The comparison of serum biomarkers in patients with osteoarthritis and rheumatoid arthritis

Comparacion de biomarcadores sericos en pacientes con osteartritis y artritis rematoidea

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ABSTRACT

Introduction: This study aims to assess the status of serum vitamin D, parathyroid hormone, type II collagen, calcium, phosphate, albumin, and alkaline phosphatase in osteoarthritis and rheumatoid arthritis patients and to study their association with rheumatoid arthritis disease activity. **Materials and Methods:** This prospective cross-sectional study was conducted at the clinical analysis department, College of Pharmacy, Hawler Medical University in 2017. They study samples were collected at Rizgary Teaching Hospital during the period September 2015 to January 2016. A total of (N=156) participants were included: (N=53) patients with rheumatoid arthritis (RA), (N=53) with osteoarthritis (OA), and (N=50) healthy controls. Enzyme Linked Immuno Sorbent Assay kits determined serum vitamin D, parathyroid hormone, and type II collagen; and serum albumin, calcium, phosphate and alkaline phosphatase, were determined by standard colorimetric methods. **Results and Discussion:** Statistically significant higher levels of parathyroid hormone and type II collagen, with lower levels of Vitamin D, were found in the osteoarthritis group than the rheumatoid arthritis group and the healthy controls (P=0.007, P<0.001, P= 0.005) respectively. Multiple linear regression showed a statistically significant difference in serum type II collagen as a dependent variable, in patients suffering from RA or OA compared to the healthy control group; after adjusting for the effect of other independent study variables, there was a mean increase of (45.90 nmol/L, P<0.001) in RA patients, and OA patients showed greater levels of type II collagen (73.950 nmol/L) than the health control group (P<0.001). **Conclusions:** Elevated type II collagen levels, in conjunction with a low vitamin D status, may be strong discriminator between osteoarthritis and rheumatoid arthritis patients.

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INTRODUCCTION

Osteoarthritis (OA) and rheumatoid arthritis (RA) are the two most common rheumatic diseases, accounting for a large percentage of disability worldwide. RA is an autoimmune disease of unknown etiology, characterized by systemic polyarthritis that affects both small and large joints [(1)]. RA occurs in all ethnic groups and throughout the world, with a female to male ratio of 3:1[(2)]. Rheumatic disease incidence increases with age, such that 56% of those over 65 years will suffer from joint complaints. The most common type of arthritis is OA. OA and RA have different etiologies; however, destruction of the articular cartilage is the combined feature, and the cause of the typical joint dysfunction [(3)].

Vitamin D plays an essential role in calcium and phosphate metabolism, bone formation, and mineralization homeostasis, and is known to play a role in the maintenance of immune homeostasis,[(4)] reduced vitamin D intake has been linked to increased susceptibility to the development of RA and found to be associated with disease activity in patients with RA [(5)]. In OA, it is hypothesized that insufficient levels of vitamin D impact knee joint cartilage and lead to the development and progression of OA of the knee,[(6)] given the influence of vitamin D on bone quality [(7)].

In RA, the presence of bone erosion correlates with high parathyroid hormone (PTH) levels and low bone mineral density (BMD). These findings suggest that treatments to prevent bone loss or to suppress PTH levels might positively affect the progression of bone erosion in RA [(8)]. In patients with OA, PTH has an inhibiting role regarding terminal differentiation of chondrocytes and in turn, suppresses OA progression [(9,10)].

Cartilage destruction is a crucial aspect of both types of arthritis and is characterized by the degradation of cartilage proteins, proteoglycans, and type II collagen, which are embedded within the extracellular matrix. Degradation of type II collagen results in irreversible damage and appears to be a candidate as a marker of cartilage degradation and disease activity in arthritis [(11)]. The aim of the study is to evaluate the status of vitamin D, parathyroid hormone, type II collagen, calcium, phosphate, albumin, and alkaline phosphatase in patients with OA and RA, and to study their association with RA disease activity.

