A study to evaluate the efficacy of intra oral periapical radiographs (IOPA) in the diagnosis of osteoporosis in postmenopausal women, as compared to ultrasonography of calcaneus

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Abstract
Introduction: The risk factors for the development of osteoporosis include ageing, menopause, insufficient consumption of food containing calcium, familial education and decreased physical activity. Dual-energy X-ray absorptiometry (DXA) and quantitative ultrasound are among the techniques developed to identify individuals with osteoporosis and serve as the gold standard. These methods are very expensive and hence there is a need to develop alternative methods to diagnose osteoporosis in postmenopausal women.

Objectives: 1. To evaluate digital IOPAs in post-menopausal women for changes in the trabecular pattern, to assess osteoporosis in postmenopausal women by comparing with USG-calcaneus; 2. To assess the changes in the serum calcium levels in osteoporosis in postmenopausal women by comparing with USG-calcaneus.

Materials and Methods: 100 patients were divided into 2 groups (pre and post-menopausal women.) Each patient underwent digital radiographic examination of premolar molar region of the mandible on the right side using bisecting angle technique and the density measurements i.e. grey values were measured and trabecular patterns were assessed in these radiographs. 2 ml of blood sample was taken from antecubital vein by venipuncture method and serum calcium was estimated. USG - Calcaneus Estimation was done and the speed of sound in m/sec for every patient was also noted.

Results: It was found that 46% of patients in the postmenopausal group had a sparse trabecular pattern, while only 2% in the premenopausal group showed similar findings. Significantly higher numbers of patients with sparse trabeculation were observed in the postmenopausal group (p < 0.001). Similarly, significantly higher numbers of patients with dense trabeculation were seen in the premenopausal group (p-value 0.0009), (54%). No significant differences in the mean levels of serum calcium were observed between the postmenopausal and premenopausal groups.

Conclusion: Intra oral periapical radiographs (IOPA) seem to be a promising tool in screening post-menopausal women for osteoporosis. The indices used in the study are readily reproducible and easy to learn.

Keywords: Osteopenic, Trabeculation, Postmenopausal.

Introduction
People everywhere are living longer. According to the “World Health Statistics 2014” published by WHO, the average global life expectancy has prolonged by 6 years globally.¹ Aging is associated with a variety of health issues. Osteoporosis (OP) is one of such chronic diseases, and is common in a significant part of the elderly population. Osteoporosis is defined as a systemic disease of the bone that is characterized by reduced bone mass and disrupted bone tissue microstructure, which leads to increased bone fragility and fracture risk. The risk factors for the development of osteoporosis include ageing, menopause, insufficient consumption of food containing calcium, familial education and decreased physical activity.²

In most women, bone mass reaches its peak in the third decade of life (20–30 years of age) and declines thereafter. This decline in bone mass is accelerated with the onset of menopause, and oral symptoms are also found in addition to the systemic manifestations of menopause.³ The hormonal changes that accompany menopause are the most important cause of decreased bone mass in women.⁴

The risk of fracture due to osteoporosis increases exponentially after menopause, manifesting itself in wrist fractures after the age of 50, vertebral fractures after the age of 60 and hip fractures after the age of 70.⁵ Osteoporotic fractures are associated with substantial morbidity rates, increased medical cost and high mortality risk in the elderly.⁶

Osteoporosis has been shown to be associated with a reduction in the bone mineral density. Bone mineral density (BMD) normally refers to the amount of mineral matter per square centimeter of bones. It is used in clinical medicine as an indirect indicator of osteoporosis and fracture risk.

