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Effect of phytoestrogen (isoflavones) rich soy food supplement on bone turnover among postmenopausal women

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Abstract
Osteoporosis is greater in postmenopausal women than in men. Hence, planning suitable intervention approaches, especially from natural sources might be beneficial in reducing the osteoporosis risk. The intervention study was planned with 30g of phytoestrogen rich soy food supplement containing 60mg of isoflavones which was taken with 200ml of milk along with calcium and Vitamin D_3. The findings well demonstrated the attenuation of bone mass through BMD and BMD T-score. The biochemical markers also represented reduction in bone turnover process as indicated by lowered levels of both enzymes in soy food experimental group. Whereas the condition seemed to be worsen with case control group by experiencing continuous reduction in bone density and elevated levels of bone turnover markers. The findings indicated a need of educating women to aware of appropriate strategies suitable for enhancing bone health and minimizing the further risk of osteoporosis with ageing process.

Keywords: Osteoporosis, postmenopausal women, Bone health, Bone Turnover

Introduction
Postmenopausal osteoporosis is a metabolic disease of the bone and increases the probability of bone fractures. Decreased Bone Mineral Density (BMD) and weakened structural architecture are major risk factors of osteoporosis and osteopenia. BMD decreases with age in both women and men, but women typically begin with lower BMD than men and exhibit accelerated bone loss after menopause due to ovarian hormone deficiency. Therefore, women are more susceptible to osteoporosis than men. Current available therapies for postmenopausal osteoporosis include treatment with estrogen or Hormone Replacement Therapies (HRT), calcitonin, bisphosphonates, relaxofene, strontium ranelate and anabolic agents. More importantly, HRT is the most widely recommended therapy in order to reduce the rate of postmenopausal bone loss. However, recent findings have suggested HRT is associated with higher risk of breast, endometrial and ovarian cancers, cardiovascular disease, venous thromboembolism and stroke. Hence, it would be helpful to discover a naturally occurring substance that minimizes bone loss in postmenopausal women, thus decreasing the necessity for drug therapy.

Phytoestrogen are naturally occurring plant compounds that are structurally and functionally similar to estradiol. There are four major classes of phytoestrogens: isoflavones found in soy beans and soy products, ligins found in whole grains, cereal and oil seeds, flavonoids found in some fruits and legumes and coumestans found in bean and alfafa sprouts. Isoflavones are the phytoestrogens with the most potent estrogenic activity, being genistein, diadzein and glycetin, found in soy beans, their most active components. Soy beans are leguminous plants that have been cultivated in Asia, especially in China, Korea and Japan for over 1,000 years. However, due to their outstanding nutritional properties, they have been introduced to Western diets [1].

The number of postmenopausal women is expected to increase considerably due to the prolongation of the life span and postmenopausal osteoporosis is therefore expected to become a great socioeconomic problem with increase in both mortality and morbidity. New strategies for preventing and treating postmenopausal osteoporosis are thus of interest and increased attention has been focused on soy bean-derived isoflavones [2].

Isoflavones are compounds in plant foods, particularly soy beans, that are structurally similar to the mammalian estrogens and that have received for their potential bone-sparing properties. Rates of hip fracture in Asian populations, whose traditional diets are rich in soy, are substantially lower than those in Whiter residing in the United States. However, Bone Mineral Density (BMD) in Asian populations is comparable to that in White population after adjustment for height and weight.

Nevertheless data from animal experiments provided evidence that soy protein can attenuate menopausal bone loss and it was suggested that isoflavones in soy might be responsible for protective effects on bone. Intervention trials in humans using either soy protein or isoflavone extracts generally reported protective effects on bone although sample sizes were small and trials often were relatively short.

Of the many techniques developed to assess bone mass, bone mineral or other related aspects of skeletal mass or structure, the most highly developed technically and the most thoroughly validated biologically is Dual Energy X-ray Absorptiometry (DEXA), which is regarded as the "gold standard", with which the performance characteristics of less well-established techniques can be compared. All these techniques are used for the diagnosis of osteoporosis, prognosis (fracture prediction), monitoring the natural history of the disorder and assessing response to treatment [3].

New methods of measuring osteoporosis using ultrasound have also been developed. One such ultrasound system measure is BMD at the patient's heel and takes about a minute. The ultrasound systems for testing osteoporosis are smaller and less expensive than traditional DEXA systems. These systems have recently received US Food and Drug Administration (FDA) clearance. The hope is that this more compact, lower cost system will allow this vital diagnostic test to become more widely available in the future for preliminary screening of the subjects for osteopenia and osteoporosis [4].

