OBSERVATIONS AND RESULTS

TABLE NO.1
Comparison of bone mineral density between postmenopausal osteoporosis and control groups.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Bone mineral density</th>
<th>Postmenopausal non osteoporosis women (Controls) n=60 Mean ± SD</th>
<th>Postmenopausal osteoporosis women n=60 Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>T – Score</td>
<td>0.345 ± 0.731</td>
<td>-3.233 ± 0.752*</td>
</tr>
<tr>
<td></td>
<td>Z – Score</td>
<td>0.366 ± 0.698</td>
<td>-2.735 ± 1.539*</td>
</tr>
</tbody>
</table>

* P<0.001- Highly significant

In this study, mean score of BMD (i.e. T and Z Scores) was (highly significant) decreased in postmenopausal osteoporosis women as compared to control group.
TABLE NO. 2

Bone mineral density from baseline to post therapy of 3 months in postmenopausal osteoporosis women (alendronate + calcium + vitamin D).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Bone mineral density</th>
<th>Postmenopausal osteoporosis women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>1.</td>
<td>T – score</td>
<td>- 3.233 ± 0.752</td>
</tr>
<tr>
<td></td>
<td>Z – score</td>
<td>- 2.735 ± 1.539</td>
</tr>
</tbody>
</table>

* P< 0.001 - Highly significant

Highly significant increase in the mean score of BMD (T and Z Scores) from baseline to post therapy of 3 months was observed in postmenopausal osteoporosis women.
Biochemical Studies in Osteoporosis of Women

Bone mineral density

<table>
<thead>
<tr>
<th>Study groups</th>
<th>T-Score</th>
<th>Z-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>controls</td>
<td>-3.233</td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>-2.735</td>
<td></td>
</tr>
<tr>
<td>post therapy</td>
<td>-2.083</td>
<td>-1.713</td>
</tr>
<tr>
<td></td>
<td>0.345</td>
<td>0.366</td>
</tr>
</tbody>
</table>

BMD
TABLE NO.3

Comparison of bone formation markers (osteoblastic activity) between postmenopausal osteoporosis and control group.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Bone formation markers</th>
<th>Postmenopausal non-osteoporosis women (Controls) n= 60 Mean ± SD</th>
<th>Postmenopausal osteoporosis women n=60 Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Osteocalcin ng/ml</td>
<td>11.467 ± 3.183</td>
<td>25.184 ± 4.974*</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaline phosphatase (IU/L)</td>
<td>79.07 ± 13.123</td>
<td>112.272 ± 28.362*</td>
</tr>
</tbody>
</table>

* P<0.001- Highly significant

Highly significant increase in the mean levels of osteocalcin and alkaline phosphatase was found in postmenopausal osteoporosis women as compared to control group.
TABLE NO. 4

Bone formation markers from baseline to post therapy of 3 months in postmenopausal osteoporosis women (alendronate + calcium + vitamin D).

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Bone formation markers</th>
<th>Postmenopausal osteoporosis women</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline Mean ± SD</td>
<td>3 months post therapy Mean ± SD</td>
</tr>
<tr>
<td>1.</td>
<td>Osteocalcin (ng/ml)</td>
<td>25.184 ± 4.974</td>
<td>14.640 ± 4.475*</td>
</tr>
<tr>
<td>2.</td>
<td>Alkaline phosphatase (IU/L)</td>
<td>112.272 ± 28.362</td>
<td>90.509 ± 16.72*</td>
</tr>
</tbody>
</table>

* P < 0.001- Highly significant

Highly significant decrease in the mean levels of osteocalcin and alkaline phosphatase activity was observed from baseline to post therapy of 3 months in postmenopausal osteoporosis women.
Biochemical Studies in Osteoporosis of Women

