Would14-3-3η enable an approach to personalized medicine for secondary osteoporosis in rheumatoid arthritis?

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ABSTRACT:

Background: Early identification and disease monitoring of secondary osteoporosis(OP)in rheumatoid arthritis (RA) are challenges for rheumatologists, identification of biomarkers predictive to bone mineral density (BMD) change is crucial in management. Serum14-3-3η protein is validated as a diagnostic and prognostic biomarker in RA. Meanwhilethe exact mechanism by which 14-3-3η intervenes osteoporosis is still unclear and few studies have been focused on it. Our aim wasto evaluate the association among 14-3-3η protein, inflammation, bone remodeling, osteoporosis riskand disease activity in RApatients.

Methods:

Bone mineral density was measured using dual energy X-ray absorptiometry, serum samples were collected for all participants. Quantitative enzyme-linked immunosorbent assay (ELISA) was used to determine14-3-3η,TNF-α and IL-6 levels. Meanwhile, B-CTX and PINP were measured using electrochemical luminescence immune-analyzer.The diagnostic value of each marker was determined via receiver operating characteristic (ROC) curve, and the association between 14-3-3η and osteoporosis was assessed using multiple logistic regression which identified 14-3-3η as an independent risk factor for RA-related osteoporosis.

Results:

Seventy-twoRA patients and twentyfive controls. Patients were divided into three subgroups, normal BMD, osteopenia, and osteoporotic.Serum 14-3-3 η, TNF-α,B-CTX and IL-6 level were the highest and PINP is the lowest in osteoporosis group, There were significant differences among the subgroups ($p<0.05$).Also, there were significant positive correlation between 14-3-3 η and TNF-α,B-CTX ,IL-6 ($p<0.05$) while it had significant negative correlation with both BMD,PINP ($p<0.05$).

Conclusion

Serum 14-3-3η is independent risk factor for RA-related osteoporosis. Serum 14-3-3η detection by itself or combined with other indices was helpful in predicting osteoporosis. Its effect on osteoporosis may be due to its role in adjusting inflammation and bone remodeling.

Keywords:14-3-3η protein, TNF-α,B-CTX ,IL-6 , BMD, Osteoporosis, RA , Bone markers, Cytokines.

Introduction:

Rheumatoid arthritis is one of the most common chronic autoimmune rheumatic diseases affecting approximately 1.5% of world populationcausing low BMD, silent progressive osteoporosis related fracturesand disability affecting life quality.The relative risk of fracture in RA patients is 2-fold greatercompared with sex- and age-matched subjects affecting 200 million people worldwide increasing morbidity and mortality[1]. In the year2000 alone there were an estimated 9 million new osteoporotic fracture cases.So early diagnosis with anaccurate prognostic markers is needed for effective management[2].Also,RF and anti-CCPmight not appear in early RA and/or serum-negativecases resulting in misdiagnosis. As a result,preventive intervention stillinadequate [3,4].Therefore, it was urgent to improve current diagnostic and disease-monitoring tests. Serum 14-3-3η is avalidated inflammatory biomarker with diagnostic and prognostic value that improved the sensitivity of RA diagnosis and cover the shortage of detection of RF and anti-CCP[1,5].Maksymowych et al.,found that pairing anti-CCP with RF delivered an AUC inferior to that of 14-3-3η combined with RF or to that of 14-3-3η and anti-CCP,indicating that 14-3-3η is more potential than RF and anti-CCP for RA diagnosis. So, combination of the 3 indices can yield improved diagnostic value [6,7].

Today, it is not well established how 14-3-3η protein plays a critical role in RA patients accompanied with OP? There were few previous studies about 14-3-3η level change in RA with OP. Accumulating evidence has indicated that 14-3-3η protein is elevated in RA patients,contributing to up-regulation of pro-inflammatory mediator expression and cytokines playing an important role on the pathophysiological process of joint damage and remodeling process. Based on this, 14-3-3η protein may affect occurrence and progression of osteoporosis[8,9,10].

The aim of this study was to evaluate correlation among 14-3-3ηprotein and inflammation, bone remodeling,osteoporosis. Also tosuggest a mechanism of which 14-3-3η protein affecting osteoporosis development in RA patients.

