expression of OPN has been reported to be regulated by mechanical stress, both in vitro and in vivo, and the ability of this protein to influence bone homeostasis through inhibition of mineral deposition is well known [47].

Published studies have shown that OPN is involved in both bone formation and resorption. It acts as an important regulator of inhibiting osteoblast proliferation and enhancing osteoclast differentiation, in addition, OPN upregulates osteoclasts motility, which may enhance the ability of osteoclasts to absorb bone, it works as an anchor to promote attachment of osteoclasts to bone for bone resorption causing a high turnover [20,60]. OPN is known to be a major ligand for CD44 on bone cells, which is important in mediating osteoclast function and also required for osteoclast motility. As elegantly shown by Chellaiah *et al.*, 2003 [61], the reduction in functionality of OPN-deficient osteoclasts can be attributed to decreased motility which was caused by a lack of cell surface expression of CD44. Osteopontin stimulates CD44 expression on the osteoclast surface, thereby upregulating osteoclast motility and bone resorption [60]. Furthermore, OPN binds to $\alpha_v\beta_3$ integrin a major receptor on the osteoclasts via the arginine-glycine-aspartic acid (RGD) sequence. In this way, OPN serves as an attachment for osteoclasts, activating the resorption process resulting in the decrease of bone mass [53].

Experimental animal studies of OPN-deficient mice proved resistant to bone loss, indicating a possible link between OPN and postmenopausal osteoporosis. The rate of bone mineral loss is increased after menopause [62]. Osteoclasts deficient in OPN are hypomotile and exhibit decreased capacity for bone resorption, and bones lacking OPN are less readily remodeled [63,64]. The main estrogen mechanism in preventing bone loss is by reduction of osteoclast precursors responsiveness to RANKL and by directly suppressing RANKL-induced osteoclast differentiation [65,66]. Thus, the lack of estrogen upregulates osteoclastogenesis, and OPN upregulates osteoclast motility, which may enhance the ability of osteoclasts to absorb bone causing a high turnover in postmenopausal osteoporosis [20].

The role of OPN in bone homeostasis has been investigated using OPN-deficient mice: the OPN null mice are resistant to ovariectomy-induced or age-induced bone loss compared with wild-type mice (-10% vs -60% reduction in bone volume, respectively) [60], but the skeleton does not respond properly under stress. In these mice, the osteoclasts are in a higher number but have failed to develop into mature cells, and their distribution is changed [21]. As well as, the inorganic phase of the bone matrix is disturbed as the OPN acts as an inhibitor of the hydroxyapatite formation [67]. In OPN-deficient mice, a 30% decrease in fracture risk was found, suggesting a role of OPN in preventing the fracture propagation [58]. During fracture healing, OPN deficiency delayed early vascularization, disturbed matrix organization, and delayed the time course of callus late remodeling, indicating that the presence of normal OPN level is essential for normal fracture healing [68].

In human subjects, correlation studies between OPN and BMD demonstrated that high OPN level proved to be a statistical risk factor for low BMD in postmenopausal women and these women are more vulnerable for osteoporosis to occur and subsequent fracture risk. Studies have demonstrated that estrogen deficiency reduces bone formation and bone mass, these effects are mediated by the non-collagenous matrix protein "Osteopontin". Elevated OPN levels have been reported in patients with increased bone resorption, these data point toward a specific pathophysiological role of OPN in osteoporosis [20,21,30,60,62,69].

Clinical studies have shown that excessive serum OPN have negative effects on bone health, being as a concomitant event in high bone turnover state associated with low bone mass and low BMD. In addition, published data suggested that high level of circulating OPN has been considered to be a promising biomarker for early diagnosis of PMO and fracture prediction [21,62]

The findings of many studies support the published suggestion that, BMD measurements are not the optimal determinant factor of postmenopausal osteoporosis, and must be correlated with levels of serum concentrations of markers of bone metabolism and markers of bone turnover, predominantly resorption markers, which are a mirror that reflect the true state of bone quality and may predict fracture risk independently of BMD in postmenopausal women.

Collectively, the biochemical bone marker OPN has been implicated with bone micro-architectural structure and bone turnover levels, independently of BMD. Thus, the menopausal women with high OPN level may considered the target population for the development of osteoporosis. The level of circulating OPN concentration was associated with an increased risk of incident fractures. High serum OPN levels has been demonstrated to be a significant risk factor being used as a biomarker for early osteoporosis diagnosis. The recommendation for patient at risk, must be DEXA scanning at regular intervals to assess BMD to check if osteoporosis happened.

Conclusion

Serum OPN levels acted as a significant risk biomarker for the development of osteoporosis. It is suggested that OPN is a sensitive monitoring indicator to evaluate bone metabolism in postmenopausal women and for the early diagnosis of osteoporosis. Therefore, it is recommended to use BTM – OSTEOPONTIN as a complementary tool for assessing bone quality and for early prediction of fracture risk.

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