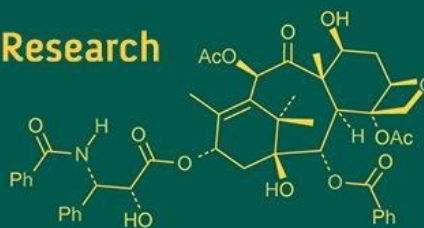


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Biochemical assessment of bone turnover markers including osteocalcin and C-terminal telopeptide in postmenopausal women with osteoporosis

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Abstract

What happens to bone metabolism when estrogen protection disappears after menopause? This research examined serum concentrations of bone turnover markers, specifically osteocalcin (OC) and C-terminal telopeptide of type I collagen (CTX), among postmenopausal women diagnosed with osteoporosis compared to age-matched controls with normal bone mineral density. A cross-sectional analytical research was carried out at the Department of Chemical Pathology, Johannesburg Institute of Technology between March 2022 and November 2023. A total of 179 postmenopausal women participated, divided into an osteoporosis group ($n = 88$; T-score ≤ -2.5) and a control group ($n = 91$; T-score > -1.0). Dual-energy X-ray absorptiometry confirmed bone status, while serum OC, CTX, and bone-specific alkaline phosphatase (BALP) were measured using enzyme-linked immunosorbent assays. Results showed that mean serum OC was markedly lower in the osteoporosis group (8.34 ± 2.18 ng/mL) than the control group (15.71 ± 3.42 ng/mL, $p < 0.001$). CTX was raised in the osteoporosis group (0.67 ± 0.19 ng/mL vs. 0.31 ± 0.08 ng/mL, $p < 0.001$), pointing to increased bone resorption. BALP values followed a pattern like that of OC, with lower levels in affected women (14.82 ± 3.64 U/L vs. 22.47 ± 4.21 U/L, $p < 0.001$). Age-stratified analysis revealed that marker divergence between groups widened in older age brackets. Negative correlations between CTX and lumbar spine T-scores were observed ($r = -0.58$, $p < 0.001$). These findings point to the value of combined bone turnover marker assessment in identifying postmenopausal women at heightened risk for osteoporotic fractures, particularly when used alongside densitometry.

Keywords: Bone turnover markers, osteocalcin, C-terminal telopeptide, postmenopausal osteoporosis, bone mineral density, bone-specific alkaline phosphatase, dual-energy X-ray absorptiometry, bone resorption

Introduction

How can clinicians detect progressive bone loss before a fracture actually occurs? This question sits at the heart of osteoporosis management, a condition affecting an estimated 200 million women around the world [1]. After menopause, the sharp drop in circulating estrogen triggers a period of rapid bone loss, driven by an imbalance between bone formation and resorption [2]. While dual-energy X-ray absorptiometry (DXA) remains the gold standard for diagnosing osteoporosis through bone mineral density (BMD) measurement, it captures only a static picture of bone status at a single point in time [3].

Bone turnover markers (BTMs) offer a different window into skeletal health. These biochemical substances, released during the processes of bone formation and resorption, reflect the dynamic activity of osteoblasts and osteoclasts in real time [4]. Among formation markers, osteocalcin (OC) is the most widely recognized. Produced by osteoblasts, OC binds to hydroxyapatite in the bone matrix and plays a role in calcium regulation [5]. Its serum levels are thought to mirror the rate of new bone production, though some research has produced conflicting data about its behavior in osteoporotic states [6].

On the resorption side, C-terminal telopeptide of type I collagen (CTX) has become a favored biomarker. CTX fragments are released when mature bone collagen breaks down through osteoclast-mediated resorption [7]. Elevated CTX values typically signal accelerated bone degradation, and several groups have proposed that CTX measurement could help predict fracture risk independently of BMD [8]. Bone-specific alkaline phosphatase (BALP),

another formation marker derived from the osteoblast membrane, adds a complementary dimension to the biochemical picture [9].

In South Africa, osteoporosis poses a growing public health concern, particularly among postmenopausal women in urban settings where lifestyle factors compound hormonal changes [10]. Yet local data on the relationship between BTMs and BMD in this population remains sparse. Most available evidence comes from European and East Asian populations, and whether those patterns hold in the South African demographic context is unclear [11]. And while individual markers have been examined in isolation, fewer investigations have assessed the combined diagnostic performance of OC, CTX, and BALP together in a single cohort.