The field of bone turnover markers has developed considerably in the recent times. Biochemical monitoring of bone metabolism depends upon measurement of enzymes and proteins released during bone formation and of degradation products produced during bone resorption. 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 sensitive and useful for the individual monitoring of osteoporotic patient treated with antiresorptive agents [5].

Changes of bone turnover with aging are responsible for bone loss and play a major role in osteoporosis. Although an increase of bone turnover has been documented at the time of menopause, the subsequent abnormalities of bone resorption and formation and their potential role in determining bone mass in the elderly have not been investigated. To address their issue, the present study focused on identifying the osteopenic and osteoporotic postmenopausal women using the diagnostic method of QUS bone densitometry measured at calcaneous bone. The intervention was planned with natural isoflavone rich soy food supplement. The efficacy of the intervention was evaluated by assessing the change in bone turnover in terms of measuring bone mineral density by DEXA and biochemical bone turnover markers measured in serum.

Materials and Methods

Tirupati town located at Chittoor of Andhra Pradesh from South India was purposively selected as the study sample. The women from major well established areas of Tirupati town comprised of urban sample and the women from slum area belong to urban slum sample. Four different age groups Viz., 35-44 years (n=60,); 45-54 years (n=80); 55-64 years (n=80) and 65-74 years (n=40) both from urban (n=260) and urban slum (n=260) areas with a total sample of 520 members constituted the preliminary screening sample to identify postmenopausal osteopenic and osteoporotic women.

The BMD testing was carried out using QUS bone densitometry measured at calcaneous bone for the women who voluntarily participated in the study during free BMD campaigns at local orthopedic hospitals. The bone health status was evaluated through WHO criteria based on BMD T-score standard deviation against young normal adult mean. The women with BMD T-score up to -1.0 SD were considered as normal, -1.0 to -2.5 SD as osteopenia and more than -2.5 SD as osteoporosis.

The intervention study was planned for osteopenic and osteoporotic postmenopausal women with 30g of soy food supplement containing 60mg of isoflavones along with 500 mg of calcium and 250 IU of Vitamin D_3 (n=30). The soy food supplement was taken with 200 ml of milk. The efficacy of the soy food supplement was tested against case control group (n=30).

Food and Drug Administration (FDA) approved 'Quantitative Ultra Sound (QUS)' bone densitometry as only for screening the patients for osteopenia and osteoporosis but not for testing therapeutic practices and efficacy of therapeutic and curative intervention approaches. In this regard, the dual energy X-ray absorptiometry (DEXA) remained the golden standard method of assessing BMD, identifying disease condition and for treatment of the disease. Hence, in the present study DEXA technique with a model of HOLOGIC – Discovery A (S/N 82730) available at Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati was employed to evaluate the changes in bone density and impact of soy food supplement. The BMD was examined at Lumbar spine (AP) L_1-L_4 for pre and post testing.

The extent of bone turnover was also assessed using the important serum biochemical bone turnover markers analyzed by the enzymes, serum alkaline phosphatase [6] and serum acid phosphatase [7]. Similar to that of BMD, the serum bone turnover markers were evaluated for pre and posttest changes of the intervention study.

Results and discussion

The bone mineral density and BMD T-score were measured at Lumbar spine (AP) position(L1-L4) using the golden standard technique, DEXA both in control and food supplement groups at baseline and after completion of six months intervention period. Simultaneously, the pre and posttest evaluation was continued for the selected serum biochemical bone turnover markers. The results obtained were interpreted and presented separately under different heads.

1. Effect of supplementation on BMD and BMD T-scores using DEXA bone densitometry

Determination of bone mineral density was the direct measure of evaluating the bone health. The changes of Lumbar spine BMD measured at AP Site (L1- L4) in course of time among Soy isoflavone food supplementation experimental group of women against case control group were depicted in table no-1. The level significance for the alterations in BMD was identified through paired t-test and denoted in the same table. Similarly, the pre-post intervention changes against control group in the corresponding lumbar spine BMD T-score were also noted and presented in table no-2.

Table 1: Pre and post test changes at Lumbar spine (AP) L1-L4 BMD- Effect of supplementation among osteopenic and osteoporotic postmenopausal women

Experimental group	DEXA-Lumbar spine BMD (AP): L1-L4		
	Pre-Treatment (Initial)	Post-Treatment	Calculated t-value
Control (n=30)	0.69 ± (0.08)	0.63 ± (0.07)	12.40**
Soy Food Supplement (n=30)	0.69 ± (0.09)	0.80 ± (0.10)	13.32**

Note: **=1 Percent level

The findings indicated a significant increase in bone density in six months intervention period of Soy food supplement along with a tablet containing calcium and vitamin D. The level of significance noticed to be at one percent level indicating a significant improvement in bone health and reduction in bone turnover as evidenced by increase in Lumbar spine BMD. In case of case control group who were not provided either soy food supplement or any other means of supplementation or medication experienced a significant reduction in BMD denoting an increase in bone resorption.