Methods:

The present study was a cross-sectional case control study that included seventy-two RA patients and twenty-five controls. Demographic and clinic-pathological characteristics of the patients were collected,Disease activity was recorded as the diseaseactivity score in 28 joints (DAS28).Patients were diagnosed as RA based on the 1987 revised criteria of the American College of rheumatology. RA patientsBased on BMDT score, were divided into three subgroups of normal, osteopenia, and osteoporotic.According to world health organization (WHO) definition, i.e., BMD ≥ 2.5 standard deviations below the young adult mean (or T score ≤- 2.5) was defined as osteoporosis, and osteopenia was defined as BMD ≤- 1.0 SD and >- 2.5 SD.Bone mineral density (BMD) was measured using dual energy X-ray absorptiometry (DEXA) at lumbar spine L2-4 and proximal femur for all the participants ,BMD was automatically calculated from bone area (cm2) and bone mineral content (g) and expressed in gram per square centimeter .Excluded from the study,Patients with acute or chronic infectious disease, endocrinal disease, drugs (androgens, steroids, anticonvulsant,estrogen, alcohol users, anticoagulant), or other causes which lead to secondary osteoporosis or any drug that affectsbone metabolism within 1 month before blood-specimen collection. Serum samples were collected,separated immediately and stored. RF and C-reactive protein (CRP) concentrations were measured by nephlometry on IMMAGE 800 (Beckman Coulter, Inc.). Erythrocyte sedimentation rate (ESR)by Westergren method. Quantitative enzyme-linked immunosorbent (ELISA) was used for 14-3-3η,TNF-α, and IL-6 assay. Anti CCP and bone-turnover markers, including 25-hydroxy vitamin D3 (25[OH] D3), parathyroid hormone (PTH), procollagen type I N-propeptide (PINP) and β-crosslaps (β-CTx) were tested using Cobase601 electrochemiluminescence immunoassay(F.Hoffman-La Roche Ltd.). All assays were performed in strict accordance with manufacturer provided protocols. The study had local Research Ethics approval.

Statistical analysis:

The analysis of the data was carried out using the IBM SPSS 20.0 statistical package software (IBM; Armonk, New York, USA) and MedCalc software, version 15.0 (MedCalc Software). Normality of the data was tested using the Shapiro-Wilk or Kolmogorov-Smirnov tests. Data were expressed as mean±SD, minimum and maximum of range for quantitative parametric measures, median, interquartile range (IQR) for non-parametric data, in addition to both number and percentage for categorized data.

The Student *t*-test for parametric data and Mann-Whitney U test for non-parametric data were used for comparison between two independent group, while Kruskal-Wallis (KW) was used for comparison between independent groups for non-parametric datafollowed by Dunn's post-hoc test assess intergroup differences. The *Chi‑square test or Fisher's exact test* were used to compare categorical variables.

Pearson's correlation was used to estimate the strength of linear relationship between two continuous variables. Receiver operating characteristic (ROC) curve was computed and the area under the ROC curve was used to evaluate the ability of different biomarkers to diagnose early RA. A p-value less than 0.05 was considered significant.

Results:

The general characteristics of subjects are summarized in (**Table 1**). There were significant difference between patients and controls with respect to all parameters except sex. The results of our study confirm the observation of previous studies that serum concentrations of inflammatory markers are elevated in the majority of patients with RA.

Out of 72 RA patients included in our study, 9 with early RA patients, 63 with established RA. The median level of 14-3-3η in early RA group was significantly elevated than that in the established RA group., While the other inflammatory markers are lower (**Table 2**). Using serum 14-3-3η assessment in detection of early RA had the upper hand over the other inflammatory markers indicating its role in initiating inflammatory process in the early phase resulting in new joint involvement and progression of damaged one which could point out to patients at high risk of joint damage. Also, the persistent high level of 14-3-3η protein level in established group despite treatment indicates Systemic low grade inflammation that is generally not detectable by routine CRP testing.

RA patientsBased on BMD T score, were classified in **Table 3** into three subgroups. Significant differences for age, disease course and DAS28 were detected among the three groups ($p<0.05$) and the osteoporotic group presented with a significantly elder age and longer disease course ($p<0.05$). Serum 14-3-3η, IL-6,TNF-α and β -CTX level were the highest in osteoporosis group and there were significant difference among the normal BMD, osteopenia and osteoporosis groups which means that higher expression of 14-3-3 proteins is associated with the development of osteoporosis in RA patients and less favorable outcomes in concentration-dependent manner that is capable of inducing both pro-inflammatory cytokines involved in the degradation of cartilage and bone. PINP value decreased gradually from normal group to osteoporosis group and were significantly different among the groups.