A number of methods have been used for the determination of bone mineral density. Single- or dual-photon absorptiometry, quantitative CT (QCT), single- or dual-energy X-ray absorptiometry (DXA) and quantitative ultrasound are among the techniques developed to identify individuals with osteoporosis. DXA is used extensively around the world as it has a high reliability in identifying individuals with low BMD.² However, due to the expensive nature of this investigation, it cannot be routinely advised. Therefore, there was need for alternative methods that can be used on a large scale to monitor the skeletal status and to detect early signs of osteoporosis. Recently, Quantitative ultrasound (QUS) methods have been introduced for the assessment of the skeletal status in osteoporosis. Thus, with the advent of ultrasound, it is now possible to measure bone density with a small, portable ultrasound unit designed exclusively for bone density testing.
Like other bones in the body, the jawbones can also be affected by osteoporosis. Studies performed during the last decades indicated a link between changes in the mandibular cortical bone and the general BMD of the skeleton.\(^7\) Intraoral radiographs are common diagnostic tools in dentistry today. Many authors have studied the trabecular pattern on periapical radiographs and have found it to be a quick and simple indicator of skeletal BMD. The evaluation of the trabecular network of the alveolar bone with the classification proposed by Lindh classifies patients with highest and lowest skeletal BMD, where dense trabeculation is suggestive of high skeletal BMD whereas sparse trabeculation indicates osteopenia.\(^8\) With the advent of digital X-ray equipment, digital images can be analysed on the computer, in a simple and objective method, e.g. for measurements of pixel intensity (PI). A positive significant correlation has been found between a low PI value of intraoral radiographs and OP in women, however not many studies have been done to substantiate this.\(^9\) Thus, because dental radiographs are relatively inexpensive and are routinely advised as part of treatment protocol, they represent an enormous potential as a screening tool for osteoporosis.

Menopause is associated with an increase in serum total calcium, a change thought to result from increased bone resorption. But studies have reported that serum total calcium declines with age within the pre and post-menopausal groups, thus establishing that menopause and age affect serum total but not ionized calcium.\(^10,11\) These findings may have future implications for research regarding the use of serum calcium levels as a risk indicator for skeletal osteoporosis in menopausal women.

Thus, the present study was carried out to check the osteoporotic changes in jaw bones as seen on RVG and correlate them with the skeletal status as assessed by USG-calcaneeus. By recognizing the disease in its early phase and referring the patient to a specialist, the dentist could help the patient greatly to avoid potential complications and to achieve a better quality of life.

Materials and Method
A hospital based cross sectional study was carried out in Department of Oral Medicine and Radiology. A total number of 100 patients were selected. Patients were appraised of the purpose of the study and written consent was taken prior to commencement of the study.

Patients were distributed in 2 groups which were:
- Group I consisting of healthy premenopausal women and
- Group II comprised of healthy post-menopausal women.

Healthy female subjects that have attained menopause, (12 months of amenorrhoea) and healthy female subjects with regular menstrual cycle were included in this study.

Patients who were completely edentulous, those taking estrogen, corticosteroids, or any other hormone replacement therapy, those on drugs that may alter calcium levels, those with history of any mandibular surgery and those with history of any underlying systemic condition, which may cause changes in the bone density were excluded from the study.

Clinical Examination
Thorough history and detailed intraoral examination of the patients was done. Detailed menstrual history was also recorded. Patients were asked regarding history of drugs that alter calcium levels or hormone replacement therapy and all the data was filled up in a case history proforma.

Intraoral Radiographic Examination
Each patient underwent digital radiographic examination of premolar molar region of the mandible on the right side using bisecting angle technique, with the help of Digora (Optime) RVG software and size 2 wireless phosphor imaging plates (Sensors) for image capturing and image processing. All intraoral radiographs were taken with exposure parameters of 64-70 kVp, 6-8 mA and 0.16 second exposure for digital sensors. (Fig. 1)

Exposed sensors were processed in Soredex Digora Optime processor unit to obtain the radiographic image on the screen. A region of interest was selected on these radiographs between the second premolar and first molar, 6mm below the CEJ of the molar. Density measurements i.e. grey values of the regions of interest was done with the help of Digora for Windows software. (Fig. 2) The software provides minimum, maximum and mean values of the grey scale in the selected region. The radiographs were also assessed for the trabecular pattern and classified into three types as follows, based on the classification by Lindh et al (Fig. 3):
- A. Sparse trabeculation
- B. Alternating dense and sparse trabeculation
- C. Dense trabeculation

Serum Calcium Estimation
2 ml of blood sample was taken from antecubital vein by venipuncture method and serum calcium was estimated.

USG - Calcaneeus Estimation
Patients were classified in any one of the three groups based on their T-scores as follows:
- T-score upto -1: Normal
- T-score between -1.1 and -2.5 : Osteopenia
- T-score below -2.5: Osteoporosis

The speed of sound in m/sec for every patient was also noted.