MATERIALS AND METHOD

Study Groups: This was a prospective cross-sectional study comprising (50) healthy volunteers individuals were chosen randomly; these had no history or clinical sign and symptoms of RA and OA, and were aged 21 to 74 years, both genders were included and 106 patients: 53 patients with OA, and 53 with RA. Disease Activity Scores (DAS 28) were assessed for all RA patients by a specialist, Disease Activity Score 28 **(**DAS 28) involved calculating number of tender and swollen joint in 28 joints includes shoulders, elbows, wrists, knee, small joints of hands (metacarpophalangeal (MCP) joints, interphalangeal (PIP) joints, interphalangeal (IP) joints of thumps), visual analogue scale (VAS) and ESR or CRP.

All patients were aged between 21 to 75 years, and both genders were included. The participants were patients who were admitted to, or received consultations at, Rizgary Teaching Hospital between September 2015 and January 2016, and who agreed to complete a questionnaire on disease manifestations.

All participants were diagnosed and evaluated by a specialist in the Rheumatology department, who used American College of Rheumatology criteria (2010) [(12)]. The study was approved by the Ethics Committee of the College of Pharmacy, Hawler Medical University, and participants consent was obtained in verbal form.

Sample Collection: Eight milliliters (mls) of venous blood were collected, without tourniquet, from each subject. The blood was allowed to clot, and centrifuged for 15 minutes at 3000 rounds per minute. The resultant obtained sera were dispensed into four eppendorf tubes and immediately frozen at -20 °C till the time of assay.

Exclusion Criteria: Patients suffering from liver diseases and renal diseases; Systemic Lupus Erythematosus (SLE); hyperparathyroidism; and hypoparathyroidism; as well as those who had undergone joint replacement; and those receiving estrogen therapy, or calcium, vitamin D or glucose amine supplementation, were excluded.

Study Protocol: A detailed history from both patient groups (RA and OA) and the HC group, were taken and monitoring of disease activity for RA patients, using DAS 28, was performed.
Statistical Analysis: Statistical analyses were performed using SPSS (Statistical Package for Social Sciences) version 21-computer software, in addition to special formulas developed using Microsoft Excel 2016.

Analysis of Variance (ANOVA) was used to test the statistical significance of differences in mean of a normally distributed variable between more than two groups. Further exploration of the statistical significance of the difference in mean between all possible paired combinations of groups was explored by a least significant difference test (LSD) [13]. 'P' value of ≤ 0.05 was considered as statistically significant.

RESULTS

The results were based on analysis of study biomarkers for 53 OA patients and 53 RA patients (N=106), and (N=50) healthy controls. Study biomarkers were evaluated for their relationship to RA disease activity. As shown in table 1, a statistically significant differences were observed in mean age between the studied groups. This (mean ± SD) was significantly higher in OA patients compared to both the HC and RA groups, at (56.6 ± 10.3) years, (47 ±13.7) years, and (45.8 ± 13.3) years respectively, with P< 0.001. The difference in mean age failed to reach statistical significance between the RA and HC groups, with a P value = 0.61. The mean BMI showed a statistically significant difference, and was higher (31.2 ± 4.7Kg/m^2) in the OA group, compared to the HC and RA groups (28.2 ± 5.1 Kg/m^2) and (26.6 ± 5.3 Kg/m^2) respectively, with a significance of P< 0.001.

Table 1: Differences in (mean ± SD) age and Body Mass Index between the studied groups.