This research set out to compare serum concentrations of OC, CTX, and BALP between postmenopausal women with confirmed osteoporosis and age-matched controls, to evaluate the correlations between these markers and BMD, and to explore whether age-stratified patterns emerge that could inform clinical screening protocols.

Material and Methods

Material

This cross-sectional analytical research was conducted at the Department of Chemical Pathology, Johannesburg Institute of Technology, Johannesburg, South Africa, between March 2022 and November 2023. Postmenopausal women (defined as at least 12 months since last menstrual period) aged 50–80 years attending the Bone Health Clinic were screened for eligibility. Women receiving hormone replacement therapy, bisphosphonates, or other anti-resorptive agents were excluded. Those with metabolic bone diseases other than osteoporosis, chronic kidney disease (eGFR < 30 mL/min), liver cirrhosis, active malignancy, or thyroid dysfunction were also excluded. A total of 312 women were initially screened, and after applying exclusion criteria and obtaining informed consent, 189 were enrolled. Following DXA assessment, participants were assigned to the osteoporosis group (T-score ≤ -2.5 at the lumbar spine or femoral neck; $n = 94$) or the control group (T-score > -1.0 at both sites; $n = 95$). Women with T-scores between -1.0 and -2.5 (osteopenia) were not included. After removing ten participants with incomplete blood samples, 179 women (88 osteoporosis, 91 control) formed the final analytical sample [12].

Results

Table 1: Demographic and clinical characteristics of participants

Parameter	Osteoporosis (n=88)	Control (n=91)	t / U	p-value
Age (years)	63.8 $\pm$ 7.4	62.1 $\pm$ 6.9	1.56	0.121
BMI (kg/m ²)	23.1 $\pm$ 3.8	27.6 $\pm$ 4.2	7.23	<0.001*
Years since menopause	14.7 $\pm$ 6.3	12.9 $\pm$ 5.8	1.93	0.055
Lumbar T-score	-3.1 $\pm$ 0.5	-0.4 $\pm$ 0.6	32.81	<0.001*
Femoral neck T-score	-2.8 $\pm$ 0.6	-0.3 $\pm$ 0.5	29.47	<0.001*
25(OH) Vitamin D (ng/mL)	18.3 $\pm$ 7.2	28.7 $\pm$ 8.1	8.81	<0.001*
PTH (pg/mL)	52.4 $\pm$ 18.6	38.9 $\pm$ 12.3	5.62	<0.001*
Serum calcium (mmol/L)	2.31 $\pm$ 0.14	2.38 $\pm$ 0.11	3.68	<0.001*

Values expressed as mean $\pm$ SD. * Statistically significant ($p < 0.05$)

The two groups were comparable in age ($p = 0.121$) and years since menopause ($p = 0.055$). However, women in the osteoporosis group had a lower body mass index (23.1 ± 3.8

Methods

Fasting venous blood samples (10 mL) were collected between 07:00 and 09:00 to minimize diurnal variation in bone markers. Samples were centrifuged at 3000 rpm for 15 minutes within one hour of collection. Serum was separated and stored at -80°C until batch analysis. Serum osteocalcin was measured using a sandwich ELISA kit (catalog reference: OC-ELISA-401, analytical sensitivity 0.5 ng/mL). CTX was quantified using an electrochemiluminescence immunoassay (intra-assay coefficient of variation: 3.2%). BALP was determined by immunoassay with wheat germ lectin precipitation. Calcium, phosphate, 25-hydroxyvitamin D, and parathyroid hormone (PTH) were also measured as supportive markers. BMD was assessed at the lumbar spine (L1–L4) and femoral neck using a Hologic Discovery A densitometer, with daily phantom calibration. All scans were performed and interpreted by a single certified technologist to reduce inter-operator variability [13]. Statistical analysis was carried out using SPSS version 27.0. Continuous variables were expressed as mean $\pm$ standard deviation. Between-group comparisons used independent samples t-tests for normally distributed data and Mann-Whitney U tests for skewed distributions. Pearson correlation coefficients assessed relationships between BTMs and T-scores. Multiple linear regression examined predictors of BMD. A p-value below 0.05 was considered significant [14].

