Research Article

Study of bone formation marker levels in rheumatoid arthritis

Abhishek Dubey, Davina Hijam, Oinam Prabita Devi, Wahengbam Diana Devi, Suman Debnath

Department of Biochemistry, Regional Institute of Medical Sciences, Imphal, Manipur, India

Correspondence to: Davina Hijam, E-mail: davina hijam@yahoo.co.in

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ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune disease of unknown cause that affects the joints principally. The disease affects between 0.5% and 1% of the adult population worldwide. Two to three times as many women as men suffer from the disease. Osteocalcin (OC) is a small protein of 49 amino acids long. OC is the most abundant non-collagenous protein in bone. OC originates from osteoblasts and is deposited into bones or released into circulation, where it correlates with histological measures of bone formation. Bone alkaline phosphatase (ALP) is a glycoprotein that is found on the surface of osteoblasts. This enzyme reflects the biosynthetic activity of these bone-forming cells. The presence of OC and ALP in the circulation may, therefore, provide a specific chemical index of osteoblastic activity. **Objectives:** This study was undertaken to estimate the values of serum OC and ALP among patients with RA and healthy control groups and to compare and find out any changes in levels of serum OC and ALP between the study and control groups. **Materials and Methods:** It was a case–control study done on 76 RA patients and 76 age- and sex-matched healthy individuals. Serum OC and serum ALP values were evaluated among all 76 cases and 76 controls. Serum OC was measured using immunoenzymatric assay and ALP was measured by colorimetric method. Statistical analysis was performed and results were tabulated and analyzed. **Results:** Mean ± standard deviation of serum OC level is significantly higher ($P < 0.001$) among cases (18.50 ± 8.72 ng/ml) than controls (9.98 ± 7.68 ng/ml). Similarly, the values of ALP are higher ($P < 0.001$) among cases (216.22 ± 59.96 IU/L) than controls (164.17 ± 50.70 IU/L). A significantly positive correlation was found between serum OC and serum ALP levels. Patient with the highest mean value of serum OC also has the highest values of ALP. The values of serum ALP and OC levels increase significantly in both early and late stages when compared with control values. **Conclusions:** A significant difference between the values of serum OC and ALP among cases and controls was seen in the study. Levels of both these parameters are elevated in subjects with RA compared to controls. Furthermore, the levels of serum OC correlated with the levels of serum ALP. This study demonstrates that increased bone formation is associated with RA together with bone resorption.

KEY WORDS: Rheumatoid Arthritis; Osteocalcin; Alkaline Phosphatase

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disorder that may affect many tissues and organs but principally affect the joints. This process is based on imbalance between bone-resorbing osteoclasts and bone-forming osteoblasts. The imbalance is caused by enhanced expression of inflammatory cytokines, such as tumor necrosis factor, which foster the differentiation of osteoclasts and hamper the formation of osteoblasts. In consequence, repair of bone erosion is limited, with localized deposition of bone at the base of erosions (sclerosis)[1] and growth of small bony spurs.[2] The pathological findings in the joints include chronic non-suppurative, proliferative, and inflammatory synovitis with the formation of a pannus, which erodes cartilages,

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bones, ligaments, and tendons. In the acute phase, effusion and other manifestations of inflammation are common. In the late stage, organization may result in fibrous dysplasia. Although the cause of RA remains unknown, autoimmunity plays a principal role in chronicity and progression.[3]

Remodeling of bone is a continuous process throughout life. Resorption and formation of bone are normally tightly coupled. Changes leading to decreased rates of bone formation or increased rates of bone resorption or both may cause a decrease in bone mass. This involves a fine balance of the activity of bone depositing cells (osteoblasts) and bone resorbing cells (osteoclasts).[4] Osteocalcin (OC) originates from osteoblasts and is deposited into bones or released into circulation, where it correlates with histological measures of bone formation.[5]

On catabolism of OC, its characteristic amino acids, ϒ-carboxyglutamic acid (Gla) is secreted into the urine. Both serum OC and urine Gla are currently being used for the clinical assessment of bone diseases. Serum OC measurement provides a non-invasive marker of bone metabolism.[6]

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate group from many types of molecules including nucleotide, proteins, and alkaloids. As the name suggests, ALP is most effective in an alkaline medium. Bone ALP is the bone-specific isoform of ALP, a glycoprotein that is found on the surface of osteoblasts. Bone ALP reflects the biosynthetic activity of these bone-forming cells. It has been shown to be sensitive and specific marker of bone metabolism.