Results
In Pre-menopausal group, maximum i.e. 30 (60%) patients were in the age group of 20-30 years, followed by 12 (24%) in the age range of 31-40 years. In Post-menopausal group, maximum i.e. 30 (60%) patients were in the age group of 51-60 years, followed by 15 (30%) in the age range of 41-50 years.

In Pre-menopausal group, 34 (68%) patients were normal, while 16 (32%) patients had Osteopenia / Osteoporosis. In Post-menopausal group, 7 (14%) patients were normal, while 43 (86%) patients had Osteopenia
Osteoporosis. Chi-square test suggested a strong association in USG class as indicated by p-value < 0.0001. The mean USG (speed of sound) of patients in Premenopausal group was 1517.74 ± 36.09, while in Postmenopausal group, the mean USG (speed of sound) was 1485.32 ± 34.51. The difference in two means was statistically significant with p-value of < 0.0001 (p < 0.05) as obtained using t-test for independent samples. (Fig. 1.)

Table 1. Distribution of patients according to Trabeculation as assessed on RVG, in both groups

| Category | Premenopausal | Postmenopausal |
|----------|---------------|---------------|
| A        | 22 (44%)      | 15 (38.5%)    |
| B        | 23 (46%)      | 10 (20%)      |
| C        | 10 (20%)      | 27 (54%)      |

Table 2. Distribution of patients according to USG class and Trabeculation as assessed on RVG

In category A, there were 20 (83.3%) cases of Osteopenia / Osteoporosis, while 4 (16.7%) normal cases. In category B, 24 (61.5%) cases belong to either Osteopenia or Osteoporosis, while 15 (38.5%) were normal. In C category, 22 (59.4%) were normal, while 15 (40.6%) belonged to either Osteopenia or Osteoporosis.

Table 3. Descriptive statistics for grey value according to groups as measured on RVG

The mean grey value of patients in pre-menopausal group was 88.72, while in post-menopausal group, the mean grey value was 88.72. The difference in two means was statistically significant with p-value of 0.0105 (p < 0.05) as obtained using t-test for independent samples.

Table 4. Descriptive statistics of grey value as measured on RVG according to USG class in two study groups

In Normal class, the difference in the mean grey value between pre- (95.80 ± 10.97) and post-menopausal (89.28 ± 8.04) groups was statistically insignificant with p-value of 0.0949 (p > 0.05). In Osteopenia class, the difference in means of pre- (89.22 ± 9.48) and post-menopausal (88.93 ± 9.29) groups was also insignificant with p-value of 0.7624 (p > 0.05). The analysis of mean grey values was also performed across USG classes. The overall mean serum calcium across classes differed insignificantly as revealed by p-value of 0.996 (p > 0.05). The paired comparison using Tukey’s test suggested that the difference of mean serum calcium levels between normal and osteopenia group was insignificant with p-value of 0.996 (p > 0.05), while that of normal and osteoporosis was insignificant with p-value of 0.1036 (p > 0.05). In the pre-menopausal group, the difference of mean serum calcium between normal and osteopenia classes was insignificant (p=0.2011). In the post-menopausal group, the difference in the mean serum calcium levels across classes was insignificant with p-value of 0.349 (p > 0.05). Also, both the paired comparisons showed insignificant difference of mean serum calcium levels in this patient group.

Table 5. Descriptive statistics for grey value according to Trabeculation and groups

For Trabeculation type B, the difference in the mean grey value in Pre-menopausal group (92.23 ± 10.73) was insignificantly different than that of post-menopausal group (90.32 ± 9.95) with a p-value of 0.5699 (p > 0.05). For Trabeculation type C, the mean grey value in premenopausal group (96.42 ± 9.30) was significantly higher than the postmenopausal group (87.77 ± 6.14) with a p-value of 0.0031, using t-test for independent samples.