Study variables	HC N =50	OA N=53	RA N=53	P (t-test)
Age (years)				< 0.001
Mean ± SD	47±13.7	56.6±10.3	45.8±13.3	
P (LSD) for difference in mean between::				
Rheumatoid arthritis and Healthy controls				0.61 [NS]
Rheumatoid arthritis and Osteoarthritis				<0.001
Osteoarthritis x Healthy controls				<0.001
Body Mass Index (kg/m2)				<0.001
Mean ± SD	28.2±5.1	31.2±4.7	26.6±5.3	
P (LSD) for difference in mean between				
Rheumatoid arthritis x Healthy controls				0.11[NS]
Rheumatoid arthritis x Osteoarthritis				<0.001
Osteoarthritis x Healthy controls				0.002

NS: not significant

A statistically significant difference was observed in the mean of vitamin D across the study groups. This was shown to be higher in the HC group (24.75 ± 16.58 ng/ml) compared to the RA and OA groups,

for which the means were (14.84 ± 12.74 ng/ml) and (9.31 ± 7.79 ng/ml) respectively with a P value = 0.005. The difference in the mean also reached statistical significance when comparing the HC and RA groups, with a P value = 0.007; between the RA and OA groups, with a P value = 0.003; and between the HC and OA groups, with a P value = 0.008 (table 2).

A highly statistically significant difference was observed in the mean of PTH between the groups. This was shown to be higher in the OA group (39.56 ± 28.08 Pg/ml) than in the RA group (26.67 ± 15.49 Pg/ml) and HC group (25.53 ± 15.84 Pg/ml) respectively, with a P value<0.001. There was no statistically significant difference between the RA and HC groups in this regard, with a P value = 0.78 (see table 2).

Similarly, the mean for serum type II collagen was statistically higher in the OA group (190 ± 5.1 nmol/L) compared to the RA and HC groups, for which the mean was (162 ± 5.1 nmol/L) and (116 ± 4.1 nmol/L) respectively, with a P value < 0.001. The differences in mean also reached statistical significance when comparing the RA and HC groups in this regard, with a P value <0.001 (see table 2).

Table 2: Differences in mean of selected serum parameters between the groups.

Study variables	HC N = 50	OA N = 53	RA N = 53	P(T-test)
Vitamin D (ng/ml)				0.005
Mean ± SD	24.75 ± 16.58	9.31 ± 7.79	14.84 ± 12.74	
P (LSD) for difference in mean between:				
Healthy controls and rheumatoid arthritis				0.007
Rheumatoid arthritis and Osteoarthritis				0.003
Healthy controls and Osteoarthritis				0.008
Parathyroid hormone (Pg/ml)				<0.001
Mean± SD	25.53 ± 15.84	39.56 ± 28.08	26.67 ± 15.49	
P (LSD) for difference in mean between:				
Rheumatoid arthritis and healthy controls				0.78[NS]
Rheumatoid arthritis and Osteoarthritis				0.002
Osteoarthritis and healthy controls				<0.001
Type II collagen (nmol/L)				<0.001
Mean± SD	116±4.1	190±5.1	162±5.1	
P (LSD) for difference in mean between:				
Rheumatoid arthritis and healthy controls				<0.001
Rheumatoid arthritis and Osteoarthritis				<0.001
Osteoarthritis and healthy controls				<0.001

NS: not significant

Regarding the other study biomarkers, alkaline phosphatase (ALP) showed a significantly higher mean in the OA group (8.51 ± 4.07 KAU/dl) compared to the RA group (3.68 ± 2.61 KAU/dl) and HC groups (4.78 ± 3.91 KAU/dl), with a P value < 0.001 (see table 3). The presence of statistically significant higher mean serum total calcium, free calcium, phosphate, and albumin were detected in the HC group than the RA and OA groups. The mean value for free calcium in the HC group was (5.13 ± 0.27 mg/dl), for the RA group (4.89 ± 0.18 mg/ml), and for the OA group (4.75 ± 0.37 mg/dl), with a P value < 0.00) (see table 3). The mean values for serum phosphate were, for the HC group (5.55 ± 1.52 mg/dl), the OA group (5.43 ± 1.15 mg/dl), and the RA group (4.3 ± 2.25 mg/dl), with a P value < 0.001. A similar finding was

observed for serum albumin: the mean for the HC group was (4.87 ± 0.66 g/dl), (4.5 ± 0.46 g/dl) for the OA group, and (4.73 ± 0.78 g/dl) for the RA group, with a P value of 0.015 (see table 3).

