

Analysis of Plasma Osteocalcin Levels, Status of Oral Disease and Alteration in Mandibular Bone Density in Postmenopausal Women

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Abstract: ***Aim:** The present study was undertaken for assessing Plasma Osteocalcin levels, status of Oral Disease and alteration in Mandibular Bone Density in Postmenopausal Women (PMW). **Materials & methods:** 80 premenopausal women and 80 PMW were enrolled. For analysing the oral dryness, clinical score of oral dryness (CSOD) on a scale of upto 10 was used in all each subject. Complete dental profiling of all the subjects was done. Digital panoramic radiographs were taken for all the subjects. Measurements were made on each PAN and following indices were evaluated for assessing the mandibular BMD: Mandibular cortical index (MCI), Mandibular cortical index (MCI), panoramic mandibular index (PMI), Mandibular cortical width (MCW), and Fractal dimension (FD). Osteocalcin levels were evaluated with ELISA technique. All the results were recorded and analysed. **Results:** Mean osteocalcin levels of PMW (453.12 ng/mL) were significantly higher in comparison to the premenopausal subjects (249.28 ng/mL). PMW had significantly higher clinical score of oral dryness and number of peri - apical radiolucencies in comparison to premenopausal women. Bone mineral density as assessed by mandibular cortical index were found to be negatively and significantly correlated with oral disease status and osteocalcin levels. Only mandibular cortical index varied significantly statistically in between the two study groups. **Conclusion:** There is significantly higher prevalence of oral lesions along with oral dryness in postmenopausal women. Also, thinning of mandibular cortex is significantly higher in postmenopausal women. Higher plasma osteocalcin levels helps in predicting osteopenia/osteoporosis at an early stage in such subjects. **Clinical significance:** Special considerations should be made in postmenopausal women while doing treatment planning for prosthetic purposes (dental implants) as edentulous bone is at higher risk of osteoporosis in such patients.*

Keywords: Osteocalcin, Postmenopausal, Mandibular bone density

1. Introduction

Menopause is invariable accompanied with a series of physical alterations. Most of these alterations are directly linked to estrogen loss, including vaginal dryness and bone demineralization. Some authors have also reported slight higher prevalence of cardiovascular pathologies in postmenopausal women (PMW). Among PMW, osteoporosis is a prime concern resulting in considerable morbidity and mortality. It has also been reported that more than half of women over the age of sixty five years have a compression fracture. Hence; it is necessary to maintain bone mass for preventing the osteoporosis. Vertebral fractures (due osteoporosis in PMW) can also result in loss of height, kyphosis and lordosis (postural changes).¹⁻³

It is probably inappropriate to analyse the incidence and statistical data in relation to menopause as it is a female's physiologic alteration. However; it increases the risk for development of different pathological lesions. Occurrence of osteoporosis involving the mandible in these patients increases the risk for development of pathologies involving oral soft and hard tissues. This can result in loosening of

teeth thereby increasing the chances of tooth loss. Loss of teeth in the posterior tooth region can lead to loss of neuromuscular stability of the mandible, decrease in masticatory efficiency, poor aesthetics etc.⁴⁻⁵

For appreciating the pathophysiologic pathways for bone metabolic pathologies, different biochemical markers of bone metabolism are considered as significant tools. The assessment of the protein fragments produced by osteoblasts (osteocalcin, enzymes produced during osteogenesis: alkaline phosphatase) are of significant importance for analysing the osteoblastic activity.⁶⁻⁷ Hence; the present study was undertaken for assessing Plasma Osteocalcin levels, status of Oral Disease and alteration in Mandibular Bone Density in Postmenopausal Women.

