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Comparison of Serum Calcium, Phosphorus, and Calcitonin Levels Between Postmenopausal and Premenopausal Iraqi Women

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Abstract

Background:

Menopause is associated with hormonal changes that may affect mineral metabolism and bone health. In Iraq, data on serum calcium, phosphorus, and calcitonin patterns in postmenopausal women compared with premenopausal women are still limited.

Objectives:

To compare serum calcium, phosphorus, and calcitonin levels between postmenopausal and premenopausal Iraqi women, and to evaluate the ability of these markers to discriminate postmenopausal women who may be at increased risk of osteoporosis.

Methods:

The study involved participants from Al Diwaniyah, Iraq: 120 participants total (32 postmenopausal women; 102 male participants). Samples were collected to measure total serum calcium, inorganic phosphorus and calcitonin levels using standard lab techniques. Comparisons between the groups were made using independent samples t-test and ROC curve analysis to evaluate how well calcium, phosphorus, and calcitonin levels can tell apart cases (premenopausal women) and controls (postmenopausal women)..

Results:

Compared to premenopausal controls, postmenopausal patients have significantly lower average phosphorus and calcium levels (with p values of < 0.001 and 0.0024, respectively). The calcium ROC analysis yielded a modest AUC of 0.67 with a cut-off of < 8.55 mg/dL (sensitivity 78%, specificity 52%). Conversely, the phosphorus ROC analysis yielded an AUC of 0.69 with a cut-off of < 3.85 mg/dL (sensitivity 74%, specificity 58%). In contrast, calcitonin showed a high level of discrimination with an AUC of 0.88 and a cut-off of ≤ 27.84 pg/mL (sensitivity 95%, specificity 82%.(

Conclusion:

Post-menopausal Iraqi female patients indicated lower serum calcium and phosphorus levels, with lower

serum calcitonin levels compared to pre-menopausal females. Calcium and phosphorus alone were not accurate in identifying those patients who are good candidates for screening; however, calcitonin demonstrated superior discrimination. Together, all three markers demonstrated an overall lower accuracy when interpreted alone; however, when considered collectively, the use of these biomarkers may assist clinicians in determining osteoporosis risk status.

Keywords: postmenopausal women, Premenopausal women, Serum calcium, Serum phosphorus, Calcitonin.

Introduction

Women experience menopause as a natural progression through which the female reproductive cycle ends and is marked by the complete stop of menstruation due to the failure of the ovaries to follow through with egg production (Menopause – The Endocrine Connection, n.d.). As estrogen declines, the body has numerous effects that will occur because of the absence of the hormone. There is a decrease in women's bone mass as a result of accelerated bone turnover and the disruption of the ratio between the process of building bones and breaking down bones (Cheng et al., 2022). As women transitional through menopause, the progressive loss of bone mass will place them at an increasing risk of developing osteopenia or osteoporosis after they have gone through menopause (Cheng et al., 2022; Rani et al., 2023). As women age, they are experiencing longer periods of time spent in the state after menopause, which increases the likelihood that they will experience a brittle bone condition. In Iraq, this increase in brittle bone condition is becoming an immediately important public health issue (Ilesanmi-Oyelere et al., 2019 Jassim & Mohammed Jawad, 2023 Liang et al., 2025.(

Calcium and phosphorus are essential minerals necessary for bones' makeup and normal functions of the body's systems (Shaker & Deftos 2023). Calcium makes up most of the composition of mineralized bone and plays important roles in things like brain function and muscle movement, coagulation (creating the mixture that helps your blood clot), and sending messages between cells (Calcium - Health Professional Fact Sheet n.d.). Phosphorus also exists mainly as the phosphate ion, and it is the primary molecule that makes up (hydroxyapatite) crystals, which give strength and rigidity to bone (Michigami & Ozono 2019). These two minerals have a tightly controlled homeostasis via several different hormone actions (parathyroid hormone, vitamin D and calcitonin) and through renal handling and intestinal absorption (Shaker & Deftos 2023.(

Calcitonin is a peptide hormone that is secreted from the parafollicular cells of the thyroid gland, and it plays an important role in helping to maintain normal levels of calcium and phosphorus in the body (McLaughlin et al. 2023). Calcitonin works by inhibiting osteoclastic resorption from bone tissue, thereby decreasing the amount of calcium and phosphate released from bones into circulation (Hsiao et al. 2020). Calcitonin also increases the amount of calcium and phosphate excreted through the kidneys, opposing the actions of parathyroid hormone (McLaughlin et al. 2023). Calcitonin secretion and or activity around menopause may have an impact on the turnover of minerals and lead to changes in bone remodeling dynamics and bone strength (Hsiao et al. 2020). Thus, including calcitonin as a variable in studies regarding bone metabolism may provide an enhanced understanding of how hormones function to regulate bones metabolism and postmenopausal bone health (Shaker & Deftos 2023.(

