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Original Research Article

An Observational Study for the Comparison of Mineral Status (Ca, Mg & P) and Alkaline Phosphatase Levels in Middle Aged Rheumatoid Arthritis Patients with Age-Matched Healthy Individuals

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Conflict of interest: Nil

Abstract:

Background: Rheumatoid arthritis (RA) is a progressive, systemic autoimmune disease characterized by chronic inflammation of synovial joints, systemic manifestations, and progressive joint and bone destruction. The current study was conceptualized to assess serum calcium, magnesium, phosphorus, and alkaline phosphatase levels in middle-aged patients with RA.

Methodology: A total of 30 RA patients and 30 healthy controls (matched by age and sex) were recruited. After ethical clearance and informed consent, participants were subjected to clinical examination, routine blood investigations, and specific tests to estimate: Serum Calcium – Arsenazo III method, Serum Magnesium – Calmagite method, Serum Phosphorus – Ammonium molybdate method, Serum ALP – IFCC kinetic method.

Results: Among all the variables studied, mineral metabolism markers (calcium, phosphorus, magnesium, ALP) and liver function abnormalities were significantly associated with RA positivity, whereas demographic and lifestyle factors were not.

Conclusion: These findings may aid clinicians in early recognition of systemic involvement in RA and promote comprehensive patient management strategies.

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Introduction

Rheumatoid arthritis (RA) is a systemic, chronic, inflammatory autoimmune disorder that primarily targets the synovial joints and is characterized by persistent synovitis, systemic inflammation, and the presence of autoantibodies such as rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies. The disease typically results in progressive joint damage, bone erosion, and functional disability, which significantly impacts the quality of life of affected individuals [1]. While clinical diagnosis of RA relies on ACR/EULAR criteria, including joint counts and serological markers, there is growing interest in using biochemical parameters as adjunctive tools for assessing disease severity and systemic involvement.

Among these parameters, Alkaline Phosphatase (ALP) stands out as a marker of bone turnover. ALP is produced by osteoblasts and is essential for bone mineralization. Elevated serum ALP levels have been observed in various bone-related disorders and are often reflective of increased osteoblastic activity or bone remodeling [2]. RA patients with active disease may show altered ALP levels, indicating ongoing bone metabolism dysregulation.

Serum Calcium (Ca), Magnesium (Mg), and Phosphorus (P) are essential minerals for bone and muscle function. Calcium is critical for bone strength and neuromuscular signaling, phosphorus is a major component of hydroxyapatite crystals in bones, and magnesium serves as a cofactor in numerous enzymatic reactions, including those involving ATP metabolism [3].

Rheumatoid Arthritis is a systemic autoimmune disease with significant implications on bone health and mineral homeostasis. The disease pathogenesis is intricately linked to inflammatory processes that affect calcium, magnesium, phosphorus levels, and ALP activity. Despite their potential diagnostic and

prognostic relevance, these biochemical parameters are not routinely monitored in RA patients in India. This study seeks to explore and quantify the biochemical differences in mineral status and ALP levels between RA patients and healthy individuals, contributing to a better understanding of RA-related metabolic alterations. The findings may pave the way for incorporating simple, inexpensive biochemical tests into standard RA evaluation protocols.

Materials and Methods

Study Design and Type: This study is designed as a hospital-based observational comparative study conducted at a tertiary care teaching hospital. The primary objective is to evaluate and compare serum levels of calcium (Ca), magnesium (Mg), phosphorus (P), and alkaline phosphatase (ALP) in middle-aged rheumatoid arthritis (RA) patients versus age- and sex-matched healthy controls.

Study Setting and Duration

Institution: Department of Biochemistry, in collaboration with Department of Clinical Immunology and Rheumatology, S.M.S. Medical College, Jaipur and Geetanjali Medical college, Udaipur, Rajasthan, India.

Study Duration: 16 months (September 2023 to December 2024).

Study Phases: Preparatory (2 months), Sample collection (6 months), Laboratory processing (2 months), Data analysis and report writing (6 months).

Ethical Clearance and Institutional Permissions

Before initiation, this study was submitted to and approved by the following bodies:

- Institutional Ethics Committee (IEC), S.M.S. Medical College, Jaipur.
- Research Review Board (RRB)
- Head of Department, Clinical Immunology and Rheumatology

Informed written consent was obtained from all participants in both English and Hindi languages, following the Declaration of Helsinki guideline. The consent form included details on confidentiality, voluntary participation, withdrawal rights, and absence of risks associated with blood sampling.

Study Population: Cases**:** Diagnosed RA patients, based on 2010 ACR/EULAR classification criteria, attending OPD or admitted in IPD of the Department of Clinical Immunology and Rheumatology. **Age group:** 40–60 years. **Gender:** Both male and female. **Disease duration:** ≥6 months

Controls: Apparently healthy individuals without any known chronic illness, preferably first- degree relatives or accompanying attendants of the cases. Matched for age (±2 years) and sex. Belonging to the same geographic and socioeconomic background.