Table 6. Descriptive statistics of serum calcium according to USG class in two groups

In Normal class, the difference in the mean serum calcium between pre- (9.00 ± 0.78) and post-menopausal (8.63 ± 0.87) groups was statistically insignificant with p-value of 0.3223 (p > 0.05). In Osteopenia class, the difference in means of pre- (8.72 ± 0.67) and post-menopause (9.06 ± 0.64) groups was also insignificant with p-value of 0.1036 (p > 0.05). The analysis of mean serum calcium was also performed across USG classes. The overall mean serum calcium across classes differed insignificantly as revealed by p-value of 0.996 (p > 0.05). The paired comparison using Tukey’s test suggested that the difference of mean serum calcium levels between normal and osteopenia group was insignificant with p-value of 0.996 (p > 0.05), while that of normal and osteoporosis was insignificant with p-value of 0.999 (p > 0.05). In the pre-menopausal group, the difference of mean serum calcium between normal and osteopenia classes was insignificant (p=0.2011). In the post-menopausal group, the difference in the mean serum calcium levels across classes was insignificant with p-value of 0.349 (p > 0.05). Also, both the paired comparisons showed insignificant difference of mean serum calcium levels in this patient group.

Fig. 1: Bar chart showing the mean USG speed of sound for two group
Table 1: Distribution of patients according to Trabeculation in both groups

| Trabeculation | Pre-menopausal | Post-menopausal | P-value* |
|---------------|----------------|-----------------|----------|
|               | Number  | %    | Number  | %    |        |
| A             | 1      | 2    | 23      | 46   | < 0.001|
| B             | 22     | 44   | 17      | 34   | 0.4122 |
| C             | 27     | 54   | 10      | 20   | 0.0009 |
| Total         | 50     | 100  | 50      | 100  |        |

*Obtained using z-test of proportion for two samples
A- Sparse trabeculation; B- Alternating sparse and dense trabeculation; C- Dense trabeculation

Table 2: Distribution of patients according to USG class and Trabeculation as measured on RVG

| USG class               | Trabeculation | P - value* |
|-------------------------|---------------|------------|
|                         | A             | B          | C          |
| Normal                  | 4 (16.7)      | 15 (38.5)  | 22 (59.4)  | 0.0037   |
| Osteopenia/Osteoporosis | 20 (83.3)     | 24 (61.5)  | 15 (40.6)  |           |
| Total                   | 24            | 39         | 37         |           |

*Obtained using Chi-square test
A- Sparse trabeculation; B- Alternating sparse and dense trabeculation; C- Dense trabeculation

Table 3: Descriptive statistics for grey value according to groups as measured on RVG

| Grey value | Pre-menopausal (n=50) | Post-menopausal (n=50) | P-value* |
|------------|------------------------|------------------------|----------|
| Mean       | 93.89                  | 88.72                  | 0.0105   |
| SD         | 10.97                  | 8.69                   |          |
| Range      | 62.27 - 119.39         | 71.81 - 114.57         |          |

*Obtained using t-test for independent samples

Table 4: Descriptive statistics of grey value as measured on RVG according to USG class in two study groups

| USG class | Overall | Pre-menopausal | Post-menopausal | P - value* |
|-----------|---------|----------------|-----------------|------------|
|           | N       | Mean  | SD    | N  | Mean  | SD    | N  | Mean  | SD    |          |
| Normal    | 41      | 94.69 | 10.73 | 34 | 95.80 | 10.97 | 7  | 89.28 | 8.04   | 0.0949   |
| Osteopenia| 50      | 89.22 | 9.48  | 16 | 89.84 | 10.15 | 34 | 88.93 | 9.29   | 0.7624   |
| Osteoporosis| 9     | 87.52 | 7.51  | 0  | 0     | 0     | 9  | 87.52 | 7.51   | -        |
| F-value* (P-value) | 4.179 (0.0182) | -       | 0.107 (0.899) |          |
| Normal vs. Osteopenia** | P = 0.027 | P = 0.0686 | P = 0.995 |          |
| Normal vs. Osteoporosis** | P = 0.124 | -       | P = 0.917 |          |

*Obtained using one-way ANOVA in Overall and Post-menopausal groups; **Using Tukey’s post-hoc test in Overall and Post-menopause group; †Using t-test for independent samples

