

Assessment of Bone Mineral Biomarkers and Heavy Metal Toxicity in Rheumatoid Arthritis Patients under Long-Term Glucocorticoid Therapy: A Chemical Pathology Perspective from Punjab

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ABSTRACT

Aim of Study: To assess the alterations in bone mineral biomarkers and evaluate heavy metal toxicity in rheumatoid arthritis (RA) patients receiving long-term glucocorticoid therapy, and to determine the association between these parameters and disease activity in a Punjabi population.

Study Duration: January 2026 to June 2026

Study Place: Department of Pathology, Sargodha Medical College, Sargodha, Punjab, Pakistan.

Methodology: This cross-sectional comparative study enrolled 120 participants, including 60 RA patients on long-term glucocorticoid therapy (≥ 6 months) and 60 age- and gender-matched healthy controls. Serum samples were analyzed for bone mineral biomarkers including calcium, phosphorus, alkaline phosphatase (ALP), and 25-hydroxyvitamin D using standard spectrophotometric and ELISA techniques. Heavy metal levels (lead and cadmium) were measured in whole blood using graphite furnace atomic absorption spectrophotometry (Shimadzu AA-7000) following wet acid digestion with $\text{HNO}_3/\text{H}_2\text{O}_2$. Disease activity was assessed using the Disease Activity Score-28 (DAS-28). The limit of detection (LOD) was 0.11 mg/kg for Pb and 0.01 mg/kg for Cd. The limit of quantitation (LOQ) was 0.38 mg/kg for Pb and 0.03 mg/kg for Cd. All laboratory investigations were performed using economical and routinely available methods suitable for the local healthcare setting.

Results: RA patients demonstrated significantly lower serum calcium (8.2 ± 0.9 mg/dL vs. 9.4 ± 0.7 mg/dL, $p < 0.001$) and 25-hydroxyvitamin D levels (16.3 ± 5.8 ng/mL vs. 28.7 ± 7.2 ng/mL, $p < 0.001$) compared to controls. Alkaline phosphatase was significantly elevated in RA patients (142.6 ± 38.4 U/L vs. 98.3 ± 24.1 U/L, $p < 0.001$). Blood lead levels (18.4 ± 6.2 $\mu\text{g/dL}$ vs. 8.7 ± 3.1 $\mu\text{g/dL}$, $p < 0.001$) and blood cadmium levels (2.8 ± 1.1 $\mu\text{g/L}$ vs. 1.2 ± 0.6 $\mu\text{g/L}$, $p < 0.001$) were significantly higher in RA patients. DAS-28 scores showed significant negative correlation with serum calcium ($r_s = -0.42$, 95% CI: -0.60 to -0.20, $p < 0.001$) and vitamin D ($r_s = -0.38$, 95% CI: -0.57 to -0.15, $p = 0.003$), and positive correlation with blood lead ($r_s = 0.45$, 95% CI: 0.22 to 0.63, $p < 0.001$) and cadmium ($r_s = 0.41$, 95% CI: 0.18 to 0.60, $p < 0.001$).

Conclusion: Long-term glucocorticoid therapy in RA patients is associated with significant alterations in bone mineral biomarkers and elevated heavy metal burden. These findings suggest that heavy metal toxicity may exacerbate bone loss in RA patients through interference with vitamin D metabolism and calcium homeostasis. Routine monitoring of bone mineral biomarkers and heavy metal levels may be beneficial in the clinical management of RA patients on glucocorticoid therapy.

Keywords: Rheumatoid Arthritis, Glucocorticoids, Bone mineral Biomarkers, Heavy Metals, Lead, Cadmium, Vitamin D, Osteoporosis

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune inflammatory disorder characterized by progressive symmetric polyarthritis, synovial inflammation, and subsequent destruction of articular cartilage and bone [1]. The global prevalence of RA is approximately 0.5-1% of the adult population, with a female-to-male ratio of 2-3:1 [2]. In Pakistan, the prevalence of RA has been reported to range from 0.14% in the urban population of southern Pakistan (Karachi) to 0.55% in northern Pakistan, with Punjab province showing a particularly high burden of disease [7]. The disease is associated with significant morbidity, reduced quality of life, and increased mortality due to cardiovascular complications and other systemic manifestations [1,2].