2. Materials & Methods

The present study was conducted with the aim of assessing Plasma Osteocalcin levels, status of Oral Disease and alteration in Mandibular Bone Density in Postmenopausal Women. Among these, 80 subjects were premenopausal women while the remaining 80 were PMW. Only those

subjects were included in the PMW group, in which there was a minimum of 2 years history of start of menopause at the time of study. Written consent was obtained from all the subjects after explaining in detail the entire research protocol. Recording of complete demographic and clinical details of all the patients was done. Blood samples were obtained from all the patients and complete lipid and haematological profile was recorded. From the medical record files, body mass index (BMI) plus waist circumference was recorded. Subjects with body mass index of more than equal to 23 Kg/m² along with waist circumference of more than or equal to 80 cm were categorised as obese. Complete oral examination was done in all the subjects using mouth mirror, probe and tweeter for identifying presence of any oral lesion (if any) and dryness. For analysing the dryness, clinical score of oral dryness (CSOD) on a scale of upto 10 was used in all each subject, based on criteria described previously in the literature. Scoring of CSOD was as follows⁸:

- Score of 1 to 3 (Low CSOD score): Mild dryness
- Score of 4 to 6 (Medium CSOD score): Moderate dryness, and
- Score of 7 to 10 (High CSOD score): Extreme dryness

Complete dental profiling of all the subjects was done, which included recording of the number of missing teeth (except for third molar) and thorough periodontal examination using Hu - Friedy 12 UNC colour - coded periodontal probe. Along the six clinical sites of a tooth (distobuccal, midbuccal, mesiobuccal, distolingual, midlingual, and mesiolingual), measurement of bleeding on probing (BOP), probing depth (PD) and clinical attachment loss (AL) was done for diagnosing periodontal pathologies.

After carrying out the oral examination, digital panoramic radiographs were taken for all the subjects during the same visit. An experienced and radiologist analysed the presence of peri - apical lesions, alveolar bone loss and mandibular bone mineral density indices. After examining digital panoramic radiographs and carrying out clinical examination, diagnoses of diseases teeth was established based on presence of peri - apical radiolucencies and clinical appearance.⁹ Alveolar bone loss (ABL) was assessed radiographically by evaluating the mesial and distal surfaces of six teeth and thereby calculating the ABL% based on criteria previously described in literature.¹⁰

Measurements were made on each PAN and following indices were evaluated for assessing the mandibular BMD:

- Mandibular cortical index (MCI)
- Mandibular cortical index (MCI)
- Panoramic mandibular index (PMI)
- Mandibular cortical width (MCW)
- Fractal dimension (FD)

MCI¹¹

As per classification given by Klemetti et al, MCI (inferior mandibular cortical thickness) is classified as follows (**Figure 1: a, b and c**):

C1: Sharp and even endosteal margin of the cortex

C2: Presence of lacunar resorption/ cortical residues on endosteal margin on one or both sides,

C3: Presence of heavy endosteal residues on the cortical layer

PMI: It refers to the ration obtained by dividing mandibular cortex thickness with distance among mental foramen's inferior margin and the inferior mandibular cortex.¹²

MCW: It refers to the line that was at right angle to the mandible's inferior border at the middle of the mental foramen.¹³

FD: Digital radiograph were opened with PixelStyle software and Image J software and FD was calculated based on criteria described previously in literature.^{14,15}

Haematological analysis

All the patients were recalled in the morning and peripheral venous blood samples were obtained after overnight fasting. All the samples were stored at - 80 degree centigrade and were sent to pathology department for further analysis. ELISA technique (BGLAP ELISA kit, USA) was used for evaluating the osteocalcin levels as per manufacturer's instructions.

All the results were recorded in Microsoft excel sheet and were analysed by SPSS software. Mann - Whitney U test, Spearman correlation coefficient and student t test were used for assessment of level of significance. P - value of less than 0.05 was taken as significant.