Bone turnover may be assessed by the measurement of enzymes or matrix proteins produced by osteoblasts or osteoclasts. The presence of OC and ALP in the circulation may, therefore, provide a specific chemical index of osteoblastic activity. In fact, the level of serum OC and ALP is increased in disorders characterized by accelerated bone turnover.

For a long time, the diagnosis of RA was mainly based on clinical manifestations. However, it is often difficult to diagnose RA in very early phase of the disease, and in many cases, irreversible damage had occurred by the time diagnosis which is confirmed. Therefore, laboratory tests which are sensitive and specific early in the disease course are desirable to allow earlier diagnosis and intervention. Bone biomarkers may add useful information for assessing fracture risk and for monitoring osteoporosis in RA patients.[7]

The aim of our study is to estimate the values of serum OC and ALP among the control and study groups of RA and to compare and find out any changes in the levels of serum OC and ALP between the study and control groups.

MATERIALS AND METHODS

A case–control study was carried out in the Department of Biochemistry in collaboration with the Department of Medicine, Regional Institute of Medical Sciences, Imphal, Manipur, India, between October 2016 and September 2018. The study population consists of patients with the age group of 18 and above who were coming from different areas of Manipur and attending rheumatology clinic of medical OPD or admitted in the medical ward of RIMS, Imphal. Seventy-six diagnosed cases of RA aged 18 years and above, irrespective of sex, caste, and creed, who were willing to participate in the study voluntarily were included in this study while patients with Paget's disease of bone, those suffering from osteoarthritis, psoriatic and reactive arthritis, hyperthyroidism, or hyperparathyroidism were excluded from this study. Seventy-six age- and sex-matched healthy subjects aged 18 years or above irrespective of sex, religion, and socioeconomic status were included as controls for the present study.

A detailed history including name, age, sex, sociodemographic data, duration of disease, and age of onset of disease was collected from each participant. Five milliliters of venous blood were drawn from each individual in a sterile plain vial for the estimation of serum OC and ALP. Hemolyzed samples were discarded. The blood collected in the plain vial was centrifuged immediately and was stored at 2–8°C. Before use, all the samples were kept at room temperature and tests were done within 24 h. Serum OC was measured using immunoenzymatric assay for *in vitro* quantitative measurement of intact human OC (OST) in serum as described by Power and Fottrell.[8] Serum ALP was measured from patient blood using colorimetric method using RX IMOLA by Randox Laboratories Ltd. based on the method described by Bowers Jr and McComb.[9] Cases were divided into two groups. Those cases with the duration of <5 years were categorized as early-stage RA cases while those with duration more than 5 years were categorized as late-stage RA.

All the data collected were tabulated thereafter and statistical analysis was done using IBM SPSS statistics version 20 software. Data were expressed as mean ± standard deviation (SD). Statistical tests such as χ^2-test, independent *t*-test, ANOVA (F-test), and Pearson's correlation coefficient "r" were applied whenever found suitable and necessary. $P < 0.05$ was considered statistically significant.

Approval was sought from the Institutional Ethical Subcommittee, RIMS, Imphal. Consent was taken from each individual before taking blood samples. Confidentiality was maintained.

RESULTS

The mean age of the cases was 54.57 ± 11.42 years, and for the controls, it was found to be 51.91 ± 11.88 years and majority

of the patients belonged to the age group of 51–60 years (53.95%) followed by 61–70 years (15.79%).

Table 1 shows sex-wise distribution of controls and RA cases. Numbers of females are more in both the groups. Among cases, 54 (71.05%) were female as compared to 22 (28.95%) males. Among controls, the number of males and females was 27 (35.52%) and 49 (64.48%), respectively.

Table 2 shows mean (± SD) for serum OC and ALP among RA cases and controls. Serum OC (cases – 18.50 ± 8.72 ng/ml and controls – 9.98 ± 7.68 ng/ml) and ALP levels (cases – 216.22 ± 59.96 IU/L and controls – 164.17 ± 50.67 IU/L) are higher among cases than controls. This difference is found to be statistically significant ($P < 0.001$).