These results suggested that this molecule might also enable us to further sub-classify the disease so could be a new addition for a rheumatologists diagnostic and treatment strategy in RA. Further work on these abilities can provide more effective targeted patient care.

The associations of 14-3-3η to other markers in RA patients are summarized in **Fig 1** There were positive correlation among 14-3-3η and (IL-6, β -CTX and TNF). Meanwhile, we found a significantly negative correlation between PINP and 14-3-3η ($p<0.05$). Also, significant negative correlation was observed between DAS28 with (14-3-3η,IL-6,TNF- α,B-CTX) and positive correlation with PINP . Regarding PTH, There were significant positive association with (IL-6 & TNF-α and B-CTX). However, vitamin D was negatively correlated with PTH in all subjects. There was a positive correlation between serum 14-3-3η level, inflammatory cytokines and DAS28.These findings suggest that 14-3-3η is important mediator of inflammation playing a pivotal role in the development and progression of RA and may serve as a novel marker for monitoring disease activity.

Subjects with severe vitamin D deficiency was 26.4% of the studiedcases(< 10 ng/ml 25(OH)D, whereas 58.3% had vitamin D insufficiency or borderline [25(OH)D 10 to < 20 ng/ml. Subjects with normal (vitamin D sufficient) represented 15.3% [25(OH)D ≥ 20 ng/ml. Results showed statistically significant difference in the prevalence of vitamin D abnormalityin osteoporosis subjects. When PTH levels were

compared in the three groups, levels were significantly higher in subjects with severe vitamin D deficiency ($p < 0.06$) when compared to vitamin D sufficiency or vitamin D insufficiency subjects indicating accelerated bone loss in patients with inflammatory disorders, exacerbated with Vitamin D deficiency.

Significant positive correlation between serum 14.3.3 protein and RF & Anti-CCP ($p < 0.05$) were found making sense that combination of those biological biomarkers may increase the accuracy of early diagnosis of rheumatoid arthritis. In addition, To assess the diagnostic performance of various biomarkers in RA group, The Receiver operating curve (ROC)analysis was done**figure 2**. Serum 14.3.3 protein had the best diagnostic performance and can be used to discriminate between patients and controls at a cutoff level of > 0.1, with 100% sensitivity, 100% specificity. Pairing anti-CCP and RF delivered an AUC (0.907,95%CL 0.757-0.979) inferior to that of 14-3-3η if combined withRF(1.0,95%CL 0.897-1.0) and to that of 14-3-3η when combined with anti-CCP(1.0,95%CL 0.897-1.0),indicating that 14-3-3η is a RA-specific marker which ismore potential than and superior to RF and anti-CCP complementing them for RA diagnosis. Combination of these indices increase their diagnostic value thereby preventing those patients from unfavorable disease outcomes caused by diagnostic delay.This further would enable an approach to the idea of personalized medicine, whereby the patient would have all these biomarkers measured and thus allocated a specific treatment regimen.

To determine which variables could predict BMD level, also to investigate the association between laboratory characteristics and the presence of osteopenia or osteoporosis in patients with RA, multiple regression was performed on all variables (**Table4**). The results showed that both age and disease duration of the participants appeared to be significant determinants of BMD. Univariate analysis revealed that among all measurements, 14-3-3η, TNF – α and IL6 were associated with RA-related osteoporosis, Whereas bone-turnover markers were not identified to be highly significant except PINP. Then, these five indices were selected into multivariate analysis and 14-3-3η were identified as an independent risk factor for RA-related osteoporosis (OR,1.271 ; % CI,1.031-1.906 ; P <0.01).

Our results strongly suggest the pathogenic and diagnostic role of serum 14-3-3η in RA which can be explained by the ability of 14-3-3η protein to provoke inflammatory and degenerative factors.

Table 1: Demographic, clinical and laboratory characteristics of the study participants

	RA patients (N=72)	Control (N=25)	p value
Age (y)	48.2±7.5 (35-62)	43.5±6.4 (35-56)	0.006*
Sex			
Male	36 (50%)	13 (52%)	0.863
Female	36 (50%)	12 (48%)	
Disease duration (m)	53±50.6 (3-244)		
Early RA (≤12 m)	9 (12.5%)		
Established RA (>12 m)	63 (87.5%)		