3. Results

In the present study, 80 PMW and 80 premenopausal subjects were enrolled. Mean age of the PMW and premenopausal subjects was 53.12 years and 39.36 years respectively. Mean fasting blood glucose levels of PMW and premenopausal women was found to be 88.6 mg/dL and 80.4 mg/dL respectively (**Graph 1**). While analysing the plasma osteocalcin levels, it was observed that mean osteocalcin levels of PMW (453.12 ng/mL) were significantly higher in comparison to the premenopausal subjects (249.28 ng/mL) (p - value < 0.05) as shown in **Table 1**. Average number of missing teeth in postmenopausal women was 7 while in premenopausal women were 2; on comparing the results were found to be statistically significant (**Table 2**). Mean attachment loss was 3.1 mm and 2.2 mm in postmenopausal women and premenopausal women respectively. Mean ABL% was 27.8% and 20.4% in postmenopausal women and premenopausal women respectively. On comparing statistically, it was observed that PMW had significantly higher clinical score of oral dryness and number of peri - apical radiolucencies in comparison to premenopausal women. Plasma osteocalcin levels among PMW with mandibular cortical index of C2 - C3 (435.1 ng/mL) were significantly higher in comparison to PMW with mandibular cortical index of C1 (321.8 ng/mL) as shown in **Table 3**. It was also seen that mean ABL%, mean number of missing teeth and mean value of clinical attachment loss was significantly higher in PMW with mandibular cortical index of C2 - C3 in comparison to subjects with index of C1 (p - value < 0.05). Also, it was observed that BMD as assessed by mandibular cortical index were found to be negatively

and significantly correlated with oral disease status and osteocalcin levels as shown in **Table 4**. On comparing the different indices and parameters of BMD of mandible between PMW and premenopausal women, it was seen that only mandibular cortical index varied significantly statistically in between the two study groups (p - value < 0.005) as shown in **Table 5**.

4. Discussion

“Menopause” word is of Greek origin referring to month and cessation. At times, the term is used for describing climacteric, which denotes happenings associated with “the changes in life. It has been seen that with advancing age, bone mass decreases in humans. In the approximately thirties and forties, human bones show decline in density along with increasing porosity. This is specifically exaggerated in women after the occurrence of menopause. Hence; significantly higher risk of osteoporosis is associated in such women. Mineral levels of the skeleton have been shown to affect the BMD of mandible, thereby resulting in generalized bone resorption.^{16, 17}

Until the last decade, the association between energy metabolism and osseous tissue was regarded as unidirectional in which there are multifaceted communications between leptin, adiponectin, and neuropeptides. Literature from the animal studies has demonstrated that osseous cells control glucose and fat metabolism through osteocalcin. Osteocalcin is a protein in nature and is found to be present mainly in bone and dentine tissue. It is known to play a crucial role in regulating calcium homeostasis throughout the bone mineralization process. After its formation, a significant proportion of it is assimilated into the bone’s extracellular matrix. However, in systemic circulation, a small proportion of it is released. Hence; it is regarded as a specific marker for demonstrating osteoblastic activity.^{18 - 22} Hence; the present study was undertaken for assessing Plasma Osteocalcin levels, status of Oral Disease and alteration in Mandibular Bone Density in Postmenopausal Women.

In the present study, 80 PMW and 80 premenopausal subjects were enrolled. Mean osteocalcin levels of PMW were significantly higher in comparison to the premenopausal subjects as shown in **Table 1**. Also PMW had significantly higher clinical score of oral dryness and number of peri - apical radiolucencies in comparison to premenopausal women (**Table 2**). Our results were in concordance with the results obtained by Kalaiselvi VS et al and Thanakun S et al who also reported similar findings in their respective studies. In the study conducted by Kalaiselvi VS et al, authors analysed correlation between bone remodelling biomarkers and osteocalcin with BMD in post - menopausal non osteoporotic and osteoporotic women. A total of 30 non - osteoporotic (Group 1) and 30 osteoporotic subjects were (Group 2) were enrolled in their study and serum osteocalcin and BMD was evaluated. In PMW, they observed a negative association among osteocalcin level and the BMD. Also significant results were obtained by the authors while comparing the mean serum osteocalcin and BMD values among both the study groups.^{23, 24} In another study conducted by Thanakun S et al, authors compared the

osteocalcin levels, oral pathologies, and mandibular BMD alterations among PMW and premenopausal women. After analysing 92 subjects, they observed significantly higher osteocalcin levels in PMW in comparison to premenopausal women. Similar to our study, they also reported significantly higher incidence of oral lesions in PMW. However; in PMW they reported significantly lower mandibular BMD.²⁴