The decline in estrogen levels following menopause is linked to changes in the body's ability to regulate calcium and phosphorus levels through multiple mechanisms (Rasul et al. 2025). Decreased estrogen levels may result in the mobilization of calcium and phosphorus from the bone into the blood stream through increased bone resorption (Cheng et al. 2022). Changes in parathyroid hormone levels and alterations in the metabolism of vitamin D after menopause may also affect intestinal absorption, renal excretion and skeletal mobilization of calcium and phosphorus (Bhattarai et al. 2020). As a result of these changes, measurements of serum calcium and phosphorus may differ between postmenopausal and premenopausal women even though these measurements are within the normal reference range (Al-Hamdani 2010). Understanding these changes will allow for the identification of women who may be at risk for significant bone loss (Shaikhomar et al. 2022; Ying et al. 2022.(

When assessing serum calcium and phosphorus levels, post-menopausal women can provide insights into their mineral metabolism, as well as help the clinician assess their overall bone health when used along with a patient's clinical history/assessment and other biochemical or densitometric tests (Panchagnula & Amarnath, 2023). Routine, cheap laboratory tests may be most useful when used within a local healthcare environment, particularly when there is limited access to special imaging techniques or more advanced assays (Lewiecki, 2024; Steiner et al., 2023). Recognizing the difference in mineral profile of post-menopausal versus pre-menopausal women, even if it is slight, can help provide some guidance for preventive services and assist in the establishment of general clinical decisions in both primary care and outpatient settings (Sabri et al., 2022; Thériault et al., 2023.(

Few published studies exist addressing the serum calcium and phosphorus patterns of postmenopausal women in Iraq compared to premenopausal women (Hamdani, 2010; Al-Khakani et al., 2018). Most of the available data that have been reported originate from studies conducted outside of Iraq and do not provide enough information regarding the effects of the genetic, dietary, environmental, and lifestyle characteristics local to Iraq (Gheita & Hammam, 2018). Local evidence is needed on mineral metabolism to better understand how to develop contextually relevant preventive and therapeutic strategies related to bone disorders in Iraqi women (Gheita & Hammam, 2018; Makarem et al., 2025.(

On this basis, the aim of the present study is to compare serum calcium, phosphorus and calcitonin levels between postmenopausal and premenopausal Iraqi women.

Methods

Study Design and Setting

This research was commissioned by the Association of American Medical Colleges (AAMC) and conducted by the National Academy of Sciences (NASEM). The project's goals are to address common issues affecting post-menopausal women such as poor mental and physical health and screen for risk factors and symptoms of postmenopausal disease. The study includes two groups of females: one group comprised of post-menopause (aged 47-79 years), and the second group has pre-menopause (aged 21-45 years). Women in the post-menopause group were recruited from a number of facilities throughout the Al Diwaniyah province of Iraq; specifically, Women & Children's Hospital, 3rd Al-Sada Health Centre, and the Street Doctor's Clinic. Women in both groups must be residents of the Al-Diyaniyah province of Iraq and give informed consent in order to participate in the study. All females must have been diagnosed with

post-menopausal conditions by a doctor and agreed to provide urine samples at no cost. A total of 120 participants will be recruited into the study. collection.

Participants

All the participants of this study included two groups of females, 60 of them were postmenopausal (the patient group) and 60 of them were premenopausal (the control group). The two groups were reported with respect to their demographic characteristics on a mean ± standard deviation basis. The postmenopausal women had a mean age of 54.79 years ± 6.03 years versus 29.27 years ± 6.89 years for the females in the control group. For body mass index (BMI) values, the postmenopausal women had a mean BMI of 26.56 kg/m² ± 2.67 kg/m² versus 25.48 kg/m² ± 2.56 kg/m² for the premenopausal women in the control group. All the participants in the study were Iraqi females from the Al Diwaniyah Governorate and met the subject's inclusion criteria. All participants were excluded from the study if the subjects were currently using any hormonal replacement therapy, had any chronic systemic disease, or had any condition known to alter bone metabolism.

Sample Collection and Handling

Blood samples (3 mL) from all subjects (both from patients and controls) were taken via standard venipuncture after overnight fasting. The remaining 3 mL were drawn into gel separator tubes, allowed to clot at room temperature for approximately 15 minutes, and then centrifuged at 3000×g for 10 minutes for the separation of the serum. The resulting serum was then divided into multiple Eppendorf tubes and frozen at -80°C until they were analyzed.