Table 3 shows the distribution of serum OC among RA cases and controls according to sex. Among controls, males have slightly higher OC level (10.21 ± 7.80 ng/ml) compared to females (9.85 ± 7.69 ng/ml) while among RA cases, females (18.98 ± 9.22 ng/ml) have more level compared to males (17.34 ± 7.80 ng/ml). These differences among both the groups are statistically insignificant. Difference between OC (mean ± SD) levels among males of both the groups is statistically significant and among females of both the groups is also statistically significant ($P < 0.001$).

Table 4 shows the mean ± SD values of ALP in both the groups according to sex. Females have higher value of ALP among both controls and RA cases (169.90 ± 54.23 IU/L and 217.28 ± 64.07 IU/L, respectively) compared to males (153.76 ± 42.55 IU/L and 213.61 ± 49.66 IU/L respectively). However, this difference according to sex is not found to be statistically significant. However, this difference is significant between males of both the groups ($P < 0.001$). Difference between mean (± SD) is also significant between females of both the groups ($P < 0.001$).

Table 5 shows the correlation between mean (± SD) serum OC and ALP in RA cases. A positive correlation is found between the values of OC and ALP. ALP levels are showing a trend to increase with increasing S. OC levels. This correlation is found to be statistically significant ($r = 0.411$, $P < 0.001$).

Table 6 shows the values of serum ALP and serum OC among controls, early and late stages of RA. The values of serum ALP and serum OC increase significantly in both early and late stages when compared with control values.

Table 1: Sex-wise distribution of controls and cases

Sex	Controls	RA cases
	n (%)	*n (%)*
Male	27 (35.52)	22 (28.95)
Female	49 (64.48)	54 (71.05)
Total	76 (100)	76 (100)

RA: Rheumatoid arthritis

DISCUSSION

In the present study, RA is found to be most commonly prevalent among the adults in the age group of 51–60 years. Furthermore, females outnumbered males. The prevalence of RA was higher among females as compared to males.

The mean ± SD values of serum OC and serum ALP were significantly higher in cases compared to controls. These findings are similar to the findings of Magaro *et al*.,[10] Aschenberg *et al*.,[11] and Vaithialingam *et al*.[12] but contradictory to the findings of Batmaz *et al*.,[13] Jacobs *et al*.,[14] and Gheita *et al*.[15] OC and ALP are markers of bone formation. Increased bone turnover is a feature of RA. Bone formation and resorption form a coupling process of bone metabolism, in which both components follow each other continuously.[16] When resorption is increased, so is formation. Imbalance between these processes is responsible for increased or decreased bone mineral density and chances of complications due to it. RA is inflammatory disease. It is characterized by chronic inflammation of the synovium, particularly of small joints, which often leads to the destruction of articular cartilage and juxta-articular bone. With cytokines mediated increased resorption of bone, bone formation also increases. This increased bone formation is correlated with increased markers of bone formation (i.e., OC and ALP) in patient serum.

Table 3 compares serum OC levels of controls and cases according to sex. Among the controls, males exhibited highest value (10.21 ± 7.80 ng/ml) of serum OC level than females (9.85 ± 7.69 ng/ml), but this difference is insignificant. However, the study cases showed higher OC levels among females (18.98 ± 9.22 ng/ml) compared to male value (17.34 ± 7.42 ng/ml). The mean ± SD value of serum OC among the study group indicated significantly higher levels ($P < 0.001$) in both males and females when compared with the corresponding mean ± SD values of controls. In human medicine, the sex differences in bone markers are manifested

Table 2: Summary of biochemical parameters in controls and RA cases

Parameter	Controls (Mean±SD)	RA cases (Mean±SD)	*t*	*P*-value
Osteocalcin (ng/ml)	9.98±7.68	18.50±8.72	6.40	<0.001
Alkaline phosphatase (IU/L)	164.17±50.70	216.22±59.96	5.78	<0.001

SD: Standard deviation, RA: Rheumatoid arthritis

Table 3: Comparison of serum OC (Mean±SD) of the controls and RA cases according to sex

Sex	Serum OC a level (ng/ml) (Mean±SD)		*t*-value	*P*-value
	Controls	RA		
Male	10.21±7.80	17.34±7.42	3.25	<0.001
Female	9.85±7.69	18.98±9.22	5.42	<0.001

SD: Standard deviation, RA: Rheumatoid arthritis, OC: Osteocalcin

Table 4: Comparison of serum alkaline phosphatase level (Mean±SD) in controls and RA cases by sex