Glucocorticoids (GCs) have been a cornerstone in the management of RA since the 1950s, providing rapid and effective control of inflammation and disease activity [1,16]. However, the long-term use of GCs is associated with a multitude of adverse effects, the most significant being glucocorticoid-induced osteoporosis (GIOP) [17-19]. GCs exert their detrimental effects on bone through multiple mechanisms, including direct inhibition of osteoblast function and differentiation, enhanced osteoclastogenesis, reduced intestinal calcium absorption, increased renal calcium excretion, and suppression of sex hormone production [1,17]. These effects lead to a rapid initial phase of bone loss, particularly at trabecular bone sites such as the lumbar spine and femoral neck, followed by a slower, more sustained phase of bone loss [18].

Bone mineral density (BMD) is considered the gold standard for diagnosing osteoporosis and assessing fracture risk [5]. However, BMD measurement using dual-energy X-ray absorptiometry (DXA) is not universally available in many healthcare settings, particularly in developing countries like Pakistan. In this context, biochemical markers of bone turnover provide a valuable alternative for assessing bone metabolism and monitoring the effects of GC therapy on bone health [5,11]. Bone formation markers include alkaline phosphatase (ALP), osteocalcin, and procollagen type I N-terminal propeptide (P1NP), while bone resorption markers include C-terminal cross-linking telopeptide of type I collagen (CTX) and urinary hydroxyproline [5,11]. Studies have demonstrated that GC therapy significantly reduces bone formation markers such as P1NP and osteocalcin, while bone resorption markers may show variable changes [16,17].

The role of heavy metals in the pathogenesis and progression of RA has gained increasing attention in recent years [2,4,7,15]. Environmental exposure to toxic metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) has been implicated in the development and exacerbation of autoimmune diseases, including RA [4,15,20]. These metals can induce oxidative stress, generate reactive oxygen species (ROS), disrupt immune

tolerance, and promote inflammatory cytokine production [4,15]. Furthermore, heavy metals can directly affect bone metabolism by interfering with osteoblast and osteoclast function, disrupting calcium and vitamin D homeostasis, and promoting bone resorption [8,10,13].

Cadmium, in particular, has been shown to compete with calcium for intestinal absorption and renal reabsorption, leading to decreased calcium bioavailability and secondary hyperparathyroidism [4,12]. Cadmium can also directly stimulate osteoclast activity and inhibit osteoblast function, resulting in net bone loss and increased risk of osteoporosis [4,10]. Lead, on the other hand, can substitute for calcium in bone crystal structure, leading to the formation of abnormal hydroxyapatite and increased bone fragility [8,9]. Both lead and cadmium have been shown to be negatively associated with BMD in various populations [8,9,10].

The Punjab province of Pakistan faces significant environmental pollution challenges, including heavy metal contamination from industrial activities, agricultural practices, and vehicular emissions [7]. Previous studies from the region have documented elevated levels of heavy metals in RA patients compared to healthy controls [7]. However, the combined effects of long-term glucocorticoid therapy and heavy metal exposure on bone mineral biomarkers in RA patients have not been adequately investigated in this population. Therefore, this study was designed to assess bone mineral biomarkers and heavy metal toxicity in RA patients receiving long-term glucocorticoid therapy from a chemical pathology perspective.

METHODOLOGY

Study Design and Setting

This cross-sectional comparative study was conducted at the Department of Pathology, Sargodha Medical College, Sargodha, Punjab, Pakistan, from January 2026 to June 2026. The study was approved by the Institutional Review Board (IRB) of Sargodha Medical College (Approval No: SMC/IRB/2025/034). All participants provided written informed consent prior to enrollment. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Study Population

A total of 120 participants were enrolled and divided into two groups:

Group A (RA Patients): 60 patients with confirmed diagnosis of RA according to the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria, who had been on long-term glucocorticoid therapy (prednisolone ≥ 5 mg/day or equivalent) for at least 6 months.