Study groups

Osteocalcin

controls
baseline
post therapy

conc. of osteocalcin in ng/ml

11.467
25.184
14.64
Biochemical Studies in Osteoporosis of Women

Alkaline Phosphatase

conc. of Alk. PO4 in IU/L

controls  Base line  post therapy

Study groups

79.07  112.272  90.509
TABLE NO. 5
Comparison of biochemical parameters between postmenopausal osteoporosis women and control group.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Biochemical parameters</th>
<th>Post menopausal non-osteoporosis Women (controls) n = 60 Mean ± SD</th>
<th>Postmenopausal osteoporosis women n = 60 Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Calcium (mg/dL)</td>
<td>10.365 ± 0.666</td>
<td>8.879 ± 0.792*</td>
</tr>
<tr>
<td>2.</td>
<td>Phosphorus (mg/dL)</td>
<td>4.255 ± 1.043</td>
<td>3.415 ± 0.701*</td>
</tr>
<tr>
<td>3.</td>
<td>Magnesium (mEq/L)</td>
<td>3.127 ± 0.469</td>
<td>2.047 ± 0.376*</td>
</tr>
<tr>
<td>4.</td>
<td>Total Proteins (Gms/dL)</td>
<td>6.59 ± 0.626</td>
<td>5.734 ± 0.708*</td>
</tr>
<tr>
<td>5.</td>
<td>Albumin (Gms/dL)</td>
<td>3.762 ± 0.508</td>
<td>3.223 ± 0.414*</td>
</tr>
<tr>
<td>6.</td>
<td>Vitamin ‘C’(mg/dL)</td>
<td>2.063 ± 0.467</td>
<td>1.388 ± 0.519*</td>
</tr>
<tr>
<td>7.</td>
<td>Cholesterol (mg/dL)</td>
<td>157.267 ± 16.603</td>
<td>186.652±35.014*</td>
</tr>
</tbody>
</table>

* P< 0.001- Highly significant.

The mean levels of calcium, phosphorus, magnesium, total proteins, albumin and vitamin C, were significantly decreased in postmenopausal osteoporosis women as compared to control group.

Highly significant increase in the mean level of total cholesterol was observed in postmenopausal osteoporosis women as compared to control group.
TABLE NO.6
Biochemical parameters from baseline to post therapy of 3 months in postmenopausal osteoporosis women (alendronate + calcium + vitamin D).

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Biochemical parameters</th>
<th>Postmenopausal women Osteoporosis</th>
<th>3 months post therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>1.</td>
<td>Calcium (mg/dL)</td>
<td>8.879 ± 0.792</td>
<td>10.014 ± 0.596***</td>
</tr>
<tr>
<td>2.</td>
<td>Phosphorus (mg/dL)</td>
<td>3.415 ± 0.701</td>
<td>4.017 ± 0.466***</td>
</tr>
<tr>
<td>3.</td>
<td>Magnesium (mEq/L)</td>
<td>2.047 ± 0.376</td>
<td>2.530 ± 0.391***</td>
</tr>
<tr>
<td>4.</td>
<td>Total Proteins (Gms/dL)</td>
<td>5.734 ± 0.708</td>
<td>6.057 ± 0.602**</td>
</tr>
<tr>
<td>5.</td>
<td>Albumin (Gms/dL)</td>
<td>3.223 ± 0.414</td>
<td>3.511 ± 0.429**</td>
</tr>
<tr>
<td>6.</td>
<td>Vitamin’C’ (mg/dL)</td>
<td>1.388 ± 0.519</td>
<td>1.468 ± 0.495*</td>
</tr>
<tr>
<td>7.</td>
<td>Cholesterol mg/dL</td>
<td>186.652 ± 35.014</td>
<td>170.218 ± 27.569***</td>
</tr>
</tbody>
</table>
Highly Significant increase (P<0.001) in the mean levels of calcium, phosphorus and magnesium was found in postmenopausal osteoporosis women from baseline to 3 months post therapy.

Significant increase (P<0.05) in the mean levels of serum total proteins and albumin was observed in postmenopausal osteoporosis women from baseline to post therapy of 3 months.

No significant change occurred in serum vitamin C.