14-3-3η (ng/ml)	0.42±0.28 (0.07-1.17)	0.11±0.05 (0.03-0.19)	<0.001*
TNF-α (pg/ml)	15.1±5.6 (6.8-30.1)	8±1.9 (4.3-11.1)	<0.001*
IL-6 (pg/ml)	10.1±3.5 (3.3-18.2)	7.6±2.4 (3.3-12.3)	0.002*
B-CTX (ng/ml)	0.41±0.2 (0.17-0.81)	0.14±0.04 (0.06-0.19)	<0.001*
PINP (ng/ml)	40.8±11.1 (17.7-62.2)	47.2±5.8 (21.3-58.3)	<0.001*
Anti-CCP (U/ml)	100.3±23.9 (63.9-162.1)	65.9±14.1 (43.2-94.3)	<0.001*
RF (U/ml)	97±70.9 (29.1-278.1)	7.8±2.5 (3.2-14.2)	<0.001*
ESR (mm)	59.2±30.3 (16-151)	5.8±2.4 (2-9)	<0.001*
CRP (mg/L)	25.7±13 (6.5-53.6)	5.4±1.6 (2-8.2)	<0.001*
DAS28	4.7±0.6 (3.1-5.8)	-	
25(OH)D (ng/ml)	14±5.6 (3.5-28.4)	31.3±6.3 (19.7-44.3)	<0.001*
Normal (sufficiency)	11 (15.3%)	24 (96%)	<0.001*
Insufficiency (borderline)	42 (58.3%)	1 (4%)	
Severe deficiency	19 (26.4%)	0 (0%)	
PTH (pg/ml)	40.8±10.1 (22.7-65.6)	23.5±6.3 (12.6-36.9)	<0.001*
DEXA			
Normal	39 (54.2%)	25 (100%)	<0.001*
Osteopenia	17 (32.6%)	0 (0%)	
Osteoporosis	16 (22.2%)	0 (0%)	

**** p-value considered significant at <0.05- Numerical data expressed as mean, standard deviation (SD) and range***

Table 2: Early and Established RA

	Early RA (≤12 m) (N=9)	**Established RA (>12 m) (N=63)**	**p value**
	Median (*IQR*)	**Median (*IQR*)**	
14-3-3η (ng/ml)	0.28 (0.26-0.64)	0.14 (0.11-0.15)	<0.001*
TNF-α (pg/ml)	10.4 (9.7-11.9)	15.9 (11.3-18.7)	0.007*
IL-6 (pg/ml)	8.1 (5.7-8.9)	10.4 (7.8-13.2)	0.013*
B-CTX (ng/ml)	0.22 (0.2-0.25)	0.38 (0.26-0.63)	0.002*
PINP (ng/ml)	55.5 (51.2-57.2)	38.9 (31.5-45.8)	<0.001*
Anti-CCP (U/ml)	88.9 (81.4-97.3)	94.7 (84.9-112.4)	0.16
RF (U/ml)	41.3 (34.4-43.2)	70.4 (50.4-158.5)	<0.001*

ESR (mm)	30 (27-36)	57 (42-87)	<0.001*
CRP (mg/L)	15.5 (11.4-16.7)	23.1 (17.3-38.5)	0.002*
DAS28	4.8 (4.7-4.9)	4.5 (4.1-5.2)	0.865
25(OH)D (ng/ml)	18.6 (13.1-22.3)	13.2 (9.3-16.7)	0.027*
Normal (sufficiency)	5 (55.5%)	7 (11.1%)	0.031*
Insufficiency (borderline)	3 (33.4%)	38 (60.3%)	
Severe deficiency	1 (11.1)	18 (28.6%)	
PTH (pg/ml)	32.5 (30.1-37.2)	40.1 (33.6-48.7)	0.017*
DEXA			
Normal	5(65.3%)	30 (47.6%)	0.013*
Osteopenia	3 (33.3%)	17 (27%)	
Osteoporosis	1 (1.1%)	16 (25.4%)	

**** p-value considered significant at <0.05***

Numerical data expressed as median and interquartile range (IQR)