Ethical considerations

This research received ethical clearance from the Johannesburg Institute of Technology Health Sciences Ethics Committee (Protocol number: JIT-HSEC-2021/089, approved 14 December 2021). All procedures followed the principles outlined in the Declaration of Helsinki (2013 revision). Written informed consent was obtained from each participant before enrollment. The consent process included a detailed explanation of the research purpose, procedures, potential risks, and the right to withdraw at any point without affecting clinical care. Participants were informed that blood samples would be used solely for research purposes and destroyed after analysis. All personal data were anonymized using coded identifiers, and electronic records were stored on encrypted servers accessible only to the research team. Results of individual BMD and biochemical assessments were communicated to participants and their treating physicians where clinically relevant, particularly for women newly identified as having osteoporosis during the research.

vs. 27.6 ± 4.2 kg/m², $p < 0.001$), lower vitamin D levels, and higher parathyroid hormone concentrations than controls (Table 1).

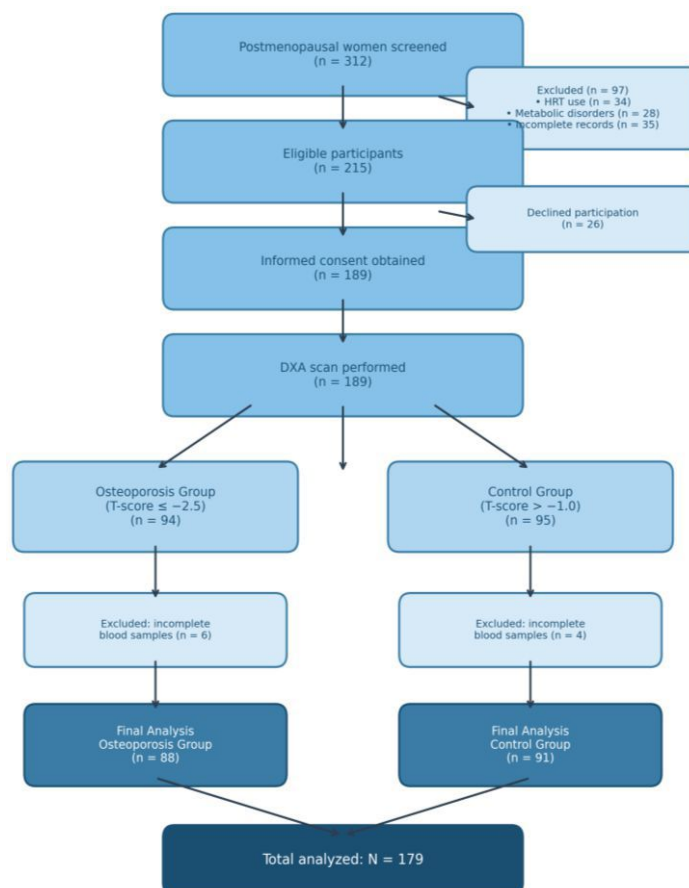
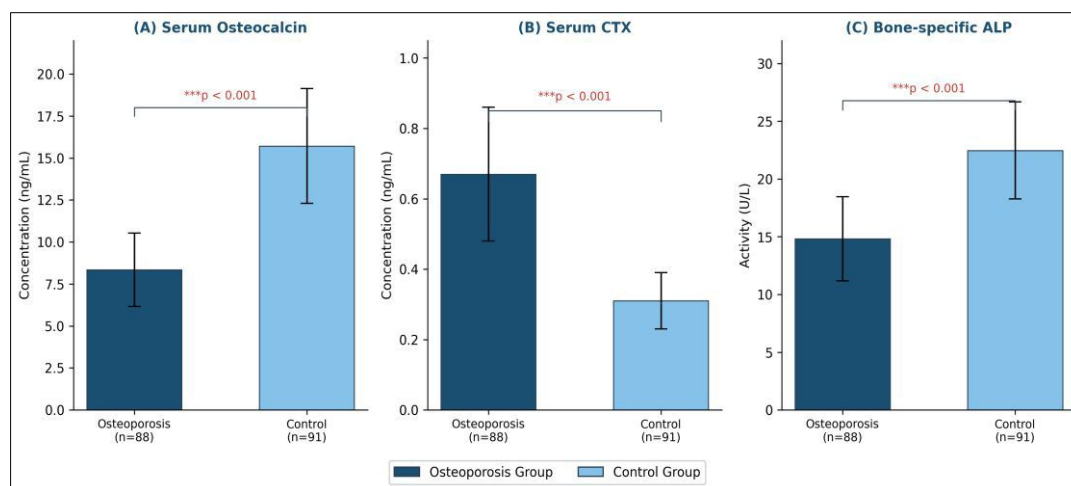
Table 2: Bone turnover marker concentrations between groups