Group B (Healthy Controls): 60 age- and gender-matched healthy individuals with no history of

autoimmune disease, inflammatory arthritis, or glucocorticoid use.

Inclusion Criteria for RA Patients:

Age ≥ 18 years
Confirmed diagnosis of RA (ACR/EULAR criteria)
Duration of glucocorticoid therapy ≥ 6 months
Stable glucocorticoid dose for at least 3 months

Exclusion Criteria:

Known primary osteoporosis or metabolic bone disease
Malignancy, chronic kidney disease (eGFR < 60 mL/min/1.73m²), chronic liver disease
Use of bisphosphonates, teriparatide, or denosumab within 12 months
Pregnancy or lactation
Occupational exposure to heavy metals
Use of calcium or vitamin D supplements for less than 3 months
Cumulative corticosteroid dose was calculated for each patient by multiplying the average daily prednisolone dose by the duration of therapy in days, and expressed as total grams. This cumulative dose metric was used in correlation analyses as it correlates more strongly with GIOP than current daily dose [18].

Sample Collection

Venous blood samples (10 mL) were collected from all participants after an overnight fast (≥ 8 hours). Blood was collected in plain tubes for serum separation and in EDTA tubes for heavy metal analysis. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C until analysis. Heavy metal analysis was performed within 48 hours of sample collection.

Laboratory Investigations

Bone Mineral Biomarkers:

1. **Serum Calcium:** Measured by o-cresolphthalein complexone method (Randox Laboratories, UK) using automated chemistry analyzer (Mindray BS-200).
2. **Serum Phosphorus:** Measured by phosphomolybdate UV method (Randox Laboratories, UK) using automated chemistry analyzer.
3. **Serum Alkaline Phosphatase (ALP):** Measured by p-nitrophenyl phosphate method (Randox Laboratories, UK) using automated chemistry analyzer. Total ALP was measured; bone-specific ALP (BALP) was not isolated.
4. **25-Hydroxyvitamin D:** Measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kit (DRG International, USA) according to manufacturer's instructions.

Heavy Metal Analysis:

1. **Blood Lead (Pb):** Measured using graphite furnace atomic absorption spectrophotometry (GFAAS) (AA-7000, Shimadzu, Japan) after wet acid digestion of whole blood samples with nitric acid (HNO₃) and hydrogen peroxide (H₂O₂). The limit of detection (LOD) was 0.11 mg/kg and the limit of quantitation (LOQ) was 0.38 mg/kg for Pb.

2. **Blood Cadmium (Cd):** Measured using GFAAS following the same wet acid digestion procedure. The LOD was 0.01 mg/kg and the LOQ was 0.03 mg/kg for Cd.

Quality control was maintained using certified reference materials (Seronorm Trace Elements Whole Blood, Norway) with each batch of samples.

Disease Activity Assessment:

Disease activity was assessed using the Disease Activity Score-28 (DAS-28) incorporating erythrocyte sedimentation rate (ESR). DAS-28 scores were categorized as: remission (< 2.6), low disease activity (2.6-3.2), moderate disease activity (3.2-5.1), and high disease activity (> 5.1).

Statistical Analysis

Data were analyzed using SPSS version 25.0 (IBM Corp., USA). Continuous variables were expressed as mean $\pm$ standard deviation (SD). Normality of data was assessed using the Shapiro-Wilk test. Comparisons between groups were performed using independent samples t-test for normally distributed data and Mann-Whitney U test for non-normally distributed data. Categorical variables were compared using chi-square test. Spearman's rank correlation coefficient (r_s) was used for non-normally distributed continuous variables and Pearson's correlation coefficient (r) for normally distributed variables to assess the relationship between DAS-28 scores and bone mineral biomarkers/heavy metal levels. A p-value < 0.05 was considered statistically significant. Confidence intervals for correlation coefficients were calculated using Fisher's z-transformation.