Table 3: Comparison between different subgroups of BMD among RA patients

	Normal (I)	**Osteopenia (II)**	**Osteoporosis (III)**	**p value**
	(N=35)	**(N=20)**	**(N=17)**	
Age (y)	43 (39-51)	48 (46-55)	57.5 (52.5-59.5)	<0.001*
Duration (m)	18 (13-30)	52 (48-68)	93 (73-150)	<0.001*
14-3-3η (ng/ml)	0.23 (0.15-0.29)	0.46 (0.39-0.51)	0.89 (0.79-0.96)	<0.001*
TNF (pg/ml)	10.7 (8.4-13.4)	17.1 (16.4-18.2)	22.1 (19-25.5)	<0.001*
IL-6 (pg/ml)	8.7 (6.1-10.3)	10.4 (8.9-11.4)	14.2 (13.3-16.1)	<0.001*
B-CTX (ng/ml)	0.25 (0.2-0.31)	0.47 (0.4-0.56)	0.74 (0.67-0.77)	<0.001*
PINP (ng/ml)	48.8 (42.1-55.5)	34.2 (30.7-38.5)	27.9 (21-34.8)	<0.01*
Anti-CCP (U/ml)	89.4 (81.4-99.5)	91.5 (84.5-101.4)	137.3 (104.6-154.1)	<0.001*
RF (U/ml)	47.3 (38.4-58.7)	90.6 (73.8-113.5)	196.5 (173.8-251)	<0.002*
ESR (mm)	41 (30-51)	58 (46-66)	102 (89.5-111)	<0.001*
CRP (mg/L)	16.6 (12.5-19.3)	33.3 (27.6-37.7)	44.8 (39.2-48.1)	<0.001*
DAS28	4.5 (4-4.9)	4.7 (4.3-5.3)	5.3 (5.1-5.5)	<0.001*
25(OH)D (ng/ml)	14.7 (10.1-18.6)	13.6 (12.3-17.1)	9.4 (7.1-13.8)	0.008*
PTH (pg/ml)	32.9 (30.1-37.2)	45.1 (41.8-47.3)	55.4 (53.2-58.7)	<0.001*

**** p-value considered significant at <0.05***

Numerical data expressed as median and interquartile range (IQR)

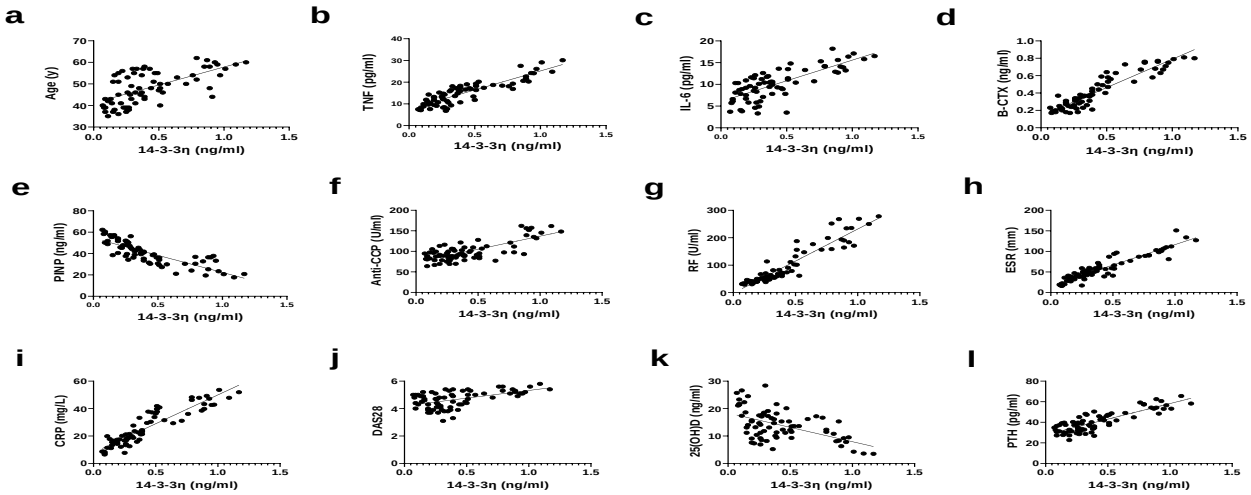

Fig 1: Scatter plots showing correlation among patients with RA

Fig 2: ROC curve for different parameters to predict cases (vs control).