Sample Size Determination: Sample size was calculated using power analysis based on previous findings by Chandrakar et al., which reported a significant difference in mean ALP levels between RA and control groups (mean difference = 61.9 ± 17.55 U/L).

Using a 95% confidence interval and 80% power, the calculated minimum sample size was 30 subjects per group (RA = 30, Control = 30), total = 60 participants.

Inclusion and Exclusion Criteria

Inclusion Criteria:

Cases: Clinically diagnosed RA (ACR/EULAR 2010 criteria)

Age 40–60 years, either sex, Willing to provide written consent

Controls:

- Age and sex-matched healthy individuals
- No clinical signs or history of RA or chronic illness
- Not on any regular medication
- Willing to participate voluntarily

Exclusion Criteria (for both groups)

- Diabetes mellitus
- Chronic liver or renal diseases
- Alcoholics
- On medications affecting mineral levels: anti-tuberculars, antiepileptics, diuretics
- Diagnosed cases of osteoporosis
- Pregnant or lactating women
- Acute or chronic infections (e.g., tuberculosis)
- Other inflammatory or autoimmune diseases

Data Collection Tools:

Clinical Proforma

- A structured clinical proforma was used to record:
- Demographics: Name, age, sex, occupation, socioeconomic status
- Clinical history: Duration of RA, drug history, comorbidities
- Physical examination: Weight, height, BMI, blood pressure
- Routine blood tests

Sample Collection and Processing

Timing: Blood samples collected between 8:00 – 10:00 AM to minimize diurnal variation. Participants were instructed to fast for 8–12 hours before sample collection.

Procedure

- 7 mL of venous blood collected under aseptic precautions:
- 2 mL in EDTA vial: For CBC and ESR
- 5 mL in plain vacutainer: For biochemical tests (ALP, Ca, Mg, P)

Processing

- Plain vials were allowed to clot at room temperature for 30–60 minutes.
- Centrifugation at 3000 rpm for 10 minutes
- Serum separated, stored at 2–8°C if not immediately analyzed
- All analyses completed within 48 hours

Laboratory Investigations:

Routine Investigations

- Complete Blood Count (CBC): Hemoglobin, TLC, DLC, Platelet count
- Erythrocyte Sedimentation Rate (ESR)
- Liver Function Test (LFT): ALT, AST, Total Protein, Albumin, ALP, Bilirubin

Renal Function Test (RFT): Urea, Creatinine, Uric Acid

Special Investigations

Parameter	Method	Principle	Equipment Used
Alkaline Phosphatase	IFCC kinetic method	Continuous monitoring of p-nitrophenyl phosphate hydrolysis	Spectrophotometer
Serum Calcium	Arsenazo III end-point method	Color complex formation with Arsenazo dye	Spectrophotometer
Serum Magnesium	Calmagite method	Colorimetric detection with calmagite dye	Spectrophotometer
Serum Phosphorus	Ammonium molybdate method	Formation of phosphomolybdate complex	Spectrophotometer

Statistical Analysis: Software: SPSS Version 29.0 (IBM Corp.). Microsoft Excel for data entry and preliminary filtering was used.

Tests Used:

Type	Test
Normality check	Kolmogorov-Smirnov Test
Parametric data	Independent Student's t-test
Non-parametric data	Mann–Whitney U test
Categorical variables	Chi-square or Fisher's exact test
Correlation analysis	Pearson or Spearman coefficient

Significance Level: p-value < 0.05 considered statistically significant**,** Confidence interval set at 95%.

Observation and Results

Table 1: Distribution of Patients According to Liver Function Test

		Rheumatoid Arthritis (RA factor)		Total
		Absent	Present	
Liver Function Test (LFT)	**ALP↑**	3	7	10
	AST↑	4	14	18
	WNL	23	9	32
Total		30	30	60
Chi Square test, p value-.001, Results are Significant				

Table 1 shows that Among RA-positive patients, the most common LFT abnormality was elevated AST (14), followed by ALP (7), while only 9 had normal LFTs. In contrast, the majority of RA-negative patients (23) had normal LFTs. The Chi-square test ($p = 0.001$) shows a significant association, indicating that abnormal LFTs, especially elevated AST and ALP, are significantly related to RA positivity in this study.

Table 2: Distribution of Patients According to Serum ALP Levels

Group	N	Mean Serum ALP (IU/L)	Standard Deviation (SD)	p-value
RA Factor Present	30	144.80	15.49	0.000
RA Factor Absent	30	86.90	15.30	

Table 2 shows that the mean serum ALP level in RA-positive patients was 144.80 IU/L (SD: 15.49), significantly higher than 86.90 IU/L (SD: 15.30) in RA-negative patients. Using an independent samples t-test, the difference was found to be highly significant ($p < 0.000$), indicating that elevated ALP levels are strongly associated with RA positivity, possibly due to increased bone turnover or inflammation linked to rheumatoid arthritis.