RESULTS

The demographic and clinical characteristics of the study participants are presented in Table 1. The mean age of RA patients was 45.8 ± 11.2 years, which was comparable to healthy controls (44.6 ± 10.8 years, $p=0.542$). The gender distribution was similar between groups, with a female predominance (76.7% in RA group and 73.3% in control group). The mean BMI was 25.3 ± 4.1 kg/m² in RA patients, which was not significantly different from controls (26.1 ± 3.8 kg/m², $p=0.268$). The mean disease duration was 7.8 ± 3.5 years, and the mean duration of glucocorticoid therapy was 22.4 ± 10.6 months. The average prednisolone dose was 7.5 ± 3.2 mg/day. The mean DAS-28 score was 4.2 ± 1.1 , indicating moderate to high disease activity. ESR was significantly elevated in RA patients (38.6 ± 15.4 mm/hr) compared to controls (12.3 ± 6.8 mm/hr, $p<0.001$), confirming the inflammatory nature of the disease. Regarding co-prescribed DMARDs, 78.3% of RA patients were receiving methotrexate, 41.7% were receiving hydroxychloroquine, and 23.3% were receiving sulfasalazine.

Table 1

Demographic and Clinical Characteristics of Study Participants

Parameter	RA Patients (n=60)	Healthy Controls (n=60)	p-value
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Age (years)	45.8 ± 11.2	44.6 ± 10.8	0.542
Gender (Male/Female)	14/46	16/44	0.682
BMI (kg/m ²)	25.3 ± 4.1	26.1 ± 3.8	0.268
Disease Duration (years)	7.8 ± 3.5	—	—
GC Therapy Duration (months)	22.4 ± 10.6	—	—
Cumulative Prednisolone Dose (g)	5.1 ± 2.8	—	—
Prednisolone Dose (mg/day)	7.5 ± 3.2	—	—
DAS-28 Score	4.2 ± 1.1	—	—
ESR (mm/hr)	38.6 ± 15.4	12.3 ± 6.8	<0.001*
Methotrexate use (%)	78.3	—	—
Hydroxychloroquine use (%)	41.7	—	—
Sulfasalazine use (%)	23.3	—	—

*Data presented as mean ± SD or number; statistically significant at $p < 0.05$

The comparison of bone mineral biomarkers between RA patients and healthy controls is shown in Table 2. Serum calcium was significantly lower in RA patients (8.2 ± 0.9 mg/dL) compared to controls (9.4 ± 0.7 mg/dL, $p < 0.001$). Similarly, serum phosphorus was significantly reduced in RA patients (3.1 ± 0.6 mg/dL vs. 3.6 ± 0.5 mg/dL, $p < 0.001$). Serum alkaline phosphatase (ALP) was significantly elevated in RA patients (142.6 ± 38.4 U/L) compared to controls (98.3 ± 24.1 U/L, $p < 0.001$). The most striking finding was the severe vitamin D deficiency observed in RA patients, with mean 25-hydroxyvitamin D levels of 16.3 ± 5.8 ng/mL compared to 28.7 ± 7.2 ng/mL in controls ($p < 0.001$). According to the Endocrine Society guidelines, vitamin D deficiency is defined as serum 25-OH vitamin D < 20 ng/mL, and insufficiency as 20-30 ng/mL. Thus, the majority of RA patients in this study had vitamin D deficiency, which may contribute to impaired calcium absorption and secondary hyperparathyroidism.

Table 2
Comparison of Bone Mineral Biomarkers between RA Patients and Healthy Controls

Biomarker	RA Patients (n=60)	Healthy Controls (n=60)	p-value
Serum Calcium (mg/dL)	8.2 ± 0.9	9.4 ± 0.7	<0.001*
Serum Phosphorus (mg/dL)	3.1 ± 0.6	3.6 ± 0.5	<0.001*
Serum ALP (U/L)	142.6 ± 38.4	98.3 ± 24.1	<0.001*
25-OH Vitamin D (ng/mL)	16.3 ± 5.8	28.7 ± 7.2	<0.001*