In the present study, mean plasma osteocalcin levels among PMW with mandibular cortical index of C2 - C3 were significantly higher in comparison to PMW with mandibular cortical index of C1 (**Table 3**). Also, mean ABL%, mean number of missing teeth and mean value of clinical attachment loss was significantly higher in PMW with mandibular cortical index of C2 - C3. In the past literature, reliability of MCI as a diagnostic tool has been proved from time to time for screening PMW with osteoporosis. Occurrence of any form of cortical erosion (C2 or C3) could be considered as a valuable indicator of reduced BMD since in nearly more than three fourth of subjects, it is accompanied with at least osteopenia. In a previous study conducted by Gaur et al, authors showed that a significant relationship exists between BMD and MCI. They observed specificity and sensitivity of be 88.9% and 100% respectively.^{25, 26}

In the present study, BMD was found to be negatively and significantly correlated with oral disease status and osteocalcin levels (**Table 4**). Impact of menopause on BMD and mandibular bone width was evaluated by Munakata M et al, who evaluated 30 premenopausal and 42 postmenopausal subjects. They observed that trabecular BMD of PMW was lower in comparison to premenopausal women. They also reported negative correlation between Trabecular region width with BMD and positive correlation between cortical width and BMD. Their results showed that both bone (mandible) quality and quantity is affected by menopause.²⁷

In the present study, it was observed that only mandibular cortical index varied significantly statistically in between the two study groups (**Table 5**). Previous studies have also demonstrated negative correlation of plasma osteocalcin levels with BMI and BMD. In a study conducted by Sternfeld et al, authors demonstrated that alterations in the menopausal status was not correlated with obesity but was significantly correlated with increased waist circumference.^{28, 29} In another study conducted by Irie et al, authors evaluated the impact of menopause on mandibular bone by assessing the morphological alterations in the mandible of ovariectomized rats. However; they not only observed significantly reduction for both bone mass and width of trabecular bone but at the same time, also observed significantly augmented distances between trabeculae in ovariectomized rats in comparison to healthy controls.³⁰

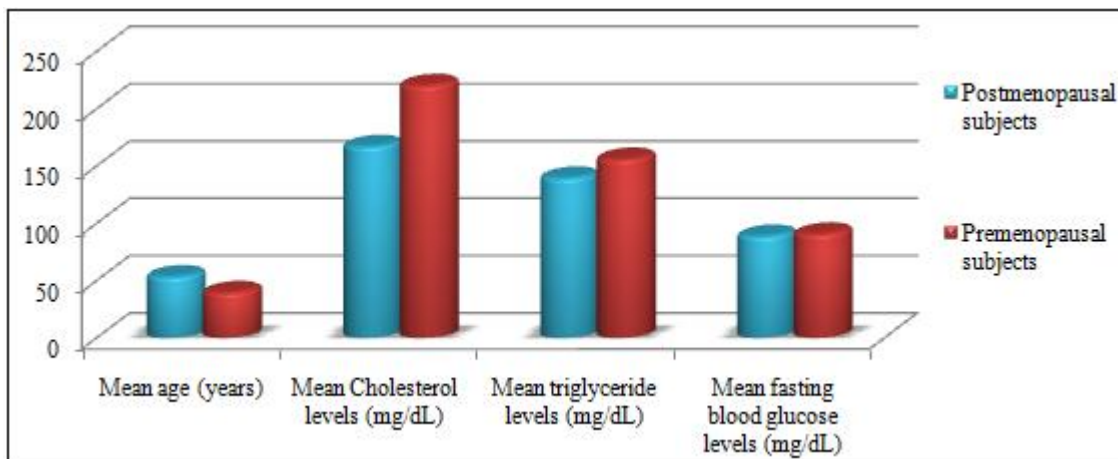
5. Conclusion

Under the light of above obtained results, the authors conclude that there is significantly higher prevalence of oral lesions along with oral dryness in postmenopausal women. Also, thinning of mandibular cortex is significantly higher in postmenopausal women. Higher plasma osteocalcin levels

helps in predicting osteopenia/osteoporosis at an early stage in such subjects. Hence; we recommend maintaining of adequate oral hygiene and regular health check at timely intervals.