Table 3: Distribution of Patients According to Serum Calcium Levels

Group	N	Mean Serum Calcium (mg/dl)	Standard Deviation (SD)	p-value
RA Factor Present	30	7.69	0.38	0.000
RA Factor Absent	30	9.22	0.51	

Table 3 shows that the mean serum calcium level was 7.69 mg/dl (SD: 0.38) in RA-positive patients and 9.22 mg/dl (SD: 0.51) in RA-negative patients. An independent samples t-test showed a highly significant difference ($p < 0.001$), indicating that lower serum calcium levels are strongly associated with RA positivity in this population.

Table 4: Distribution of Patients According to Serum Phosphorus Levels

Group	N	Mean Serum Phosphorus (mg/dl)	Standard Deviation (SD)	p-value
RA Factor Present	30	4.83	0.26	0.000
RA Factor Absent	30	3.79	0.43	

Table 4 shows that the mean serum phosphorus level was 4.83 mg/dl (SD: 0.26) in RA-positive patients and 3.79 mg/dl (SD: 0.43) in RA-negative patients. An independent samples t-test revealed a highly significant difference ($p < 0.001$), indicating that higher serum phosphorus levels are significantly associated with RA factor positivity in this population.

Table 5: Distribution of Patients According to Serum Magnesium Levels

Group	N	Mean Serum Magnesium (mg/dl)	Standard Deviation (SD)	p-value
RA Factor Present	30	1.68	0.25	0.000
RA Factor Absent	30	2.02	0.27	

Table 5 shows that the mean serum magnesium level in RA-positive patients was 1.68 mg/dl (SD: 0.25), while in RA-negative patients it was 2.02 mg/dl (SD: 0.27). An independent samples t-test revealed a highly significant difference ($p < 0.001$), indicating that lower serum magnesium levels are significantly associated with RA factor positivity in this population.

Discussion

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by chronic inflammation of synovial joints, leading to progressive disability, systemic complications, and increased morbidity. The presence of rheumatoid factor (RA factor) is a crucial diagnostic and prognostic marker, although its exact role in disease progression varies among populations. In the present study, a total of 60 patients were analyzed and equally divided into RA-positive and RA-negative groups, with detailed demographic, clinical, hematological, and biochemical comparisons. The findings provide valuable insights into factors associated with RA factor positivity, with special emphasis on biochemical derangements involving calcium, phosphorus, magnesium, and alkaline phosphatase (ALP).

The chief complaints among RA-positive patients were predominantly joint pain and fatigue. Notably, 16 patients presented with both complaints, 11 with joint pain alone, and 3 with fatigue. Among RA-negative individuals, joint pain alone was more common (19 cases), while combined complaints were seen in 9 patients. Despite these patterns, statistical analysis revealed no significant association between presenting complaints and RA factor positivity ($p = 0.117$). This suggests that while joint pain and fatigue remain hallmark clinical features of RA, they are not exclusive to seropositive disease and may also be observed in seronegative arthritis and other inflammatory conditions.

A highly significant observation emerged from drug history analysis. All RA-positive patients were on disease-modifying anti-rheumatic drugs (DMARDs), either alone or in combination with

antihypertensives, while all RA-negative patients were on non- steroidal anti-inflammatory drugs (NSAIDs) with or without antihypertensives ($p < 0.001$).

Among RA-positive patients, elevated AST and ALP were the most frequent abnormalities, while the majority of RA-negative patients had normal liver function tests. This yielded a statistically significant difference ($p = 0.001$). Elevated AST may indicate either hepatic involvement due to systemic inflammation or hepatotoxicity from medications such as methotrexate. Elevated ALP, on the other hand, reflects increased bone turnover and synovial inflammation, both characteristic of RA. The strong association between abnormal LFTs and RA positivity underscores the importance of regular biochemical monitoring in these patients.

RA-positive patients had significantly elevated ALP levels compared to RA-negative patients (144.80 vs. 86.90 IU/L, $p < 0.001$). Elevated ALP in RA is likely due to increased bone turnover, secondary to chronic synovial inflammation and osteoclastic activity. This finding indicates that ALP could serve as an additional biomarker reflecting disease activity and systemic bone involvement.

Studies supporting significantly Elevated ALP:

- B.L. Chandrakar et al. (2017) conducted a study in Durg, India, showing statistically higher ALP levels in RA patients compared to controls. 65% of female and 70% of male RA patients had elevated ALP levels [4].
- Aishwarya KP et al. found ALP levels elevated in all RA patients compared to controls, emphasizing the diagnostic potential of ALP in bone resorption processes [5].