Table 3: Differences in mean of selected serum biomarkers between the groups.

Study variables	HC N=50	OA N=53	RA N=53	P(t-test)
Alkaline phosphatase (KAU/dl)				<0.001
Mean ± SD	4.78 ± 3.91	8.51 ± 4.07	3.68 ± 2.61	
Calcium (mg/dl)				<0.001
Mean ± SD	9.97±1.06	8.56±1.34	9.05±0.65	
Free calcium (mg/dl)				<0.001
Mean ± SD	5.13±0.27	4.75±0.37	4.89±0.18	
Phosphate (mg/dl)				< 0.001
Mean ± SD	5.55±1.52	5.43±1.15	4.3±2.25	
Albumin (g/dl)				0.015
Mean ± SD	4.87±0.66	4.45±0.46	4.73±0.78	

No significant difference was observed in terms of mean age and BMI, relating to either type of disease activity (moderate activity and high activity) (see table 4).

Table 4: Differences in mean age and Body Mass Index (BMI) between those with moderate disease activity and those with high disease activity in the RA group.

	RA disease activity		
Study variables	Moderate activity N=18	High activity N=35	P(t-test)
Age (years)			0.17[NS]
Mean ± SD	42.3±14.4	47.5±12.5	
Body Mass Index (Kg/m2)			0.89[NS]
Mean ± SD	26.4±5.4	26.7±5.3	

[NS]: not significant

Only type II collagen showed a statistically significant difference between the two disease score activity levels; the mean was higher for moderate activity (164.1 ± 5.2 nmol/L) than high activity (160.9 ± 4.8 nmol/L), with a P value = 0.03. The other studied biomarkers (vitamin D, PTH) showed no statistically significant differences. (Table 5) in this regard.

Table 5: Differences in mean of selected serum parameters between those with moderate disease activity and those with high disease activity in the RA group.

RA disease activity			
Study variables	**Moderate activity N = 18**	**High activity N = 35**	**P(t-test)**
Vitamin D (ng/ml)			0.4 [NS]
Mean ± SD	16.9 ± 19.5	13.78 ± 7.39	
Parathyroid hormone (pg/ml)			0.08[NS]
Mean ± SD	21.49±14.99	29.34±15.27	
Type II collagen (nmol/L)			0.03
Mean ± SD	164.1 ± 5.2	160.9 ± 4.8	

[NS]: not significant

None of the other studied biomarkers (ALP, total calcium, free calcium, phosphate, and albumin) showed statistically significant differences in terms of disease activity scores (see table 6).

Table 6: Differences in mean of selected serum biomarkers between those with moderate disease activity and those with high disease activity in the RA group.

RA disease activity			
Study variables	**Moderate activity N = 18**	**High activity N = 35**	**P(t-test)**
Alkaline phosphatase (KAU/dl)			0.21[NS]
Mean ± SD	3.04 ± 2.22	4.01 ± 2.77	
Calcium (mg/dl)			0.31[NS]
Mean ± SD	8.92±0.57	9.11±0.69	
Free calcium (mg/dl)			0.31[NS]
Mean ± SD	4.86±0.16	4.91±0.18	
Phosphate (mg/dl)			0.25[NS]
Mean ± SD	3.81±1.76	4.56±2.45	
Albumin (g/dl)			0.36[NS]
Mean ± SD	4.87±0.78	4.66±0.77	

[NS]: not significant

A statistically significant change in serum type II collagen was detected when the patient was in the RA, as compared to the HC, group: after adjusting for the effect of other independent variables (their effect fixed to zero), there was a mean increase of (45.90 nmol/L, P<0.001). Patients with OA were seen to have more serum type II collagen at (73.950 nmol/L) than the HC group, with a significance of P<0.001; while the independent variables of: previous fracture, steroidal treatment, non-steroidal

anti-inflammatory treatment, being a smoker, BMI, age, male gender compared to female) were seen to have no significant effect on serum type II collagen, the dependent variable is type II collagen (see table 7).