Table 2: Pre and posttest changes at Lumbar spine (AP) L1-L4 BMD T-Score- Effect of supplementation among osteopenic and osteoporotic postmenopausal women

Experimental group	DEXA-Lumbar spine BMD T-Score (AP): L1-L4		
	Pre-Treatment (Initial)	Post-Treatment	Calculated t-value
Control (n=30)	-2.61 ± (0.86)	-3.03 ± (0.83)	18.59**
Soy Food Supplement (n=30)	-2.59 ± (0.82)	-2.12 ± (0.86)	19.35**

Note: **=1 Percent level

Initially at baseline, the mean BMD T-scores of the two groups were at osteoporotic level (BMD T-score: ≤ -2.5). After six months of Soy food supplementation intervention, the striking point to be noted that significant improvement was observed with a gradual shift towards osteopenia from osteoporotic condition. On the other hand, in the control group, the bone densities and BMD T-scores were worsened and the osteoporotic scores continued representing further risk status of osteoporosis and fractures.

The findings clearly supported the crucial beneficial role of isoflavone rich soy food supplementation along with calcium and Vitamin D with milk in promoting bone health of postmenopausal women. Whereas, the data highlighted a definite deterioration and increased porosity levels of bone in control group of women whose BMD T-scores found to be much deviated with ageing process due to continuous bone age-related bone loss. The results well demonstrated the potential role of isoflavones being mimic the female hormone, estradiol and to compensate the age-related decrease in estradiol which played a major role in restoration of bone mass and to avoid risk of osteoporosis.

Recent research suggested multiple benefits for women from consumption of soy protein not only in bone health but also in the areas of heart health, reducing cancer risk and relief of menopausal symptoms. The soy food fed group in the present study thus had an added advantage in providing qualitative protein along with isoflavones which will be beneficial in long run in providing better health and wellbeing of women both in general and bone health in particular. The current findings provided a clear picture for the necessity of promoting soy based foods into the regular diets to minimize the occurrence and consequences of postmenopausal osteoporosis to lead healthy and strong life.

The meta-analysis study conducted with a range of 47 to150 mg soy isoflavones from 1240 menopausal women for 6-12 months significantly increased spine BMD compared to controls. Soy isoflavone extract supplements increased lumbar spine BMD in menopausal women [8]. The present study results also demonstrated the beneficial effects of soy food supplement on bone restoration in postmenopausal women for six months period providing 60 mg isoflavones.

2. Effect of supplementation on biochemical bone turnover markers

The maximum peak bone mass is achieved by the age of 35 years and later onwards gradually the bone mass slowly declined and bone loss is accelerated at menopausal stage. The progressive bone loss with the advancing age leads to imbalance in the bone metabolic process of bone formation and resorption. The changes are being reflected in the bone related markers of serum and urine on par with the rate of bone turnover. The alterations in the bone metabolic processes in the ageing process bring imbalance in the bone remodeling with higher levels of bone resorption rather than bone formation. The phenomenon involved in the bone physiology results in an increased rate of bone turnover process. Estimating some of the important bone turnover markers will be beneficial to understand the nature of age-related changes in bone turnover process.

The bone being a compact tissue may take longer duration for the changes in bone mass and mineral density and it may be difficult to estimate immediately in response to an intervention strategy. Also, the analysis of BMD is comparatively costlier and during those conditions, the alternative strategies are required to evaluate the effectiveness of any intervention study. The biochemical marker being subjected to rapid changes may serve as the best alternative solution to assess the impact of an intervention provided.

In view of the importance of the enzymes in regulation of bone turnover process, the two important enzymes, alkaline phosphatase and acid phosphatase were analyzed for pre and posttest in the serum samples of the women subjects to study the effect of soy food supplementation against the control group who had not received any supplement. Serum alkaline phosphatase is the most commonly used marker of bone formation. Alkaline phosphatase, introduced into clinical use in 1929, was the first biochemical markers of bone turnover and is still the one most widely used in clinical practice. This enzyme is found in the plasma membrane of osteoblasts and in cells of the liver, kidney, intestine, spleen and placenta [9].