Non-Significant Difference was seen in following studies:

- Albedri Khudair et al. (2020) conducted a case-control study on 100 RA patients and 50 controls in Baghdad and found no statistically significant difference in ALP, though patients with high disease activity had slightly elevated values [6].
- Zhongxin Zhu et al. used NHANES data from the U.S. and concluded that while osteoarthritis was associated with elevated bone markers, no significant correlation between RA and ALP was observed in adjusted regression models [9]. This suggests that ALP may not be a universal marker for RA, and its utility may depend on ethnic, dietary, or disease-stage factors.

A significant reduction in serum calcium levels was noted among RA-positive patients (7.69 vs. 9.22 mg/dl, $p < 0.001$). Hypocalcemia in RA may result from chronic inflammation, impaired vitamin D metabolism, and glucocorticoid therapy, all of which contribute to bone loss and osteoporosis. The consistent lowering of calcium in RA-positive individuals suggests its potential role as a biochemical indicator of disease severity.

Numerous studies have found decreased serum calcium levels in RA patients:

- Aishwarya KP et al. reported significantly lower calcium levels in RA patients compared to healthy controls ($p<0.001$), with concurrent elevations in ALP [5].
- Najlaa Kadhim Ali et al. found calcium levels to be statistically lower in RA women with osteoporosis, suggesting a link between chronic inflammation and reduced calcium absorption or increased bone resorption [8].
- Inflammation in RA can reduce vitamin D levels, impairing intestinal calcium absorption. Glucocorticoid use further exacerbates calcium loss through renal excretion and intestinal malabsorption [8].

Conversely, serum phosphorus levels were significantly elevated in RA-positive patients (4.83 vs. 3.79 mg/dl, $p < 0.001$). Hyperphosphatemia may reflect compensatory changes in bone metabolism or altered parathyroid hormone regulation secondary to chronic inflammation. Elevated phosphorus, alongside low calcium, highlights the disturbed bone-mineral axis in RA.

- Najlaa Kadhim Ali et al. reported mildly altered phosphorus levels in osteoporotic RA patients. Though values remained within the reference range, a statistically significant difference was observed [8].
- Yazmalar et al. noted a seasonal variation in phosphate levels among RA patients, possibly related to fluctuations in vitamin D [10].

Serum magnesium levels were markedly lower in RA-positive patients (1.68 vs. 2.02 mg/dl, $p < 0.001$). Magnesium is a key modulator of inflammation and oxidative stress. Deficiency is known to enhance pro-inflammatory cytokine production, contributing to the chronic inflammatory state observed in RA. The significant reduction in magnesium among RA-positive individuals underscores its potential role in RA pathophysiology.

- Lucia M et al. observed that RA patients in stages I–II had significantly lower serum magnesium levels than controls even before the initiation of treatment [9].
- A study by Pallinti V et al. suggested an inverse correlation between serum magnesium and disease activity score (DAS28), CRP, and ESR [11].
- Mg deficiency contributes to increased levels of

pro-inflammatory cytokines like IL-1, TNF-α, and IL-6, which drive the pathogenesis of RA. Additionally, magnesium deficiency is linked with osteoporosis, further compounding skeletal damage in RA [13].

When assessed collectively, Ca, Mg, P, and ALP can offer a composite biochemical picture of the metabolic status of RA patients. Some studies have attempted to correlate all four parameters:

- Vilas U Chavan et al. noted that RA patients with elevated ALP often had concomitant decreases in calcium and magnesium, indicating compensatory bone turnover due to inflammation-induced bone resorption [14].
- Dean C et al. emphasized the "Magnesium Miracle" theory, suggesting that Mg depletion may be an initiator of both RA and osteoporosis via dysregulated immune and bone homeostasis [15].

The highly significant findings related to serum ALP, calcium, phosphorus, and magnesium emphasize the need to consider these parameters not only for diagnostic support but also for monitoring disease activity and therapeutic outcomes. Hypocalcemia, hypomagnesemia, hyperphosphatemia, and elevated ALP appear to be characteristic biochemical signatures of RA positivity in this study. Furthermore, the strong correlation between RA factor positivity and DMARD usage validates the clinical reliance on serological markers in guiding therapy. While family history trends suggest a genetic predisposition, larger studies are required to establish statistical significance.

Conclusion

This study reinforces the concept that rheumatoid arthritis is not confined to the joints—it is a systemic disease that significantly impacts mineral metabolism and bone health. The expected alterations in serum calcium, magnesium, phosphorus, and alkaline phosphatase levels suggest a metabolic component to disease activity, which can be easily tracked using routine biochemical investigations.

The data highlights the need for early detection and intervention to prevent long-term complications like osteoporosis and fragility fractures. More importantly, it opens the door to cost-effective, accessible monitoring tools that can be widely implemented, particularly in resource-limited settings like India.

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