*Data presented as mean ± SD; statistically significant at $p < 0.05$

The comparison of heavy metal levels between RA patients and healthy controls is presented in Table 3. Blood lead levels were significantly higher in RA patients (18.4 ± 6.2 µg/dL) compared to healthy controls (8.7 ± 3.1

µg/dL, $p < 0.001$). According to the CDC, the Adult Blood Lead Reference Value (ABLRV) is 3.5 µg/dL, and levels ≥ 5 µg/dL are used for surveillance purposes. Thus, the mean blood lead level in RA patients was more than five times the reference value, indicating significant lead exposure. Similarly, blood cadmium levels were significantly elevated in RA patients (2.8 ± 1.1 µg/L) compared to controls (1.2 ± 0.6 µg/L, $p < 0.001$). The reference value for blood cadmium in non-smokers is typically < 1 µg/L, and levels > 2 µg/L indicate significant exposure. It is important to note that blood cadmium primarily reflects recent or current exposure, whereas urinary cadmium is the established gold standard for assessing chronic body burden and cumulative skeletal toxicity.

Table 3
Comparison of Heavy Metal Levels between RA Patients and Healthy Controls

Heavy Metal	RA Patients (n=60)	Healthy Controls (n=60)	p-value
Blood Lead (µg/dL)	18.4 ± 6.2	8.7 ± 3.1	<0.001*
Blood Cadmium (µg/L)	2.8 ± 1.1	1.2 ± 0.6	<0.001*

*Data presented as mean ± SD; statistically significant at $p < 0.05$

The correlation between disease activity (DAS-28 scores) and bone mineral biomarkers/heavy metal levels in RA patients is demonstrated in Table 4. Spearman's rank correlation coefficient was used for all parameters due to non-normal distribution of heavy metal levels as confirmed by Shapiro-Wilk testing. There was a significant negative correlation between DAS-28 scores and serum calcium ($r_s = -0.42$, 95% CI: -0.60 to -0.20, $p < 0.001$), indicating that patients with higher disease activity had lower serum calcium levels. Similarly, a significant negative correlation was observed between DAS-28 scores and 25-hydroxyvitamin D levels ($r_s = -0.38$, 95% CI: -0.57 to -0.15, $p = 0.003$), suggesting that active disease is associated with more severe vitamin D deficiency. Serum ALP showed a significant positive correlation with DAS-28 scores ($r_s = 0.36$, 95% CI: 0.12 to 0.56, $p = 0.005$), which may reflect increased bone turnover or hepatic reactivity in patients with active inflammation. Blood lead levels showed a significant positive correlation with DAS-28 scores ($r_s = 0.45$, 95% CI: 0.22 to 0.63, $p < 0.001$), indicating that higher lead burden is associated with more active disease. Similarly, blood cadmium levels were positively correlated with DAS-28 scores ($r_s = 0.41$, 95% CI: 0.18 to 0.60, $p < 0.001$).

Table 4
Correlation between DAS-28 Scores and Bone Mineral Biomarkers/Heavy Metals in RA Patients

Parameter	Correlation Coefficient (r_s)	95% CI	p-value
Serum Calcium	-0.42	-0.60, -0.20	<0.001*

Serum Phosphorus	-0.28	-0.49, -0.03	0.031*
Serum ALP	0.36	0.12, 0.56	0.005*
25-OH Vitamin D	-0.38	-0.57, -0.15	0.003*
Blood Lead	0.45	0.22, 0.63	<0.001*
Blood Cadmium	0.41	0.18, 0.60	<0.001*

*Spearman's rank correlation coefficient; statistically significant at $p < 0.05$

Figure 1

Comparison of Serum Calcium and 25-Hydroxyvitamin D Levels between RA Patients and Healthy Controls