Biochemical Analysis

Human calcitonin ELISA kit (Catalog No. E1002Hu, Lot no. 202511015) was used for serum calcitonin analysis. Serum total calcium and inorganic phosphorus levels were measured using the FUJI Dri Chemsdry chemistrhaanalyser (FUJIFILM Corp, Japan). The Ca P III slide was used for the determination of serum calcium and was based on the end-point colorimetric method. The IP P slide was used to determine inorganic phosphorus and was based on the colorimetric photometric method. Each assay was performed utilizing internal quality control and instrument calibration per the manufacturer's guidelines in order to produce accurate analytical results.

Statistical Analysis

GraphPad Prism (version 9) was employed for data analysis. Continuous variables were presented as mean ± standard deviation (SD). The Shapiro–Wilk test assessed whether they were normally distributed. The independent samples t test compared the two groups. The diagnostic potential of serum calcium and phosphorus was determined using receiver operating characteristic (ROC) curves, and a p value < 0.05 was based on the significance level used to determine a statistically significant difference between the two groups of subjects in our hypotheses..

Results

The summary of demographic characteristics and biochemical data shows that there is an age difference between the two groups (mean ages were found to be significantly different with a p value of less than

0.001); however, there were no differences in body mass index (BMI) between the groups (p value is equal to 0.094). With regards to biochemical indicators of bone health, both groups were different statistically for the total serum phosphorus levels (p was equal to < 0.001, with the patient group having a significantly lower total serum phosphorus level than the control) and for calcium levels, since the patient group had significantly lower serum calcium levels than the control (p value of 0.0024). Lastly, calcitonin levels were found to be greater in the postmenopausal patient group than those of premenopausal controls (p value < 0.001), and therefore it was determined that calcitonin levels are also affected by the osteoporotic condition..

Table 1. Baseline Demographic and Biochemical Parameters of Study Participants

Parameter	**Patient Group (n=60) Mean ± SD**	**Control Group (n=60) Mean ± SD**	**P-value**
Age (years)	54.79 ± 6.03	29.27 ± 6.89	<0.001***
BMI (kg/m²)	26.56 ± 2.67	25.48 ± 2.56	0.094 (NS)
Phosphorus (mg/dL)	3.87 ± 0.46	4.19 ± 0.48	<0.001***
Calcium (mg/dL)	8.61 ± 0.70	8.97 ± 0.55	0.0024**
Calcitonin (pg/mL)	58.96 ± 38.1	17.78 ± 6.96	<0.001***

*Independent Samples t-test; **: significant at p <0.01; **: significant at p <0.001; NS: not significant.

ROC Curve Analysis for Serum Calcium and Phosphorus and Calcitonin

Calcium: Cut-off of < 8.55 mg/dL; Performance: Sensitivity = 78%, Specificity = 52%; AUC = 0.67 (p = 0.006, poor but statistically valid discrimination). Phosphorus: Cut-off of < 3.85 mg/dL; Performance: Sensitivity = 74%, Specificity = 58%; AUC = 0.69 (p = 0.003, also poor overall diagnostic accuracy although with acceptable levels of sensitivity.(

As a result, both mineral markers have limited potential as independent screening markers but do exhibit statistically significant AUC values that suggest some degree of diagnostic capability that could be improved when evaluated in conjunction with other osteoporosis markers .

When using serum calcitonin, the cut-point of ≤27.84 pg/mL provides excellent sensitivity (95%) and specificity (82%). The AUC (Area Under the Curve) of the ROC analysis for serum calcitonin is 0.88 (95% CI: 0.82 − 0.96, p < 0.001), indicating that it has an excellent ability to differentiate postmenopausal osteoporotic patients from premenopausal controls..