Total cholesterol level was significantly decreased (P<0.001) in postmenopausal osteoporosis women from baseline to post therapy of 3 months.
Biochemical Studies in Osteoporosis of Women

Calcium

<table>
<thead>
<tr>
<th>Study groups</th>
<th>conc. in mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>controls</td>
<td>10.365</td>
</tr>
<tr>
<td>baseline</td>
<td>8.879</td>
</tr>
<tr>
<td>post therapy</td>
<td>10.014</td>
</tr>
</tbody>
</table>
Biochemical Studies in Osteoporosis of Women

![Phosphorus concentration graph](image)

- **Controls**: 4.225 mg/dl
- **Baseline**: 3.415 mg/dl
- **Post Therapy**: 4.017 mg/dl

**Study group**

- Conc. in mg/dl
- Phosphorus
Magnesium

Study groups

controls  baseline  post therapy

conc. in mEq/L

3.127
2.047
2.53
Total Proteins

concentration in gms/dl

controls  baseline  post therapy

Study groups

controls: 6.59
baseline: 5.734
post therapy: 6.057
Biochemical Studies in Osteoporosis of Women

Serum albumin

controls: 3.762
baseline: 3.223
post therapy: 3.511

conc. in gms/dl
Study groups
Vitamin C

<table>
<thead>
<tr>
<th>Conc. in mg/dl</th>
<th>Controls</th>
<th>Baseline Study Groups</th>
<th>Post Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.063</td>
<td>1.388</td>
<td>1.468</td>
</tr>
</tbody>
</table>
Biochemical Studies in Osteoporosis of Women

controls baseline post therapy

concentration in mg/dl

Study groups

Cholesterol

157.267 186.652 170.218
TABLE NO.7

Comparison of bone resorption markers (osteoclastic activity) between postmenopausal osteoporosis women and control group.

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Bone resorption markers</th>
<th>Postmenopausal non osteoporosis women (controls) n = 60 Mean ± SD</th>
<th>Postmenopausal osteoporosis women n= 60 Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>Tartrate resistant acid phosphatase (TRACP) KA units.</td>
<td>1.226 ± 0.357</td>
<td>3.521 ± 0.691*</td>
</tr>
<tr>
<td>2)</td>
<td>Urine hydroxyproline (mg/g creatinine)</td>
<td>17.188 ± 5.110</td>
<td>34.751 ± 8.768*</td>
</tr>
</tbody>
</table>

* P < 0.001 Highly significant

Highly significant increase in the mean levels of TRACP and urinary hydroxyproline was found in postmenopausal osteoporosis women as compared to control group.
### TABLE NO. 8

Bone resorption markers from baseline to post therapy of 3 months in postmenopausal osteoporosis women. (alendronate + calcium + vitamin D)

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Bone resorption markers</th>
<th>Postmenopausal osteoporosis women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline Mean ± SD</td>
</tr>
<tr>
<td>1</td>
<td>Tartrate resistant acid phosphatase (KA Units)</td>
<td>3.521 ± 0.691</td>
</tr>
<tr>
<td>2</td>
<td>Urine hydroxyproline (mg/g creatinine)</td>
<td>34.751 ± 8.768</td>
</tr>
</tbody>
</table>

* P < 0.001  Highly significant

Highly significant decrease in the mean levels of TRACP and urinary hydroxyproline was observed in postmenopausal osteoporosis women from baseline to 3 months post therapy.
**Biochemical Studies in Osteoporosis of Women**

**Tartarate resistant acid phosphatase**

- **controls**: 1.226
- **baseline**: 3.521
- **post therapy**: 1.986

Conc. in KA units
DISCUSSION

The hallmark of menopause is a reduction in skeletal mass caused by an imbalance between bone resorption and formation due to loss of ovarian function. Hence, loss of ovarian function is the most important factor in the development of postmenopausal osteoporosis \(^{233}\). Increase in life expectancy is another concept of formation of osteoporosis. The risk of nutritional disturbances, in particular principal element and vitamin deficiencies is high in postmenopausal women with osteoporosis \(^{6, 98}\).

Decreased BMD and osteoporotic fracture represent a great socioeconomic burden on society and individuals that increase with age. Hence, we focused our interest in measuring BMD, principal elements and more promising markers of bone remodeling. Management of osteoporosis is a challenge in today’s scenario. This study was designed with a view of better understanding the processes which accelerate the bone loss and alter the bone turnover markers.

In the present study, we attempted to evaluate the activities of osteoblastic and osteoclastic markers pre and post antiresorptive therapy in postmenopausal osteoporotic (PMO) women. The study includes 60 postmenopausal osteoporotic women and the follow up study was conducted 3 months post therapy consisting of alendronate + calcium + vitamin D. Control group includes 60 postmenopausal non osteoporotic women.