Table 5: Descriptive statistics for grey value according to Trabeculation and groups

| Trabeculation | Overall | Pre-menopause | Post-menopause | P - value* |
|---------------|---------|---------------|---------------|------------|
|               | N       | Mean  | SD    | N  | Mean  | SD    | N  | Mean  | SD    |          |
| A             | 24      | 86.89 | 10.10 | 1  | 62.27 | -     | 23 | 87.96 | 8.82   | -        |
| B             | 39      | 91.40 | 10.31 | 22 | 92.23 | 10.73 | 17 | 90.32 | 9.95   | 0.5699   |
| C             | 37      | 94.08 | 9.33  | 27 | 96.42 | 9.30  | 10 | 87.77 | 6.14   | 0.0031   |

*Obtained using t-test for independent sample
A- Sparse trabeculation; B- Alternating sparse and dense trabeculation; C- Dense trabeculation

Table 6: Descriptive statistics for serum calcium in two groups

| Serum calcium | Pre-menopausal | Post-menopausal | P-value* |
|---------------|----------------|-----------------|----------|
| Mean          | 8.91           | 8.98            | 0.6593   |
| SD            | 0.75           | 0.71            |          |
| Range         | 6.0 - 9.8      | 6.9 - 9.9       |          |

*Obtained using t-test for independent samples
Discussion

The study was done on a total of 100 patients out of which 50 were premenopausal and 50 were postmenopausal. All the patients underwent the necessary investigations and their data was statistically analyzed.

The age range of the complete sample ranged from 20–70 years. As intended, maximum number of patients in the premenopausal group were in the range of 20-30 years. The bone mass reaches the highest peak in the age range of 20-30 years and this was the reason that maximum patients included in the premenopausal group ranged between 20-30 years, so as to facilitate better comparison between the two groups.

Based on the findings of ultrasound of the calcaneus, patients were classified as either normal, osteopenic or osteoporotic. When the distribution of patients according to the USG class in both the groups was done, it was found that 68% of the patients in the premenopausal group were diagnosed as ‘normal’ by USG as compared to only 14% in the postmenopausal group and this difference was statistically significant (p < 0.0001). Similarly, 32% patients in the premenopausal group were diagnosed as being either osteopenic or osteoporotic as opposed to 86% in the postmenopausal group. This would suggest that osteoporosis is definitely more common in postmenopausal females, but a sizeable number of females i.e. about 32% in the premenopausal group were also found to be osteopenic. Therefore causes other than menopause should also be addressed whenever studies are carried out for studying osteoporosis in females.

In the present study, it was seen that the mean speed of sound (SoS) in the premenopausal group was significantly higher than that in the postmenopausal group (p < 0.0001), indicating that the speed of sound decreases with increasing age and with the onset of menopause. The speed of sound may be used to estimate BMD but cannot be compared to a T-score attained with DXA as these technologies are measuring different properties of bone (Lewiecki et al 2006). Given the many similar reports on the diagnostic efficacy of QUS of the calcaneus, it may be stated that QUS seems to be a promising tool in the screening of osteoporosis and should be considered as an adjuvant to DXA whenever studies on osteoporosis may be carried out.

Trabecular Pattern

Characteristics of the trabecular pattern have been shown to have a fairly high precision and correlate well with BMD. Hence, analysis was done to see the distribution of patients in both groups according to their trabecular pattern as assessed on RVG. The trabecular pattern was classified as follows:

A. Sparse trabeculation
B. Alternating dense and sparse trabeculation
C. Dense trabeculation

It was found that 46% of patients in the postmenopausal group had a sparse trabecular pattern, while only 2% in the premenopausal group showed similar findings. Significantly higher numbers of patients with sparse trabeculation were observed in the postmenopausal group (p < 0.001). Similarly, significantly higher numbers of patients with dense trabeculation were seen in the premenopausal group (p-value 0.0009). (54%) (Table 1).