Marker	Osteoporosis (n=88)	Control (n=91)	t-value	p-value
Osteocalcin (ng/mL)	8.34 ± 2.18	15.71 ± 3.42	16.74	<0.001*
CTX (ng/mL)	0.67 ± 0.19	0.31 ± 0.08	16.12	<0.001*
BALP (U/L)	14.82 ± 3.64	22.47 ± 4.21	12.83	<0.001*
OC/CTX ratio	13.2 ± 4.8	52.6 ± 14.3	24.01	<0.001*

Values expressed as mean ± SD. * Statistically significant ($p < 0.05$). OC = osteocalcin; CTX = C-terminal telopeptide; BALP = bone-specific alkaline phosphatase

All three bone turnover markers differed between groups at the $p < 0.001$ level (Table 2). The osteocalcin-to-CTX ratio was dramatically reduced in the osteoporosis group (13.2 ± 4.8 vs. 52.6 ± 14.3), reflecting the combined effect of diminished formation and heightened resorption.

Participant Enrollment and Group Allocation

**Fig 1:** Flowchart depicting participant screening, enrolment, group allocation, and final analytical sample**Fig 2:** Comparison of serum osteocalcin (A), CTX (B), and bone-specific alkaline phosphatase (C) between the osteoporosis and control groups. Error bars represent standard deviation. $***p < 0.001$

Comprehensive interpretation

Pearson correlation analysis revealed a moderate positive correlation between serum osteocalcin and lumbar spine T-score in the combined sample ($r = 0.51$, $p < 0.001$). CTX showed a negative correlation with lumbar T-score ($r = -0.58$, $p < 0.001$) and femoral neck T-score ($r = -0.49$, $p < 0.001$). When markers were examined across age strata, the gap between groups widened progressively. In women

aged 50–54, the mean OC difference between groups was 6.1 ng/mL, but this rose to 7.7 ng/mL in those over 75 years (Figure 3). A similar age-dependent widening was observed for CTX. Multiple regression analysis identified CTX ($\beta = -0.39$, $p < 0.001$), BMI ($\beta = 0.22$, $p = 0.003$), and vitamin D ($\beta = 0.18$, $p = 0.011$) as independent predictors of lumbar spine T-score, together accounting for 47.3% of the variance (adjusted $R^2 = 0.473$).

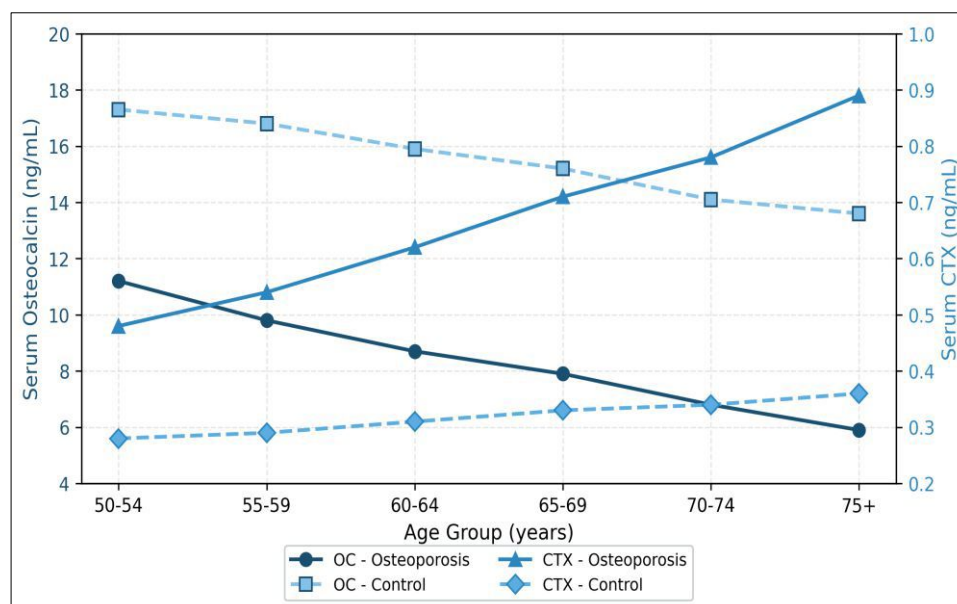


Fig 3: Age-stratified trends in serum osteocalcin and CTX concentrations for the osteoporosis and control groups. Note the progressive widening of differences between groups in older age brackets