Sex	Serum ALP levels (IU/L)		*t*	*P*-value
	Controls	RA cases		
Male	153.76±42.55	213.61±49.66	4.54	<0.001
Female	169.90±54.23	217.28±64.07	4.03	<0.001

SD: Standard deviation, RA: Rheumatoid arthritis

Table 5: Correlation between serum OC and alkaline phosphatase in RA

S. OC levels (ng/m) (Mean±SD)	Number of cases	Serum ALP levels (IU/L) (mean±SD)
3.17±1.30	4	203.32±42.43
8.24±1.57	13	189.59±49.42
12.88±1.66	9	199.80±70.94
17.22±1.71	15	210.52±46.05
22.32±1.03	18	212.84±56.52
26.61±1.42	11	216.20±28.68
35.65±5.50	6	325.97±48.38
Total	76	

r=0.411, *P*<0.001. SD: Standard deviation

Table 6: Serum alkaline phosphatase and serum OC in controls, early and late stages of RA

Parameter	Control	Early-stage RA (<5.0 years)	Late-stage RA (>5.0 years)
Alkaline phosphatase (IU/L)	164.17±50.70	212.22±56.60***	218.98±62.64***
Serum OC (ng/ml)	9.98±7.68	16.21±9.14***	20.09±8.14***

***P<0.001. SD: Standard deviation, RA: Rheumatoid arthritis

mainly due to a different skeleton size in healthy men and women.[17,18] However, these differences were not statistically significant in both the groups.[19-21]

Table 4 compares serum ALP levels in cases and controls according to both sexes. Among both the groups, females had higher values of ALP than males. However, this difference of ALP values among males and females was not significant in both the groups. However, male RA patients had significantly (<0.001) higher values of ALP than males in the control group. Similarly, the difference of ALP values among females of both the groups was also significant. These findings were in consistent with Vaithialingam *et al*.[12] who also found noticeably increased levels of ALP among females than males, but in their study, this difference was statistically significant.

In the present study, we found a positive correlation between serum OC and ALP ($r = 0.411$, $P < 0.001$), as shown in Table 5. Similar positive correlation was found in the study done by Gevers *et al*.[22] The significant correlation with OC indicates that this is caused by increase of the bone fraction and is further evidence that bone turnover is increased.

The mean ± SD value of serum ALP and serum OC increases significantly ($P < 0.001$) in both early and late stages of RA cases when compared with controls [Table 6]. In this study, values of both these parameters were higher in late stages of disease compared to early stages of the disease, but this difference was not significant. These findings are in consistent with the findings of Gevers *et al.*[22] who also did not find any correlation between OC values and disease duration or disease activity. However, Gough *et al*.[23,24] in two studies found that patients with early RA had faster bone loss both at the lumbar spine and at the hip than that observed in a control group. Moreover, bone loss was slightly greater in the 1st year as compared with the 2nd year of follow-up. Conversely, Shenstone *et al*.[25] who studied 67 patients with RA of <5 years duration and 72 controls over a 12-month period found that bone loss was low (about 1%) and comparable in patients and controls. However, they also found that bone loss at the femoral neck was faster in those patients who had experienced onset of their disease <6 months earlier, as compared with the controls and the patients who had more long-standing disease. Finally, Aman *et al*.[26] who studied 52 patients with early RA (i.e., <5 years duration) over a 24-month period did not find any bone loss.

Strength and Limitation

This is the first study of serum OC levels in Indian Manipuri population with RA. Further studies need to be done to evaluate the status of bone formation markers in patients of RA.

CONCLUSIONS

The results of this study show a significant difference between the values of serum OC and serum ALP among cases and controls. Levels of both these parameters are elevated in subjects with RA compared to controls. Furthermore, the levels of serum OC correlated with the levels of serum ALP. This correlation is found to be significant. This is the first study of serum OC levels in Indian Manipuri population with RA. This study demonstrates that increased bone formation is associated with RA together with bone resorption. It affects the bone mineral density. Increased bone turnover is

a common feature of RA. The results of this study confirm the association of the serum OC and serum ALP with RA. Thus, it can be concluded that the estimation of serum OC and ALP levels may be used as a biomarker for the diagnosis and prognosis of RA and may provide a useful tool for its management.

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