Table 7: Multiple linear regression model with type II collagen (nmol/L) as the dependent (response) variable; the role of RA and OA compared to healthy controls (HCs); and with selected confounders as explanatory (independent) variables.

Type II collagen (nmol/L)	Partial regression coefficient	P value	Standardized coefficients
RA group, compared to healthy controls	45.906	<0.001	0.700
OA group, compared to healthy controls	73.950	<0.001	1.138
Previous fracture	-0.126	0.24[NS]	-0.016
Steroidal treatment	0.226	0.86[NS]	0.003
Non-steroidal treatment	0.720	0.53[NS]	0.008
Being a smoker	-0.417	0.73[NS]	-0.005
Body Mass Index (kg/m2)	-0.075	0.37[NS]	-0.013
Age (years)	0.040	0.24[NS]	0.017
male gender compared to female	0.744	0.46[NS]	0.011

R2=0.977
P (Model) <0.001

DISCUSSION

Vitamin D in Study Groups.

Observations provide a rationale for the measurement of serum vitamin D (25(OH) D) in patients with OA and the encouragement of supplementation to raise the serum concentration to adequate levels. It is suggested that serum vitamin D measurement should be considered in any patient with symptoms suggestive of OA, even before the appearance of radiographic changes [14]. This study showed that healthy controls have higher serum vitamin D values than RA and OA groups; this corroborates the literature [15] that shows RA patients have lower vitamin D values than healthy controls. Even though the definition of normal vitamin D plasma concentration is still debated, a classification of vitamin D status on which many would agree is: deficiency 25(OH) D <20 ng/mL, insufficiency 20 – 30 ng/mL, normality >30 ng/m.[16]

The differences between study groups are significant, but the mean values in the study groups are at insufficient and deficient levels: the majority of patients with OA showed vitamin D deficiency. This may be reflective of endemic vitamin D deficiency in Iraqi people, which could be attributed to cultural practices such as clothing. Though Iraq is a very sunny country, with strong sunshine most of the year, most people wear long garments and the women usually cover their heads. Therefore, exposure to the sun is low, negatively impacting vitamin D levels.

Vitamin D deficiency in OA patients in this study are consistent with previous reports, [17,18] and adds new information to the current knowledge base. The finding that the healthy control group also had serum vitamin D insufficiency is attributed to lack of exposure to the sun, though the presence of asymptomatic OA in the control group cannot be ignored. Since the performing of radiographic examination in the asymptomatic controls was not clinically indicated, this potential study limitation should be taken into account. This issue could have lowered the mean serum vitamin D level in the control group and reduced the mean difference between the patient and control groups.

Although this was not a longitudinal study, the data demonstrated deficiency in vitamin D in the OA group associated with significantly low bone metabolic markers (serum, calcium, and phosphate) (table 3). This suggests an association between these markers and supports the idea that raising serum vitamin D to sufficient levels may exert beneficial effects in patients with knee OA, as well as the potential of vitamin D treatment in suppression of turnover markers [19].

Higher BMI values (greater than 30 kg/m2) have been shown to be associated with low vitamin D [20] and the current study is in agreement with this finding. Patients with OA are seen to have a higher BMI (31 kg/m^2), associated with low vitamin D (Table 1). Obese patients may have low serum vitamin D levels due to the decreased passage of vitamin D from skin to the general circulation.

Our study showed no significant correlation in serum vitamin D and disease activity in RA patients, this may be due to the small sample size of the RA subgroups (Table 5), this finding is agree with previous researches. [21,15] however, is contrary to some research, [22,23] which shows vitamin D levels correlate inversely with RA activity. Another study also showed decreased serum vitamin D levels in active RA, than in the 'moderate activity' group. [24]

Parathyroid Hormone in Study Groups.