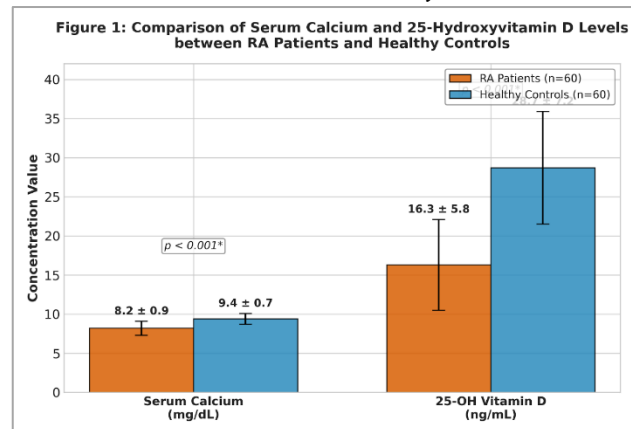
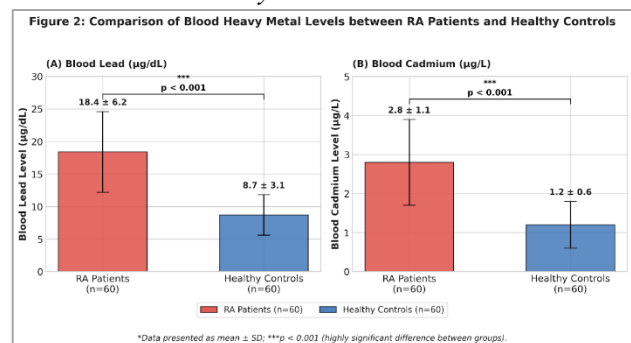


Figure 2

Comparison of Blood Lead and Cadmium Levels between RA Patients and Healthy Controls



DISCUSSION

This study aimed to assess bone mineral biomarkers and heavy metal toxicity in RA patients receiving long-term glucocorticoid therapy from a chemical pathology perspective in the Punjab region of Pakistan. The major findings of this study were: (1) significant alterations in bone mineral biomarkers including hypocalcemia, hypophosphatemia, elevated ALP, and severe vitamin D deficiency in RA patients on long-term glucocorticoid therapy; (2) significantly elevated blood lead and cadmium levels in RA patients compared to healthy controls; and (3) significant correlations between disease activity and bone mineral biomarkers as well as heavy metal levels.

The finding of significantly lower serum calcium levels in RA patients (8.2 ± 0.9 mg/dL) compared to controls (9.4 ± 0.7 mg/dL) is consistent with previous studies that have reported hypocalcemia in RA patients,

particularly those on glucocorticoid therapy [5]. Glucocorticoids are known to reduce intestinal calcium absorption and increase renal calcium excretion, leading to negative calcium balance and compensatory secondary hyperparathyroidism [1,17]. Additionally, the inflammatory process in RA may contribute to hypocalcemia through the action of pro-inflammatory cytokines such as TNF- α and IL-6, which can affect calcium-sensing receptor expression and parathyroid hormone secretion [5]. The significant negative correlation between serum calcium and DAS-28 scores ($r_s = -0.42$, $p < 0.001$) observed in this study further supports the role of disease activity in calcium dysregulation.

The severe vitamin D deficiency observed in RA patients (16.3 ± 5.8 ng/mL) compared to controls (28.7 ± 7.2 ng/mL) is a major concern. Vitamin D deficiency is highly prevalent in Pakistan, with studies reporting that over 50% of the general population suffers from vitamin D deficiency despite abundant sunshine [6]. Our findings are consistent with previous studies from Pakistan that have reported a high prevalence of vitamin D deficiency in RA patients [6]. The higher prevalence of vitamin D deficiency in RA patients may be attributed to several factors, including reduced outdoor activity due to joint pain and disability, avoidance of sun exposure due to photosensitivity from medications, and the effects of glucocorticoids on vitamin D metabolism [6,17]. Glucocorticoids have been shown to downregulate vitamin D receptor expression and inhibit the conversion of 25-hydroxyvitamin D to its active form, 1,25-dihydroxyvitamin D [17]. Additionally, heavy metals such as lead and cadmium can interfere with vitamin D synthesis and metabolism [4,13].