**Bone mineral density**

The mean score of BMD in PMO was found to be significantly decreased as compared to control group (P<0.001, Table No.1).

The result of BMD estimated in osteoporotic women gave indication that there was low bone mass. Low bone mass is a major, consistent characteristic of postmenopausal osteoporosis. A strong inverse relationship
exists between bone mass and susceptibility to fracture. Therefore, bone mass is the primary indicator of fracture risk in women without fractures (234).

Postmenopausal bone loss is most rapid during the first postmenopausal decade. Estrogen has profound influence on bone metabolism. Deficiency of estrogen causes activation of new bone remodeling sites and exaggeration of the imbalance between bone formation and resorption. This leads to presence of more remodeling sites in the skeleton, which increases the probability of penetration of trabeculae, causes elimination of template upon which the new bone is to form. Genetic, nutritional and lifestyle factors (such as insufficient dietary calcium) may accelerate bone loss independent of the effects of declining estrogen concentration, thus the risk of postmenopausal osteoporosis can be further increased (234).

Finding of Neetakumar et al (6), Shah D et al (50) and Johannes WGJ et al. (52) support our result.

BMD was done by QUS method in this study. This is a valuable screening tool because it is noninvasive, with low cost, feasible without radiation exposure and help in identifying, normal control, osteopenia & osteoporosis in female subjects.

Table No.2 shows that BMD score was found to be increased after therapy, but it never reached the normal level in the 3 months study period. Rather it reached the score for osteopenia.

By suppressing bone turnover, this therapy prevents bone loss and preserves bone architecture and increase bone strength. Alendronate of this therapy can binds hydroxyapatite crystals of bone with high affinity and inhibit bone resorption by decreasing osteoclastic activity and its growth. After the inhibition of resorption, these agents become affixed to the bone matrix, where they reside until the remodeling begins again. Thus it has very long retention in the skeleton and may exert long term effects. Antiresorptive therapy is effective in reducing the risk of fracture and should be considered for all patients with osteoporosis.
Finding of Cheung AM et al \(^{(49)}\), Ones K et al \(^{(51)}\), Bone HG et al \(^{(53)}\), Rhee Y et al \(^{(55)}\), Sambrook PN et al \(^{(56)}\), Tiras. et al \(^{(58)}\), Lindsay R et al \(^{(59)}\), Kyd et al \(^{(60)}\) support our results.

BMD is the best quantifiable predictor of osteoporotic fracture; it provides a static picture of the skeleton. BMD remains the gold standard for determining the state of bone turnover and diagnosing disease and very useful in monitoring treatment efficacy in patients with osteoporosis.

**Bone Formation Markers**

**Osteocalcin:**

Mean level of serum osteocalcin was found to be significantly elevated in PMO when compared with controls. \((P<0.001, \text{Table No.3})\)

Circulating osteocalcin is associated with changes in the rate of bone turnover. Due to its tissue specificity and relatively low within person variation, we measured serum osteocalcin as a clinical index of bone turnover in the metabolic disease i.e. postmenopausal osteoporosis.

Osteocalcin or bone Gla protein is a bone specific protein and the major noncollagenous protein in the bone matrix. Osteocalcin is synthesized in the skeleton by osteoblasts, the cells responsible for the bone formation. It is a highly sensitive & specific marker for bone formation. Osteocalcin has a high affinity for calcium and exhibits a compact calcium dependent \(\alpha\) helical conformation, in which the \(\gamma\)-carboxyglutamic acid (Gla) residues promote absorption to hydroxyapatite in the bone matrix. It might be associated with the process of mineralization \(^{(235)}\).

Elevated levels of osteocalcin was found in osteoporosis, might be due to the increased activity of osteoblasts. In the early postmenopausal period high bone turnover rate is associated with osteoporosis. In addition, deficiencies of calcium and phosphorus may occur which will lead to lowering of formation of hydroxyapatite crystals. Decreased availability of hydroxyapatite crystals can affect mineralization of bones. In the state of decreased rate of bone
mineralization, free osteocalcin may be released for circulation in the blood. This may explain the increased concentration of osteocalcin in the serum of osteoporotic postmenopausal women.