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Graph 1: Demographic and general profile

Table 1: Comparison of plasma osteocalcin levels

Osteocalcin levels (ng/mL)	Premenopausal subjects	Postmenopausal subjects	p - value
Mean	249.28	453.12	0.0001*
SD	123.48	223.75	

*: Significant

Table 2: Correlation of oral disease status with menopausal status

Parameter	Premenopausal subjects	Postmenopausal subjects	p - value
Average missing teeth number	2	7	0.001*
Mean value of attachment loss (mm)	2.2	3.1	0.003*
Mean ABL%	20.4	27.8	0.001*
Number of per - apical radiolucencies	0 (absent)	68	0.000*
	More than 1	12	
Clinical score of oral dryness	0	45	0.000*
	1 to 3	32	
	More than or equal to 4	3	

*: Significant

Table 3: Plasma osteocalcin levels, ABL%, number of missing teeth, and mean value of clinical attachment level among PMW with different mandibular cortical index

Parameter	Mandibular cortical index		p - value
	C1	C2 - C3	
Osteocalcin levels (ng/mL)	321.8	435.1	0.020*
ABL%	18.3	30.7	0.000*
Number of missing teeth	4.1	8.9	0.010*
Mean value of clinical attachment loss	2.5	3.3	0.001*

*: Significant

Table 4: Correlation of Osteocalcin levels, ABL%, Number of missing teeth and Mean value of clinical attachment loss with Mandibular cortical index in PMW

Spearman's rho	Mandibular cortical index	
Osteocalcin levels	Correlation Coefficient	- 0.471
	p - value	0.000*
ABL%	Correlation Coefficient	- 0.396
	p - value	0.003*
Number of missing teeth	Correlation Coefficient	- 0.513
	p - value	0.001*
Mean value of clinical attachment loss	Correlation Coefficient	- 0.493
	p - value	0.002*

*: Significant

Table 5: Mandibular bone density comparison

Parameter	Postmenopausal subjects	Premenopausal subjects	p - value
Right mandibular cortical index	C1	75	0.000*
	C2	5	
	C3	0	
Right mandibular cortical index	C1	75	0.001*
	C2	5	

	C3	0	3	
Panoramic mandibular index	Right	2.27	2.29	0.423
	Left	2.29	2.26	0.380
Mandibular cortical width (mm)	Right	3.42	3.57	0.721
	Left	3.49	3.63	0.873
Fractal dimension	Right	1.20	1.21	0.250
	Left	1.22	1.25	0.336