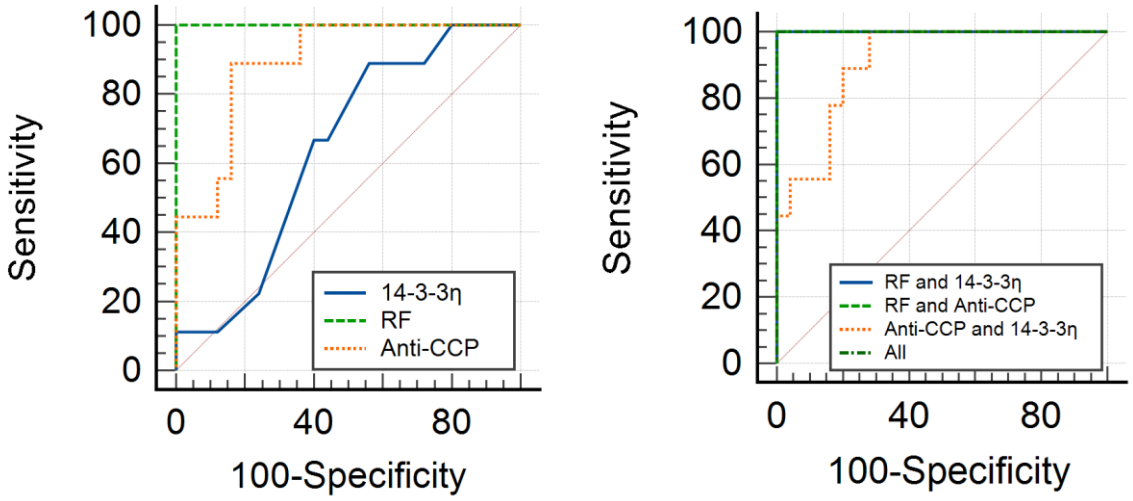

Table 4: Multiple logistic regression model of variables associated with osteopenia and osteoporosis in patients with RA.

Univariate	Osteopenia		Osteoporosis	
	OR (95% CI)	p value	OR (95% CI)	p value
Age (y)	1.1 (1.01-1.21)	0.032	1.33 (1.16-1.54)	<.001
Disease duration (m)	1.15 (1.07-1.24)	<.001	1.18 (1.1-1.27)	<.001

14-3-3η (ng/ml)	1.88 (1.28-2.77)	0.001	3.41 (1.88-6.16)	<.001
TNF (pg/ml)	2.11 (1.42-3.14)	<.001	5.37 (2.34-12.32)	<.001
IL-6 (pg/ml)	1.37 (1.06-1.78)	0.018	4.07 (2.02-8.2)	<.001
B-CTX (ng/ml)	0.38 (0.12-1.24)	0.108	0.54 (0.17-1.75)	0.306
PINP (ng/ml)	0.55 (0.38-0.81)	0.002	0.45 (0.3-0.69)	<.001
Anti-CCP (U/ml)	1.01 (0.98-1.05)	0.459	1.012 (0.999–1.013)	0.89
RF (U/ml)	0.979 (0.974–1.000)	0.73	1.24 (0.991–1.021)	0.39
ESR (mm)	1.0 (0.981–1.012)	0.96	1.07 (0.996–1.019)	0.22
CRP (mg/L)	0.998 (0.997–1.010)	0.78	1.001 (0.987–1.013)	0.62
DAS28	1.9 (0.65-5.58)	0.242	139.56 (9.43-2066.44)	<.001
25(OH)D (ng/ml)	0.99 (0.89-1.1)	0.866	0.989 (0.967–1.019)	0.758
PTH (pg/ml)	0.985 (0.949–1.010)	0.12	0.982(0.946–1.013)	0.17
Multivariate				
14-3-3η (ng/ml)	1.411 (1.125–1.920)	0.03	1.271 (1.031–1.906)	<0.01
TNF (pg/ml)	0.996 (0.958–1.035)	0.11	1.002 (1.000–1.005)	0.04
IL-6 (pg/ml)	1.037 (0.995–1.081)	0.07	1.001 (0.999–1.004)	0.26
PINP (ng/ml)	1.005 (1.002–1.007)	0.31	1.003 (1.000–1.006)	0.05

DISCUSSION

RA is a systemic inflammatory disease, If remains untreated leads to joint deformation, osteoporosis, premature disability causing reduced ability or inability to work, reduced quality of life, and even premature mortality[11,12]. There is not yet a cure that effectively reverse the disease yielding a heavy burden on the society[13].Combination of serum RF and anti-CCP with articular and radiographic changes to diagnose RA has been well established, But still patients with undifferentiated arthritis is a challenge either for the conventional diagnosis and classification criteria[14].Thus,how markers for RA diagnosis and disease monitoring works must be studied to optimize current management strategies.To the best of our knowledge, this is the first population-based study in Egyptian to examine the pathophysiological process between osteoporosis possible mechanism and 14-3-3n protein in RA.