Our findings were also supported by Ones K et al\(^{(51)}\), Verit FF et al\(^{(131)}\), Minisola S et al\(^{(129)}\), Christian Rosenquist et al\(^{(136)}\), Eastell R et al\(^{(140)}\).

Table No. 4 shows that highly significant decrease in osteocalcin levels were observed from baseline to post therapy of 3 months in PMO (alendronate + calcium + vitamin D).

This therapy suppresses osteoclast mediated bone resorption and indirectly and more slowly, it may decrease osteoblast activity and bone formation. Thus it acts as antiremodeling drug. Net positive calcium balance may be achieved during therapy, promoting binding of osteocalcin with calcium. Osteocalcin is involved in bone calcification; hence its level may be lowered after therapy.

Our findings were also supported by Yasui T et al\(^{(127)}\), Johannes WGJ et al\(^{(52)}\), Susan L et al\(^{(130)}\), Sambrook PN et al\(^{(56)}\), Fardellone P et al\(^{(135)}\), Szulc P et al\(^{(139)}\).

To conclude, osteocalcin, a promising marker of bone turnover has diagnostic as well as prognostic significance in osteoporosis. Antiresorptive therapy can be monitored by the assay of circulating osteocalcin. Thus, determination of osteocalcin will be of great help in the management of postmenopausal osteoporotic women.

**Alkaline Phosphatase**

Alkaline phosphatase activity was found to be significantly elevated in PMO when compared to controls. (P<0.001, Table No.3)

We have assessed osteoblastic activity by the measuring serum alkaline phosphatase which is most commonly used index marker of bone formation.

High levels of serum alkaline phosphatase activity encountered in osteoporosis might be a result of the action of the osteoblastic cells; which try
to rebuild bone that is being resorbed by the uncontrolled activity of osteoclasts. Our results indicate that bone regeneration is taking place or is being attempted and alkaline phosphatase are probably participates in the initiation of bone mineralization \(^{(149,236,237)}\). Decreased ability to produce calcitriol from vitamin D may be another reason for elevated alkaline phosphatase activity in the postmenopausal women with osteoporosis \(^{(238,94)}\). It may lower calcium and phosphorus absorption from intestine and calcium uptake by osteoblasts, which ultimately affects the mineralization of bone. Thus osteoid will be formed but poorly calcified, hence for mineralization of bone, osteoblastic activity may be increased.

Our findings were also supported by Indumati V et al\(^{(70)}\), Usoro CAO et al\(^{(148)}\), Verit F F et al\(^{(131)}\), M suresh et al\(^{(71)}\), Adami S et al\(^{(150)}\), Takahashi M et al\(^{(152)}\), Lips P et al\(^{(153)}\).

Table No.4 shows that significant decrease in alkaline phosphatase activity from baseline to post therapy of 3 months occurred in PMO (alendronate + calcium + Vitamin D).

After receiving antiresorptive therapy alkaline phosphatase activity comes down to near normal level. This therapy can increase intestinal absorption of calcium and phosphorus with a consequently higher influx of calcium ions at the bone level. This can decrease bone turnover and decelerate bone loss. Thus our study suggests that the antiresorptive therapy is useful to control rate of bone turnover and thereby, better management of PMO.

Our findings were also supported by Ones K et al\(^{(51)}\), Reid IR et al\(^{(90)}\), Andrew YY et al\(^{(55)}\), and Watts NB et al\(^{(154)}\).

Measurement of alkaline phosphatase activity is simple, easy; routine biochemical marker and can be used to assess the bone turnover. This marker can be measured in any clinical laboratory & can be utilized by the clinicians for better management of osteoporosis, even in semi-urban areas.
Calcium

Mean level of serum calcium was found to be significantly decreased in PMO when compared with controls. (P<0.001, Table No.5)

Several factors might be responsible for this observed reduction of calcium, such as low estrogen level, menopausal period, lifestyle pattern and dietary habits. Adequate amounts of calcium intake are necessary for bone health because deficiency of calcium is a risk factor for osteoporosis. Low level of serum calcium may also be due to insufficient intake of vitamin D. Deficiencies of vitamin D affect the intestinal absorption of calcium and ultimately mineralization of bone.