Evidence shows that a pathological increase in bone resorption arises when osteoclasts are stimulated into resorption activity at an increased rate. This upsets the normal balance between bone resorption and bone synthesis. The increase in osteoclast activity is accompanied by an increase in the synthesis and secretion of acid phosphatase. Hence in the current research, attention was focused to evaluate the bone turnover process by pre-posttest analysis of serum alkaline and acid phosphatases and the results obtained were tabulated in table no-3.

Table 3: Effect of 6-months soy food supplement on serum alkaline and acid phosphatase levels of select osteopenic and osteoporotic women groups: Pre and posttest mean scores and calculated mean values

Experimental group	Serum alkaline phosphatase (U/L)			Serum acid phosphatase (U/L)		
	Pre-Test	Post-Test	Calculated t-value	Pre-Test	Post-Test	Calculated t-value
Control (n=30)	95.32 ±17.68	99.45 ±22.88	8.14**	3.98 ±0.66	4.32 ±0.57	10.29**
Food Supplement (n=30)	92.55 ±14.93	83.60 ±12.36	7.78**	3.82 ±0.63	3.46 ±0.63	11.90**

Note: **=1 Percent level

The results from the table clearly indicated a significant ($P < 0.01$) decrease in both serum alkaline phosphatase and serum acid phosphatase in the soy food experimental group who were also additionally provided with calcium and Vitamin D along with a glass of milk. In the control group, the elevated levels of bone turn over markers were recorded in both serum alkaline phosphatase and acid phosphatase. The results supported the beneficial role of soy isoflavone in minimizing the levels of bone turnover by reducing the levels of serum alkaline phophatase and serum acid phosphatase. The possible mechanisms by which soy isoflavones positively affect bone turnover rate may be directly interacting with estrogen receptors ER-α and ER-β or indirectly by suppressing the release of proinflammatory cytokines from bone cells which are often elevated in postmenopausal condition or ovariectromy [10].

In natural ageing process, during bone remodeling process, the rate of bone resorption exceeds than formation resulting in elevated levels of bone turnover due to increased osteoclastic activity. The acid phosphatase being the important bone resorption marker gets elevated and results in bringing more porosity in the bones. To fill these excess porous osteoclastic cells, simultaneously bone structure formation process starts to repair the porosity and fill the gaps of bone, hence the alkaline phosphatase activity rises to compensate the excess porosity in the bones. Otherwise bones become too porous and brittle very easily resulting in fractures. This compensatory mechanism brings changes in the serum alkaline phosphatase by correspondingly elevated levels along with serum acid phosphatase. Once antiresorptive drugs are introduced to the patient, the levels of resorption and in turn acid phosphatase activity are reduced and consequently the levels of alkaline phosphatase are reduced. This associated metabolic interrelated changes are clearly observed in the present study by decreased levels of both bone turnover markers, serum alkaline and acid phosphatases in soy food supplemented experimental groups compared to control group, where the levels of bone resorption was being continued to be high and the trend of results indicated a significant elevated levels of the two enzymes in the serum. Hence it can be inferred that soy isoflavones are effectively able to reduce bone turnover enzyme levels and resorption rates in the bone. Several other studies expressed the usefulness of both serum alkaline and acid phosphatases [11, 12, 13].

The overall results explained the effectiveness of soy isoflavones in restoring bone as evidenced by the measurements of lumbar spine BMD and the corresponding BMD T-scores. Similarly, the two important enzymes, serum alkaline and acid phosphatases also had significant role in enhancing bone health by lowering their levels and decreasing the levels of bone turn over.

Conclusion

Osteoporosis is a growing health problem recognized in both developed and developing countries. It is associated with substantial morbidity and socio-economic burden worldwide. Morbidity and mortality associated with osteoporosis continues to be high in India primarily due to late diagnosis. Utilization of low cost and portable QUS bone densitometry technique might be helpful in early identification of both osteopenic and osteoporotic condition, to plan appropriate preventive measures in minimize ageing bone loss and further preventing the early onset of disease.

Recent strategies focused on natural therapies such as phytoestrogens to attenuate bone mass, restore bone mineral density and effectively reduce the risk of osteoporosis. The present intervention study planned with phytoestrogen rich soy food supplement in milk along with calcium and vitamin D supplementation well demonstrated the beneficial role of soy isoflavones in strengthening bone mass. On the other hand, the control group experienced continued bone loss and accelerated the condition of osteoporosis. The results thus provided a clear picture that age-related bone loss was unavoidable and hence the women should be educated to adopt healthy living and dietary practices at least to minimize the process of bone turnover for promoting bone health.

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