*: Significant

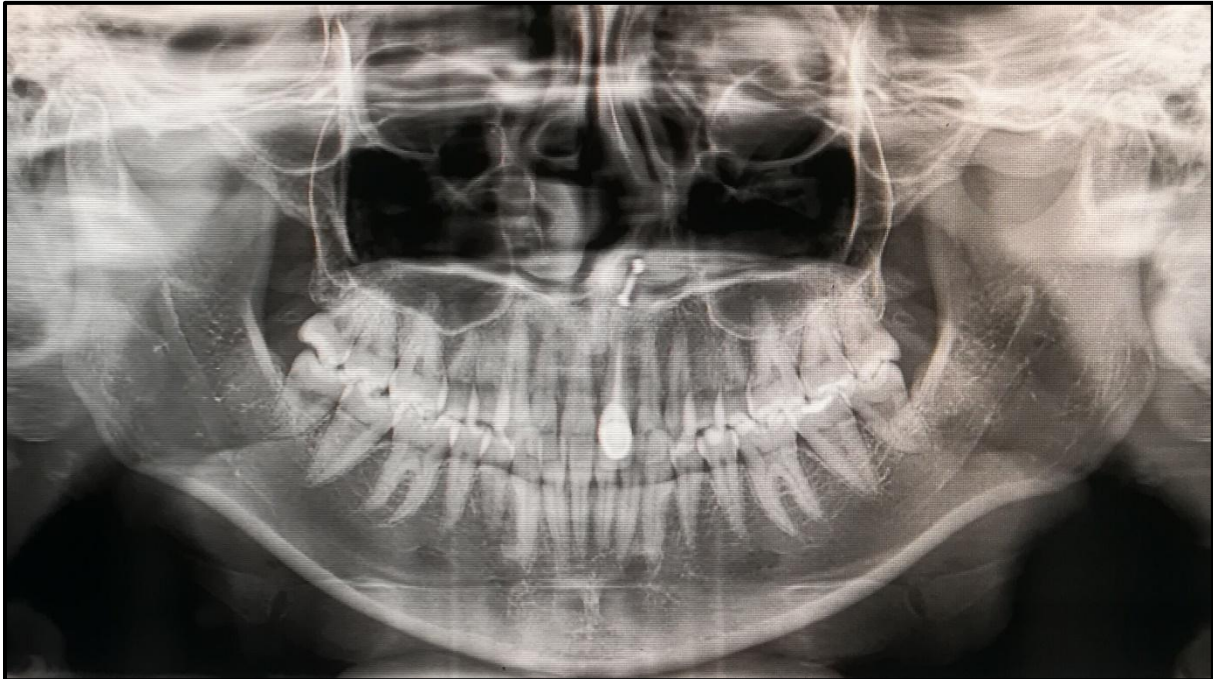


Figure 1 a): Mandibular cortical index C1

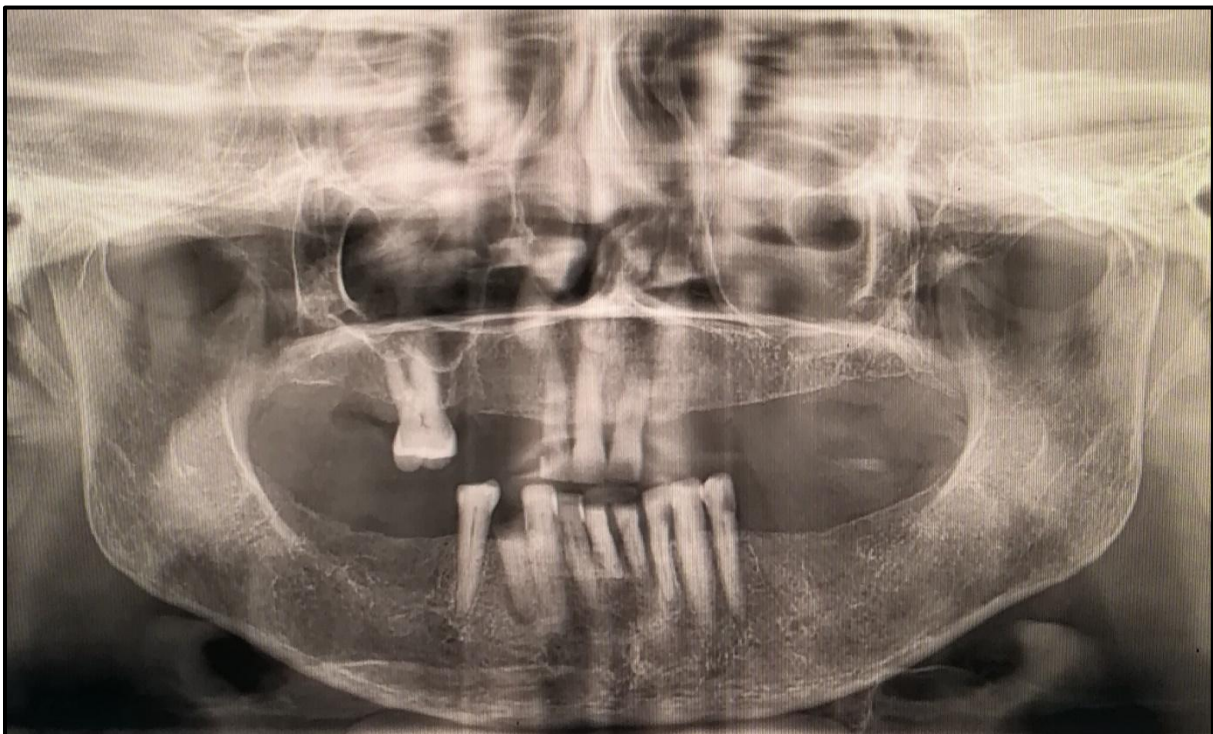


Figure 1 b): Mandibular cortical index C2



Figure 1 c): Mandibular cortical index C3