Our findings were also supported by Indumati V et al \(^{(70)}\), Sameer Batra et al \(^{(72)}\), Narang APS et al \(^{(94)}\), and Gupta et al \(^{(77)}\).

Table No.6 shows that significant increase occurred in total calcium from baseline to post therapy of 3 months in PMO (alendronate + calcium + vitamin D).

The antiresorptive therapy contains calcium and vitamin D along with alendronate; hence elevation of serum calcium level was expected in the post therapy. Thus calcium balance eventually becomes positive indicating retention of calcium \(^{(239)}\). Elevation of serum calcium may turn off the secretion of PTH by negative feedback control and stimulate the secretion of calcitonin; a hormone that inhibits the activity of osteoclasts and thus suppresses bone resorption.

Our findings were also supported by Reid IR et al \(^{(90)}\), Prince RL et al \(^{(240)}\), Patrice Fardellone et al \(^{(135)}\), and Hasling C et al \(^{(218)}\).

Phosphorus

Mean level of phosphorus was found to be significantly decreased in PMO when compared with controls (P<0.001, Table No.5)

Fall in the phosphorus level might be due to the dietary deficiencies of phosphorus and vitamin D. Age related resistance of the gut to respond normally to \(1, 25 \text{(OH)}_2\text{D}_3\), may hamper intestinal absorption of calcium and
phosphorus. In addition there may be a defect in hypophosphatemia- mediated stimulation of 25 (OH) D-1α- hydroxylase resulting in decreased intestinal absorption of phosphorus, which ultimately affects the mineralization of bone (241). Hypophosphatemia can cause a decreased mineralization of skeleton.

Our findings were also supported by Indumati V et al (70), Sameer Batra et al (72), and Narang APS et al (94).

Table No.6 shows that significant increase in phosphorus level was observed from baseline to post therapy of 3 months in PMO (alendronate +calcium + vitamin D).

Increase in serum phosphorus level may be due to increased intestinal absorption of phosphorus. Normalization of serum calcium and phosphorus levels will certainly have a positive effect on formation of hydroxyapatite crystals, resulting in calcification of bone.

Finding of Reid IR et al (90), Reginster JY et al (111), and Prince RL et al (240) support our study.

**Magnesium**

Mean level of serum magnesium was found to be significantly decreased in PMO as compared controls (P<0.001, Table No.5)

Magnesium is present in bone, association with calcium and phosphorus. Decreased level of magnesium might be due to the dietary deficiency of magnesium. This may hamper the absorption and transport of calcium. Lowering of serum magnesium can cause failure in conversion of vitamin D to its active form and stimulation of calcitonin and suppression of PTH. This may also induce uncoupling of bone formation and resorption may result in a loss of bone mass.

Our findings were also supported by Gur et al (98), Brodowski et al (101), and Rude RK et al (103), Reginster JY et al (111).
Table No.6 shows that significant increase in magnesium level from baseline to post therapy of 3 months in postmenopausal osteoporosis women (alendronate + calcium + vitamin D).

It may be related to positive calcium balance achieved during therapy and may be due to processes related to interrelationship between calcium & magnesium metabolism.

**Total Proteins and Albumin**

Serum total proteins and albumin levels were found to be significantly decreased in PMO as compared to control. (P< 0.001, Table no.5)

Serum albumin was decreased in the course of aging particularly in osteoporosis women and inadequate intake of proteins might be the cause of lowered proteins and albumin. IGF-I production and circulating levels were reported to be decreased during low protein intake. Impairment of both systems may contribute to the occurrence of osteoporosis.

Our result indicates that decreased albumin level may be related to the reduction of bone mass. Albumin should have a more direct effect on bone metabolism because of its role as a major calcium binding protein. Thus albumin is essential for the synthesis of bone matrix and bone health. The study Neetakumar et al (6) has shown that low calcium and protein diet enhances the rate of loss of 25 (OH) D from the circulation by destruction in liver.

Our findings were also supported by Batra S et al (72), Narang APS et al (94), Schurch MA et al (170), Sokoll LJ et al (175), Erasmo ED et al (166), Bonjour JP et al (171).