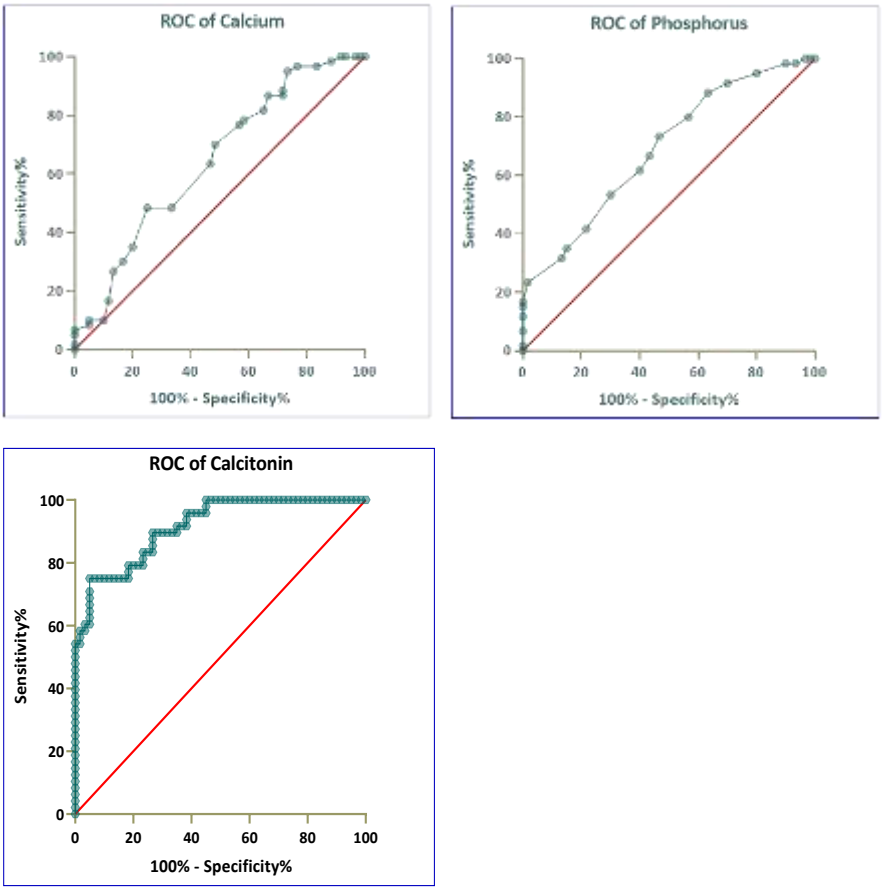

Figure 1: The ROC curve for Phosphorus and Calcium

Discussion

This study compared levels of serum calcium and phosphorus between postmenopausal women who have osteoporosis and healthy premenopausal women from Al-Diwaniyah, as well as the possible use of these simple minerals as possible screening markers. The results indicated that both calcium and phosphorus levels were significantly lower in postmenopausal women than in controls. The ROC analysis that was performed on these minerals demonstrated that there was a disturbance of minerals; however, the tests do not represent solid diagnostic tools since the discriminatory power is only moderate. In addition, serum calcitonin concentrations were significantly lower in postmenopausal women; however, the ROC analysis performed on serum calcitonin indicated that even at low concentrations, calcitonin has excellent discriminatory ability when evaluated in conjunction with conventional mineral markers.

In line with an Iraqi study by Al-Khakani et al., 2018, which found that osteoporotic women after menopause had significantly less phosphorus than controls (healthy women), the finding of reduced serum phosphorus in this population supports this conclusion, as do the results of several other regional studies indicating that postmenopausal females with diminished bone density had less phosphorus compared with age or younger matched females (Mubeen et al., 2021). In several worldwide case–control studies, phosphorus was also found to be lower in postmenopausal osteoporotic women compared to the same age nonosteoporotic counterparts; however, the magnitude of the difference between groups was not constant across studies (Saad et al., 2019). By contrast, other studies observed no major differences in phosphorus

levels when comparing osteoporotic versus non-osteoporotic women, indicating that both renal function and dietary phosphate intake as well as other comorbidities may influence phosphorus serum concentrations and sometimes mask group differences (Oliva et al., 2451). These conflicting findings indicate that phosphorus, alone, cannot be viewed as a stable diagnostic marker and that its utility as such is dependent on both clinical and nutritional context.

Our finding that postmenopausal women have much lower serum calcium levels than premenopausal women is supported by numerous research studies performed by others that found lower serum calcium levels among postmenopausal women compared to premenopausal and healthy women (Al-Khakani et al., 2018). In addition, there is substantial evidence from multicenter research on calcium levels among premenopausal and postmenopausal women that demonstrate a reduction in serum calcium in postmenopausal women and that the relationship between low serum calcium, low serum vitamin D, and low bone mineral density is especially strong in postmenopausal women with osteoporosis (Mani et al., 2024). In addition to this correlation, studies in Basrah and parts of the Middle East indicate that there is a very high prevalence of hypocalcemia and vitamin D deficiency in postmenopausal women and that the combination of hypocalcemia and vitamin D deficiency is associated with a significant reduction in bone mineral density and an increased risk of fracture (Hussein & Ibrahim, 2024). However, there are researchers who noticed that there are cases where the author mentions a normal serum calcium level but also states that bone density has been decreased significantly, stating that this can be due to the hormonal regulation of serum calcium within a normal range being tightly regulated at the cost of bone calcium storage (Fleet, 2017). The information also correlates with the conclusion that even though there is statistically significant difference between male and female athletes the actual serum calcium levels are still close to normal limit so these values cannot be used alone for diagnosis.