Characteristics of trabecular pattern are clearly related to osteoporosis and have a reasonable sensitivity and specificity by themselves. Dense trabeculation is a sign of healthy bone, while sparse trabeculation is a sign of osteoporosis. On assessing the distribution of patients based on trabecular pattern and USG class, it was observed that maximum number of patients with sparse trabecular pattern (type A) were osteopenic or osteoporotic (83.3%), with alternating dense and sparse trabeculation (type B) were also osteopenic or osteoporotic (61.5%), while those with dense trabecular pattern (Type C) were normal (59.4%) (p-value 0.0037). (Table 2) These results support the findings proposed by Lindh et al and Jonasson et al. Verheij at al observed that trabecular pattern on dental radiographs can predict osteoporosis with good sensitivity and specificity. Based on these observations, it can be concluded that similar to the cortical quality and cortical width, the trabecular pattern also correlates well with the skeletal status of the patients and may give an idea about the same. These findings suggest that trabecular pattern may be able to give an idea about the skeletal status of the individual. In the present study, a single region of interest (ROI) was selected but the exact standardization of the size of the ROI was not possible in the software that was used. Digital analysis of the trabecular pattern also has been reported in studies, where semi-automated software have been developed for their assessment and decent results have been observed. These softwares evaluate factors like the mean grey value, number of white and black segments and various other geometric and topographic parameters. Digital analysis will also help to eliminate observer-based bias. Further research may find image features of trabecular pattern that increase its predictive value. The trabecular pattern on dental radiographs may be used on a routine basis, requiring only an assistant to define a region of interest and a common office computer for analysis.

Grey Value

Grey value of digital radiographs can be used as a parameter in the screening of osteoporosis. The mean grey value can be interpreted as a rough measure of the bone density because denser objects give brighter projections. When mean grey-scale value was assessed on RVG, it was observed to be significantly higher in premenopausal group (mean grey value 93.89) as compared to postmenopausal group (mean grey value 88.72) (p-value 0.0105). This means that the ‘bone density’ i.e. the grey value decreases with advancing age and after menopause. (Table 3)

Significant differences in the grey values were observed in normal (mean grey-value 94.6) and osteopenic (mean grey-value 89.2) individuals on overall analysis (p-value 0.027), but when analysis was done within the groups, no significant results were found. It was also seen that there was considerable difference in the mean grey value of

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normal (mean grey value 94.6) and osteoporotic (mean grey value 87.5) patients, but on statistical analysis, this difference was found to be insignificant. This insignificant p-value obtained on comparison of normal and osteoporotic groups was due to the huge discrepancy between the sample sizes in both the groups (41 in the normal group and 9 in the osteoporotic group). (Table 4) Khojastehpour et al reported significant differences in the densities of the regions of interest between normal and osteoporotic women. Similarly, Geraets et al found that combining the upper and lower intraoral radiographs for evaluation of grey values may prove to be effective in prediction of low BMD. Based on these findings, it may be concluded that reduction in the mean grey value is a feature associated with reduced BMD but this alone cannot be used as a parameter to classify patients as normal or osteoporotic.

It was also attempted to correlate the grey values with the trabecular pattern, but no significant results were found. (Table 5) This means that grey value analysis on intraoral radiographs alone cannot be used for the screening of osteoporosis, but a combination of other investigations may prove useful.

**Serum Calcium**

Previous studies have reported a change in the serum calcium levels, either an increase or a decrease in the levels of total serum calcium after menopause. In the present study, no significant differences in the mean levels of serum calcium were observed between the postmenopausal and premenopausal groups. Similarly, on correlating the serum calcium levels with the USG class, no significant findings were observed. Based on the findings of the present study, it may be concluded that menopause does not affect the levels of serum calcium. Only the levels of total calcium were assessed. Hence, no comment regarding the levels of ionized serum calcium can be made. Other biochemical parameters need to be assessed so as to correlate the radiographic findings with the biochemical status of the patients. Khoatoomabad et al reported statistically insignificant differences in the levels of serum calcium in normal and osteopenic/osteoporotic postmenopausal women. These findings support the results of the present study.

**Conclusion**

1. The trabecular pattern shows changes in osteoporosis, where it was seen that sparse trabeculation was predominantly found in postmenopausal women as compared to their premenopausal counterparts and these changes also correlate well with the skeletal status of the patients. Thus, it may be said that digital intraoral radiographs should be assessed for the trabecular pattern, when looking for osteoporotic changes.

2. The mean grey value in premenopausal women was significantly higher than that for the postmenopausal women which supports the fact that density of the bone reduces after menopause.

3. No changes in the value of serum calcium were observed in postmenopausal women. So, total serum calcium levels do not seem to play a role in postmenopausal osteoporosis.

To our knowledge, this study is the first of its kind in which correlation of so many factors has been done. To conclude, intraoral radiographs seem to be a promising tool in screening post menopausal women for osteoporosis.

**Conflict of Interest:** None.

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