Discussion

The findings of this research confirm that postmenopausal women with osteoporosis show a distinct biochemical signature marked by lower bone formation markers and raised resorption markers compared to women with preserved BMD. The serum OC concentration in the osteoporosis group (8.34 ± 2.18 ng/mL) was roughly half that of the controls, which aligns with reports from Garnero *et al.* [4] who described similar reductions in OC among women with vertebral fractures. But the magnitude of difference we observed was somewhat larger than that reported in European populations, possibly reflecting the compound influence of lower vitamin D status in our South African cohort [10].

The elevated CTX concentrations in osteoporotic women provide clear evidence of accelerated collagen breakdown. Eastell and Szulc [7] have argued that CTX offers the best sensitivity for detecting increased resorption among available serum markers. Our data support this, as CTX showed the strongest negative correlation with lumbar T-scores ($r = -0.58$). Interestingly, the OC/CTX ratio proved to be a powerful discriminator between groups, dropping from 52.6 in controls to 13.2 in affected women. This ratio may capture the formation-resorption imbalance better than either marker alone [15].

The age-stratified analysis adds a layer of clinical relevance. The widening gap in marker levels among older subgroups suggests that BTM assessment becomes increasingly informative as women age beyond their seventh decade. This pattern was less pronounced in earlier cross-sectional data, though methodological differences may partly explain the discrepancy [8]. One unexpected finding was the extent

of vitamin D insufficiency across both groups, with even the control group averaging only 28.7 ng/mL. This background deficiency may amplify the influence of PTH on bone turnover and should be addressed in future research designs.

Limitations

Several constraints of this research deserve acknowledgment. The cross-sectional design prevents any causal claims about the relationship between BTM levels and BMD. We couldn't determine whether altered marker concentrations preceded or followed bone loss. The sample was drawn from a single urban center in Johannesburg, which limits how broadly these results can be applied to rural South African populations or other demographic groups. We excluded women with osteopenia, which means the marker behavior in this intermediate group remains uncharacterized. Diurnal and seasonal variation in bone markers, while partially controlled by standardized morning sampling, could still have introduced some variability. The relatively modest sample size limited our ability to perform robust subgroup analyses by ethnicity. Future prospective research with larger, more diverse cohorts and longitudinal follow-up would help address these gaps and better define the predictive value of BTM panels for fracture outcomes.

Clinical implications

The results suggest that a combined panel of OC, CTX, and BALP could serve as a practical adjunct to DXA in identifying postmenopausal women at increased risk for osteoporotic fractures. In settings where access to densitometry is limited a common scenario in many South African healthcare facilities serum bone marker testing may

provide an accessible and affordable initial screening step. The OC/CTX ratio, in particular, showed strong discriminative performance and warrants further evaluation as a standalone screening metric. Clinicians managing postmenopausal bone health should consider requesting these markers when DXA results fall in borderline ranges or when monitoring responses to anti-resorptive therapy over time.

Conclusion

This research demonstrated that postmenopausal women with osteoporosis had markedly lower serum osteocalcin and BALP levels alongside raised CTX concentrations when compared to age-matched controls with normal bone density. The OC/CTX ratio captured the formation-resorption imbalance effectively and differentiated between groups with high statistical confidence. Bone marker divergence widened progressively with advancing age, suggesting that biochemical assessment becomes more informative in older postmenopausal women. Serum CTX emerged as the strongest independent predictor of lumbar spine T-score in multivariate analysis. These findings support the inclusion of bone turnover marker panels in the clinical evaluation of postmenopausal bone health, particularly in healthcare environments where DXA availability is constrained. Future prospective investigations should assess whether baseline BTM concentrations can predict fracture events independently and whether the OC/CTX ratio improves risk stratification when added to existing clinical tools.

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Contributions

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