Osteoporosis always present in many RA patients even in the early stage, and RA-induced osteoporosis is frequent kind of secondary osteoporosis. But it was rarely reported about the value of serum 14-3-3η in osteoporosis induced by RA,In agree with Gong et al., we demonstrated that serum 14-3-3η was increased in RA patients compared to control and increased in early RA patients compared with that of established RA [2]. These results suggested that 14-3-3η was associated with the development and progression of osteoporosis in RA patients. So can be used as osteoporosis predictor in RA patients and helping in evaluation of patient management strategy needs [15,16].

In agree with Sun et al.,14-3-3nwere significantly different among RA subgroups with normal BMD, osteopenia, and osteoporosis. Additionally, 14-3-3η-positive RA patients often accompanied withhigher disease severity or worse outcomesas it was positively correlated with DAS28 score[17]. Another research found that RA patients who had a normal 14-3-3η state had lower risk and better clinical

treatment effectwhich is consistent with our findings showing that serum 14-3-3η levels in the osteoporosis and osteopenia groups were higher compared with those values in the normal bone mass group [18]. So, we could suggest that serum 14-3-3η might reflectdisease activity to a certain extent and associated with osteoporosis development in RA patients[2,6].

Detecting a high level of 14-3-3η protein extracellular in serum acts as a cell damage signal that induces pro-inflammatory cytokines and bone degrading enzymes[19].So we tried to evaluate the relationships among 14-3-3η protein and [TNF-α, IL-6, β -CTX, PINP], analyzing the mechanism of 14-3-3η protein inosteoporosis development and progression in RA patientsthrough adjusting inflammation and bone remodeling course, but the details were still not clear.Confirming this potential relationship could provide new insights for early identification of patients at risk of osteoporosis as well as support the use of cytokine-based antibody therapies as potential interventions to reduce bone loss.

In agree with Gong's study[2],There was significant negative correlationbetween 14-3-3η and BMD.Moreover,Maksymowych, stated that 14-3-3η expression was higher in patients with radiographic changes for damage and progression.This means it can affect osteoporosis development in RA patients.Meanwhile,in agreement with one study we found significant differences in the level of PINP and β -CTX among the normal BMD,osteopenia and osteoporosis group as well as a positive correlation between β -CTX and 14-3-3ηand a negative correlation between PINP and 14-3-3η ,Both β -CTX and PINP are key biomarkers of bone remodeling[17].

In agreement with Sun's study, IL-6 was negatively correlated to BMD, indicating that chronic inflammation leads to osteoporosis occurrence[18]. In the current study,we found a significant difference in the level of IL-6 and TNF-α among the three groups, Also, there were positive correlation between 14-3-3η and both IL-6& TNF-α. Subsequently, we concluded that the effect of 14-3-3η on the osteoporosis progression may be due to its adjusting inflammation and bone remodeling.

We speculated one theory for 14-3-3η induced osteoporosis,14-3-3η protein regulates proliferation and differentiation of cells, Stimulation of THP-1 cells with recombinant 14-3-3η resulted in modulating mitogen-activated protein kinase (MAPK) signaling pathway up-regulating joint damage inflammatory factors such as IL-1, IL-6 that showed a tendency of dose-dependent increase [20,21].

Pro-inflammatory cytokines usually regulated in cascades, where induction of the early cytokines serves to stimulate release of later cytokines. IL-1 stimulates IL-6, and TNF-α release, Both activate the genesis of osteoclasts, leading to bone loss[20,22]. TNF-αaffect osteoclast formation through2 mechanisms, the 1st process occurs when stromal cells are exposed to TNF-αand amplify receptor activator of nuclear factor-kappa-B ligand (RANKL), which is well known for its role in normal bone modeling and remodeling through enhancing osteoclast activation and differentiation. The 2nd mechanism suggested that TNF-α may promote osteoclast formation by directly stimulating its precursors in absence of stromal cells responsive to the cytokine, perhaps through activation of transforming growth factor [2].TNF-α is a vital mediator of bone resorption and along with other pro-inflammatory cytokines maintain bone homeostasis and remodeling by initiating osteoclastogenesis and inhibiting osteoblast [25,26].