It is well known that vitamin D influence on bone is tightly linked to PTH activity. Vitamin D requires the presence of PTH to act on bone, and it down-regulates PTH synthesis both indirectly (through increasing calcium concentration) and directly (through activating Vitamin D Responsive Elements – VDRE) in the promoting of the PTH gene. Vitamin D also inhibits parathyroid cells' proliferation[25] and modulates sensitivity to calcium, increasing the transcription of CaSR (Calcium Sensing Receptor) [26]. These molecular pathways have an important clinical implications, even though in the last decade other bone health determinants have been used to define vitamin D status. The definition of normal plasma vitamin D concentration is mainly based on the identification of the level able to suppress PTH. This threshold of normality has been set by some at 30 ng/mL (75 nmoL/L) [27].

Given the above – a vitamin D at 30 ng/ml concentration able to control and suppress PTH – this molecular pathway indicates that, vitamin D below this level may lose its ability to control PTH, this effective link clearly demonstrated in our study results, however, the mean vit D below 30ng/ml in all study groups kept PTH within normal level as well as bone biomarkers (calcium, phosphate, and ALP), thus in deficiency of vitamin D, PTH act as a global biomarker for the maintenance of serum calcium directly and phosphate indirectly but such condition enhances demineralization of the bone particularly in the OA group.

Type II Collagen in Study Groups.

The present study demonstrated a highly significant increase in serum type II collagen and PTH in the OA group. This is associated with severe vitamin D deficiency, with many potential consequences. One of the possible explanations for the results found in this study is the role of vitamin D on the cartilage degeneration marker type II collagen. A recent study [28] showed that vitamin D deficiency can directly inhibit the expression of multiple proteins in articular cartilage, such as Transforming Growth Factor– β1 (TGF–β1), Matrix Metalloproteinase-9 (MMP – 9), and Matrix Metalloproteinase-13 (MMP – 13), leading to cartilage degradation. In the absence of dramatic changes in mechanical loading, such as traumatic surgery, the mechanism above might be the main cause of induced OA in the early stages of vitamin D deficiency, which might prove another potential factor involved, in particular, in OA. Furthermore, this confirms the protective role of vitamin D on articular cartilage. The protective effects of vitamin D supplementation on knee OA were observed in the same study [28].

PTH also has an important role in collagen metabolism. One study showed the ability of PTH to inhibit the expression of COL X (an anabolic marker) while promoting the expression of type II collagen

(a catabolic marker), thereby preventing endochondral ossification. [29] This demonstrated high serum level of type II collagen in OA patients compared to healthy controls and RA patients, indicating that the rate of type II collagen degradation is increased in OA.This finding corroborates previous studies which demonstrated an increase in type II collagen breakdown products in urine, [30, 31] and cartilage explants; [32] it is also consistent with the results of the current study, in regard to the combination of vitamin D deficiency and high PTH generating a significant increase in type II collagen in the OA group. The multiple linear regression model with type II collagen showed that collagen is the potential discriminatory biomarker between the studied groups, with R2=0.977, P <0.001.

CONCLUSIONS

The study found that vitamin D deficiency is quite common in RA and OA patients, with insufficiency in the healthy control group. This issue may have been overlooked as a health problem in this region, but it is not uncommon in sunny countries. The presence of significantly high PTH and bone biomarkers (calcium, phosphate, and ALP), in the OA group appears to show the significant role of vitamin D deficiency. PTH plays a major role in the maintenance of serum bone biomarkers (calcium, phosphate, and ALP), within normal range, across all study groups, especially in OA. Type II Collagen is a potential discriminator between healthy subjects and those suffering from osteoarthritis or rheumatoid arthritis. Amoge study biomarkers only Type II Collagen showed significantly high in moderate RA disease activity.

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