The significantly elevated serum ALP levels in RA patients (142.6 ± 38.4 U/L) compared to controls (98.3 ± 24.1 U/L) require careful interpretation. Total serum ALP includes isoenzymes from liver, bone, and intestinal sources. Without isolation of bone-specific ALP (BALP) or co-measurement of gamma-glutamyl transferase (GGT), it is chemically inaccurate to attribute elevated total ALP solely to increased bone turnover [11]. Previous studies have demonstrated that the liver isoenzyme is the sole or major component of elevated total ALP in a substantial proportion of RA patients, including many with normal total ALP levels [11]. Hepatic involvement in RA, whether due to the inflammatory process itself or as a consequence of DMARD therapy (particularly methotrexate), is well-recognized and can contribute to elevated ALP [11]. Therefore, the elevated ALP observed in this study may reflect a combination of increased bone turnover and hepatic reactivity. The significant positive correlation between ALP and DAS-28 scores ($r_s = 0.36$, $p = 0.005$) supports the hypothesis that ALP may be a marker of disease activity, but further studies with BALP measurement are needed to definitively establish the bone-specific contribution.

The finding of significantly elevated blood lead levels in RA patients (18.4 ± 6.2 µg/dL) compared to controls (8.7 ± 3.1 µg/dL) is particularly concerning. According to the CDC, the Adult Blood Lead Reference Value

(ABLRV) is 3.5 µg/dL, and levels ≥ 5 µg/dL are used for surveillance purposes. The mean blood lead level in RA patients in this study was more than five times the reference value, indicating significant lead exposure. Previous studies from Pakistan have documented elevated heavy metal levels in RA patients [7]. A study conducted in Sargodha, Punjab, reported significantly higher serum levels of Pb, Cd, Cr, and Ni in RA patients compared to healthy controls [7]. The sources of lead exposure in this region may include industrial emissions, contaminated water sources, lead-based paints, and occupational exposures [7]. Lead can directly affect bone metabolism by substituting for calcium in bone crystal structure, leading to the formation of abnormal hydroxyapatite with reduced mechanical strength [8]. Lead also inhibits osteoblast function and stimulates osteoclast activity, resulting in net bone loss [8,9]. Furthermore, lead can interfere with vitamin D metabolism and calcium homeostasis, exacerbating the effects of glucocorticoids on bone [8,9].

Similarly, blood cadmium levels were significantly elevated in RA patients (2.8 ± 1.1 µg/L) compared to controls (1.2 ± 0.6 µg/L). However, it is crucial to interpret blood cadmium levels with appropriate caution. Blood cadmium primarily reflects recent or current exposure, whereas urinary cadmium is the established gold standard for assessing chronic body burden and cumulative skeletal toxicity [4,12]. Urinary cadmium mainly reflects cadmium accumulation in the kidney and is a manifestation of renal damage and osteoporosis in the later stage, whereas blood cadmium shows acute and chronic exposure [4,12]. The reliance on whole-blood cadmium levels rather than urinary cadmium measurements is a limitation of this study, as it may not accurately reflect the cumulative skeletal toxicity of cadmium [4,12]. Previous meta-analyses have suggested that while urinary cadmium concentration is related to increased risk of osteoporosis and osteopenia, blood cadmium concentration may not be [4]. Nevertheless, the elevated blood cadmium levels observed in this study indicate significant recent exposure, and cadmium has been shown to have multiple adverse effects on bone, including competing with calcium for intestinal absorption, inhibiting renal calcium reabsorption, stimulating osteoclast activity, and inhibiting osteoblast function [4,10,12,13].

The significant positive correlations between DAS-28 scores and heavy metal levels (lead: $r_s = 0.45$, $p < 0.001$; cadmium: $r_s = 0.41$, $p < 0.001$) suggest that heavy metal toxicity may contribute to disease activity in RA. This is consistent with the known immunotoxic effects of heavy metals, which can induce oxidative stress, activate inflammatory pathways, and promote the production of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 [15]. Heavy metals can also disrupt immune tolerance by affecting regulatory T cell function and promoting Th17 differentiation [15]. The combined effects of heavy metals on the immune system and bone metabolism may create a vicious cycle of inflammation and bone loss in RA patients [15,20].