All markers indicating bone turnover were significantly different among the groups.A significantly higher PTH&B-CTX and lower25(OH) vitamin D3 &PINP in the osteoporosis group was observed.Moreover, a significant positive correlation was found between PTH and IL-6 as well as TNF. Bone-turnovermarkers, including 25(OH) D3, PTH, PINP, andβ-CTX are used clinically to evaluate patient bone-metabolismstatus as they are products of bone formation and/or resorption and reflects activation of osteoblasts or osteoclast [23,24].But, Bone markers are not used for osteoporosis diagnosis because there is a great overlap between levels of osteoporotic and non osteoporotic patient. However, they can be helpful in estimating bone turnover rates [27]. PTH and 25(OH) vitamin D3indirectly stimulates bone resorption by acting upon its receptors on osteoblasts to produceIL-6.Hence, accelerated bone loss has been observed in patients with inflammatory disorders, exacerbated with Vitamin D deficiency [28 ,29].We observed low BMD with advanced age as aging is also a contributing factor to increased levels of pro-inflammatory cytokines and 25(OH) vitamin D3 deficiency [30].

In the present study, with the help of DAS28 we have measured disease activity of RA patients. DAS28 correlated significantly with 14-3-3η, Inflammation measured by hs-CRP & cytokines and radiological progression., 14-3-3η level were higher in patients with more joint damage and can be used as a marker for radiographic progression and probably a new therapeutic target. However, another study performed in Canada demonstrated that 14-3-3η levels are not correlated with DAS28 or CRP, contradicting the results obtained in the present study indicatingvariable correlation of 14-3-3η expression levels with disease activity in patients with RA [19].

In addition, the significant increase of and the positive correlation betweenCRP and cytokines levels observed in the current study indicates that cytokines and acute phase proteins reciprocally regulate each other's expression and activities. This is in agree with some previous observations,reporting communication network between fibroblasts, macrophages, lymphocytes and hepatocytes. Activation of the network results in inflammation and progressive destruction of joints [31]. In the case of RA, the presence of high concentrations of acute phase proteins in the circulation is associated with a more severe progressive course of the disease characterized by intense bone resorptionwhich reflects the higher circulating levels of cytokines observed in Osteoporosis subgroup [32].

Serum 14-3-3η levels were also observed to be positively associated with RF and ACCP levels [19]. Similar results have been obtained in other studies, which suggest that 14-3-3η expression may be correlated with RF and ACCP levels in RA patients (6,10). In the present study, the serum 14-3-3η were positively correlated with RF and ACCP levels in RA, which was consistent with the above-mentioned studies due to the fact that 14-3-3η, RF and ACCP are all involved in the release of pro-inflammatory factors, including IL-6, which are correlated with inflammation in RA [33]. Therefore, it was expected for 14-3-3η serum levels to have a positive correlation with RF and ACCP levels in patients with RA in the present study.

As for serum 14-3-3η, specificity value of 97% in comparison to44 % for RFand 84 % for ACCP, we found specificity value of 97% using 14-3-3 η levels in addition to anti-CCP and RF antibodies indicate that precision of diagnosis can be significantly enhanced, making the diagnosis more certain.Zeng and Tan (2018) stated that the diagnosis of RA patients improved through combining serum14-3- 3η protein with

rheumatoid factor and ACCP [34].More recently, they also reported that targeting 14-3-3η using an antibody-based approach in the collagen induced arthritis mouse model delayed the onset of disease and reduced the overall disease severity [35]. Therefore, probably can be considered for clinical use or we would have different guidelines.

Conclusion:

Early identification and aggressive treatment before the onset of joint erosion are important.Therefore, 14-3-3η protein providing a novel marker for predictingOP occurrence and to stratify patients according severity . It could be a core tenets in the management if combined with existing markers for more effective treatments and could be the route to personalized treatment approach.

Limitation:

Current study revealed some different results than some of other countries; this might be due to different ethnic origin, different life style,different inflammation levels, different treatments and interventions in RA patients among different researches, or related to methodological differences, cytokines assessed as well as how osteoporosis has been determined in different populations.

Declarations

Ethics approval and consent to participate

This study was approved by Minia faculty of medicine ethical committee. Written informed consent was obtained from all participants and from a parent and/or legal guardian for those who are under 16. Methods were performed in accordance with the ethical standards as laid down in the Declaration ofHelsinki and its later amendments or comparable ethical standards.

Consent for publication

- Not applicable.

Competing interests

"The authors declare that they have no competing interests

Authors' contributions

All authors read and approved the final manuscript.

Naglaa farag wrote the main manuscript text

faten ismaeil selected the cases and evaluated them clinically

mahmoud mousa collected the referances for writting +reviewed the manuscript."

eman elsayed: reviewed the manuscript

OMNIA kamal made statistical analysis , wrote the results

samar mansour : share in writting manuscript

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