The results of the ROC analysis performed in this study demonstrated AUC values of 0.67 and 0.69 for calcium and phosphorus, respectively; this value indicates a lack of discrimination between levels of the assays and is generally viewed as a statistically significant degree of discrimination overall. AUC results similar to those described above have been previously published when assessing calcium, phosphorus, and their ratios as predictors of low bone mineral density or osteoporosis amongst postmenopausal women (Sarrafi et al., 2024). They also do not fully support routine diagnostics of DEXA scan by parathyroid hormone and calcium phosphorus ratio due to the limited overall analytical reliability (Hendrianingtyas et al., 2022). There was considerable agreement between our findings and those reported in both studies in relation to the use of routine biochemical markers to provide supporting information for screening and, especially in countries where healthcare resources are limited, they should not act as a definitive diagnostic indicator on their own. The AUC was higher for calcitonin than the mean level in patients and therefore indicates that adding a hormone that directly affects the inhibition of osteoclast and renal handling of calcium may enhance laboratory based screening panels of postmenopausal women (McLaughlin et al., 2023.(

Few prior studies have examined calcitonin in relation to postmenopausal osteoporosis, most of these studies were focused on either diagnostics or therapeutic use (Tan et al, 2025). In clinical studies, using SPS or recombinant calcitonin as an anti-resorptive agent resulted in decreased biochemical markers of bone metabolism and either stabilised or showed slight increased levels of BMD in postmenopausal women with low bone density (Binkley et al., 2014). These data are consistent with evidence that abnormal calcitonin levels correlate with bone metabolism abnormalities; however, this study looked at endogenous

calcitonin levels and not the use of calcitonin as a pharmacologic agent. Conversely, many studies have reported no consistent relationship between calcitonin levels and the amount of bone loss, suggesting that inter-individual variance, methodological differences, and interactions between calcitonin and other hormones involved in calcium regulation may influence calcitonin's effect on bone outcomes (Xie et al, 2020.(

Changes in hormone production following menopause, alongside metabolic changes, explain lower calcium and phosphorus levels in postmenopausal females from a physiological point of view. The lack of estrogen increases bone resorption and likely decreases intestinal absorption of calcium, eventually resulting in a negative calcium balance and a gradual decline in bone mineral content (Cheng et al., 2022). Vitamin D deficiency is also common among postmenopausal females in Iraq and other adjacent regions and reduces the absorption of both calcium and phosphorus from the intestines and promotes the development of secondary hyperparathyroidism (Almahfooth, 2024; CAPATINA et al., 2014). Chronic release of parathyroid hormone leads to increased movement of calcium and phosphorus out of the bone, changing how they are handled in the kidney, resulting in slight decreases in serum levels and dramatically lowers bone mass (Habas et al., 2021). In this study, the low levels of calcitonin found in postmenopausal patients may reflect a lack of adequate counter-regulatory mechanisms to increased osteoclastic activity and stimulation from parathyroid hormone, contributing to continued skeletal loss. Other factors also likely contribute to the observed hormonal changes: for example, in Al Diwaniyah, patients' lifestyles tend to involve very limited exposure to sunlight and very low dairy consumption, which may worsen hormonal alterations. These findings also help to explain the relatively modest yet statistically significant reductions in serum minerals between the patient and control populations.(Khisho & Alfahad, 2025).

STUDY LIMITATIONS

There are many limitations to this research study: The research was conducted in one location: Al-Diwaniyah, and therefore cannot reliably reflect on all Iraqi women since only 120 women participated; The biochemical markers were taken at only one point in time and there was no follow-up assessment via DEXA scan for clinical evaluation, thus limiting the evaluation of the long-term impact of the condition of the bone; And calcitonin was measured as one serum value only, thus eliminating the ability for dynamic testing protocol to assess changes in bone remodeling hormones, and hence identify calcitonin's exact pathophysiological and diagnostic roles as they relate to osteoporosis post-menopause..

Conclusion

According to this study of postmenopausal Iraqi women have lower levels of serum calcium and phosphorus, along with reduced levels of serum calcitonin, when compared to premenopausal women. Overall, these results suggest that there is a change in the calcium regulating hormones and overall mineral profile within this group, indicating that there may be a change in bone metabolism. Further studies at the local level need to be done to validate these findings and determine whether measuring calcitonin for routine mineral test in postmenopausal women provides additional clinical value.

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