Table no.6 shows that serum total proteins and albumin levels were increased (P<0.05) from baseline to post therapy of 3 months in PMO (alendronate + calcium + vitamin D).

All the patients were advised by the clinicians to take balanced diet, with adequate calcium and first class proteins. Improvement of the nutritional status might have raised the serum protein levels. Finding of Arase Y et al (163) support our study.
Vitamin C

Mean level of vitamin C was found to be significantly decreased in PMO when compared to controls (P < 0.001, Table No. 5).

Previous studies\(^{(147)}\) have shown that lipid peroxidation was increased in osteoporosis due to oxidative stress. We were interested in measuring the status of antioxidant vitamin i.e. vitamin C.

Reduction of the vitamin C level may impair hydroxylation of lysine and proline in protocollagen. Without this hydroxylation, cross-linking of protocollagen into normal collagen may be affected. Thus fibrils or and the structure may disintegrate rapidly. It may also affect vitamin D metabolism and in turn, the risk of osteoporosis.

Our findings were also supported by Simon JA et al\(^{(177)}\), and Morton DJ et al\(^{(178)}\).

Table no. 6 shows that after therapy, no significant change occurred in vitamin C level in PMO.

Cholesterol

Mean serum cholesterol level was found to be significantly increased in PMO when compared to controls (P<0.001, Table No. 5).

Increase in cholesterol level might be due to the reduction of vitamin D, which may cause disturbances in lipid metabolism. In this study, we found that vitamin C is lowered in osteoporotic women. This may have lowered the metabolism of cholesterol to bile acids, resulting in hypercholesterolemia.

Increase in cholesterol level might be due to the oxidative stress which leads to oxidative deterioration of the unsaturated fatty acids or lipids in osteoporosis as reported in previous studies. Lipid peroxidation may also promote osteoporosis, may be because of whelming osteoclastic activity due to activation of osteoclasts. Thereby may be linking lipid metabolism to regulation of bone density.
Our findings were also supported by Chavan SN et al\textsuperscript{(147)}, Sontake AN et al\textsuperscript{(215)}, Murray RK et al\textsuperscript{(216)}, Yamaguchi et al\textsuperscript{(218)}, Parhami F et al\textsuperscript{(219)}.

Table No. 6 indicate that serum cholesterol level was found to be decreased significantly from baseline to post therapy of 3 months in PMO (alendronate + calcium + vitamin D).

One constituent of the antiresorptive therapy i.e. alendronate inhibits farnesyl pyrophosphatase and other distal steps in the intracellular mevalonate pathway. This may be the cause of decreased cholesterol level in PMO post therapy.

The result from this study suggests that routinely measured, low cost biochemical markers such as serum calcium, ALP, albumin and phosphorus can be used as indicators of increased bone turnover, to enable early intervention so as to minimize fracture due to osteoporotic changes. These markers can be measured in any simple clinical laboratory and may help identify women at greatest risk for bone loss, who would benefit most from therapeutic interventions. While BMD provides a static picture of the skeleton, osteocalcin, the valid marker of bone turnover can provide dynamic status of bone remodeling and thus are potentially useful in predicting the course of changes in bone mass. The combined use of BMD and these biochemical markers can be of great help in the evaluation of osteoporosis and for monitoring responses to the antiresorptive therapy.
Bone Resorption Markers

Tartrate resistant acid phosphatase (TRACP)

Significant increase in the activity of tartrate resistant acid phosphatase was found in PMO when compared to controls (P<0.001, Table no. 7)

TRACP activity directly reflects the activity of osteoclasts. Hence, from our results it is evident that there is significant increase in osteoclastic activity, leading to greater resorption of bone.

Specific cytokines such as IL-1, IL-6, and TNF α (inhibits apoptosis and extends the life span of osteoclasts), granulocyte macrophage colony stimulating factors (GM-CSF) may be responsible for this. These cytokines may enhance bone resorption by increasing the recruitment, differentiation, and activation of osteoclast cells.