A critical confounding variable that must be acknowledged is smoking status. Cadmium exposure is heavily confounded by tobacco smoke, as smoking is a major source of cadmium intake [20]. If smoking habits between Group A and Group B were not matched or adjusted for via logistic regression, the observed differences in blood cadmium levels between RA patients and controls may be partially attributable to differential smoking prevalence rather than environmental exposure alone [20]. This limitation must be highlighted as a primary consideration in the interpretation of the cadmium findings. Future studies should include detailed smoking history and adjust for this confounder in multivariate analyses.

The findings of this study have important clinical implications for the management of RA patients on long-term glucocorticoid therapy. First, routine monitoring of bone mineral biomarkers, including serum calcium, phosphorus, ALP, and vitamin D, should be considered in all RA patients on glucocorticoid therapy [1,16]. Vitamin D supplementation should be recommended for all RA patients, particularly those with documented deficiency [6,18]. The optimal dose of vitamin D supplementation in RA patients on glucocorticoid therapy is not well established, but current guidelines recommend 800-1000 IU/day of vitamin D3 in combination with calcium supplementation (1000-1200 mg/day) for the prevention and treatment of GIOP [19]. Second, heavy metal screening should be considered in RA patients, particularly those from areas with known environmental pollution, with the understanding that blood cadmium reflects recent exposure and urinary cadmium may be necessary for assessing chronic body burden [4,7]. Third, disease activity should be closely monitored and controlled, as active inflammation appears to exacerbate disturbances in calcium and vitamin D homeostasis and may increase heavy metal burden [15].

This study has several strengths, including the well-characterized study population, the comprehensive assessment of bone mineral biomarkers and heavy metal levels, the use of standardized laboratory methods with documented LOD/LOQ values, and the use of appropriate non-parametric correlation analyses for non-normally distributed data. However, the study also has several limitations. First, the cross-sectional design precludes the establishment of causal relationships between heavy metal exposure and bone mineral alterations. Second, the sample size was relatively small, which may limit the generalizability of the findings. Third, we did not measure bone mineral density (BMD) directly, which is the gold standard for assessing bone health [5]. Fourth, we did not measure bone-specific ALP or GGT, which would have allowed more definitive interpretation of the elevated total ALP [11]. Fifth, we relied on blood cadmium rather than urinary cadmium, which is the preferred biomarker for chronic cadmium body burden and skeletal toxicity [4,12]. Sixth, smoking status was not matched or adjusted for in the analyses, which is a significant confounder for cadmium exposure [20]. Seventh, we did not assess dietary calcium intake or physical activity levels, which are

important determinants of bone health. Future studies with larger sample sizes, longitudinal design, BMD measurement, urinary cadmium assessment, and adjustment for smoking status are needed to confirm our findings and to further explore the mechanisms by which heavy metals and glucocorticoids interact to affect bone health in RA patients.

CONCLUSION

In conclusion, this study demonstrates that RA patients on long-term glucocorticoid therapy have significant alterations in bone mineral biomarkers, including hypocalcemia, hypophosphatemia, elevated alkaline phosphatase, and severe vitamin D deficiency. Additionally, these patients have significantly elevated blood lead and cadmium levels compared to healthy

controls. The significant correlations between disease activity and both bone mineral biomarkers and heavy metal levels suggest that inflammation and heavy metal toxicity may interact to promote bone loss in RA patients. These findings highlight the importance of routine monitoring of bone mineral biomarkers and heavy metal levels in RA patients on long-term glucocorticoid therapy, and support the implementation of strategies to reduce heavy metal exposure and optimize vitamin D and calcium status in this population. However, the reliance on blood cadmium as a biomarker of recent exposure rather than urinary cadmium as a measure of chronic body burden, and the potential confounding effect of smoking status, must be considered in the interpretation of these results. Future research with more comprehensive exposure assessment is warranted.

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