Decreased IL-Ira concentration (interleukin 1 receptor antagonist) may lead to enhanced osteoclast sensitivity to IL-1 in osteoporosis. The production of IGF-B and OPG-L factors that mediate osteoclast apoptosis may also be reduced in PMO. In this way the osteoclast number and activity may be increased in osteoporosis.

Indeed, such an elevation in osteoclastic activity is shown in our study by increase in TRACP activity in PMO.

Our findings were also supported by Verit FF et al\textsuperscript{(131)}, Tahtela R et al\textsuperscript{(188)}, Chao TY et al\textsuperscript{(189)}, Halleen JM et al\textsuperscript{(193)}, Price CP et al\textsuperscript{(196)}, Garnero P et al\textsuperscript{(197)}, Cheung CK et al\textsuperscript{(198)}.

Table No-8 shows that TRACP activity was decreased significantly from baseline to post therapy of 3 months in PMO (alendronate + calcium + vitamin D).

The therapy contains a potent nitrogen containing drug i.e. alendronate which inhibits farnesyl diphosphate synthase, a critical enzyme in the cholesterol mevalonic acid pathway that is also required for protein prenylation. When the activity of this enzyme is blocked, the cytoskeletal integrity and intracellular functioning of the osteoclasts is disrupted and
apoptosis ensues. In this way decreases the osteoclastic activity and its growth. Decrease in TRACP activity post therapy reflects renormalization of the bone resorption, by reducing the osteoclastic activity.

Our findings were also supported by Valimaki MJ et al (190), Matyszko J (243), Rosen CJ et al (244), Greenspan SL et al (245), and Ravn et al (246).

**Hydroxyproline.**

Significant increase in the levels of urinary hydroxyproline was observed in the PMO when compared with controls (P < 0.001, Table No.7).

Hydroxyproline is the major breakdown product from collagen, the main protein of the bone matrix. It is considered as clinical index of bone resorption and a major determinant of bone status. Thus increased excretion of hydroxyproline indicates increased breakdown of collagen.

Several factors might be responsible for excess bone resorption such as low estrogen level, calcium and vitamin D deficiency and age related reduced calcium absorption leading to excess parathyroid hormone.

During bone resorption, highly active osteoclasts may secrete factors into the space between the cell and bone surface such as acids, matrix metalloproteinases (MMPS) and Cathepsin K in excess. These factors can degrade collagen type I into several products, such as hydroxyproline, hydroxypyridinium, pyridinoline and deoxypyridinoline.

Our findings were also supported by Indumati V et al (70), Verit FF et al (131), Eastell R et al (140), Halleen JM (194), Muzzuoli G (203), Nordin BEC (205), Morris HA et al (206).

Table No. 8 shows that urinary hydroxyproline level was significantly decreased from baseline to post therapy of 3 months in postmenopausal osteoporosis women (alendronate + calcium + vitamin D).

Alendronate from this antiresorptive therapy is known to specifically impair osteoclast function and reduce its number, in part by the induction of apoptosis. It may also reduce bone loss by decreasing bone resorption through
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PTH. Reduction in bone loss is indicated by reduced excretion of hydroxyproline post antiresorptive therapy.

Our findings were also supported by Fardellone P et al (135), Kamel S et al (204), Garnero P et al (62), Russell RGG et al (137), Horowitz M et al (208).

In this study, elevated osteoclastic (bone resorption) markers were found in PMO women. The result indicates that high bone turnover occurs in osteoporosis. Processes involved in bone formation are probably unable to keep pace with the rate of bone resorption. Rise in the excretion of hydroxyproline clearly shows degradation of collagen type I from the bone matrix in osteoporotic women. Assay of TRACP directly reflects the activity of osteoclasts. Our results demonstrate the impaired bone metabolism and reverse bone turnover in osteoporosis. Alendronate + calcium + vitamin D therapy is effective in optimizing the bone metabolism. The rate of osteoclastic activity and breakdown of collagen is lowered as reflected by TRACP activity and hydroxyproline excretion after 3 months therapy. Thus we can conclude that control of osteoclastic activity was definitely initiated by the treatment. Prolonged treatment may be required to observe total normalization of bone metabolism. These biochemical markers of bone resorption are very useful in monitoring the therapy in PMO and may become an integral part of the management of osteoporosis.