

## Original Article

## Level of decorin as a small leucine rich proteoglycan in knee osteoarthritis patients

Rheumatology and Rehabilitation

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### ABSTRACT

**Background:** Knee osteoarthritis is a chronic joint illness distinguished by the gradual cartilage loss of extracellular matrix and subsequent disturbance in chondrocyte biology. Decorin is a small leucine-rich proteoglycan that provides physical linkage among both molecules of aggrecan and collagen II fibrils. It is an important structural component of matrix integrity and cartilage biomechanical function. Its level helps to determine OA grading and subsequently management.

**Objective:** to measure the serum decorin levels in knee OA patients and to explore their association with clinical manifestations and disease severity.

**Methodology:** In this case-control study, 50 patients with varying grades of knee OA were compared to 50 age- and sex-matched seemingly healthy people as controls. The serum decorin level in both groups has been measured using the enzyme-linked immunosorbent assay (ELISA) technique.

**Results:** There had been a highly statistically significant elevation in serum decorin in the OA patient group, with a median of 52.4 (9.8-66.8) compared to 8.6 (6.6-9.13) in the control group, and there was a significant positive correlation ( $p < 0.05$ ) between decorin level and Western Ontario and McMaster Universities (WOMAC).

**Conclusion:** Serum decorin has been significantly elevated in the patient group and its level correlates positively with WOMAC.

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### INTRODUCTION

Articular cartilage is a hyaline cartilage with an extracellular matrix (ECM) made up of collagen, chondrocytes, and proteoglycans that carries our body weight and offers elasticity and flexibility versus tensile forces as well as compressive force resistance<sup>[1]</sup>. Proteoglycans make linkages between the collagens in the extracellular matrix (ECM)<sup>[2]</sup>. Small leucine rich proteoglycans (SLRPs), in particular, have a function in the formation, morphology, as well as bonding of the structure fibril of collagen<sup>[3]</sup>.

Decorin is an extracellular proteoglycan of the (SLRPs); it is classified as class I, together with aspirin and biglycan<sup>[4]</sup>. SLRPs are mostly present in the cartilage, especially that of decorin<sup>[5]</sup>. Decorin has pro-inflammatory and anti-fibrotic activities<sup>[6]</sup>. Decorin has an important role in fibrillogenesis as it limits the fibril diameter and regulates its structure<sup>[7]</sup>. It is also

associated with inflammatory diseases such as osteoporosis, and other conditions such as Ehlers–Danlos syndrome, corneal diseases and muscular dystrophy<sup>[8]</sup>. Therefore, the aim of this study was to measure serum decorin levels in knee OA patients and explore their association with clinical manifestations and disease severity, which subsequently important in determining appropriate management for OA.

### PATIENTS AND METHODS

This Case-control study has been conducted on 100 subjects attended the Rheumatology and Rehabilitation department's outpatient clinic at Al Zahraa University Hospital. Fifty patients with varying grades of knee OA, 3 of them were males and 47 were females as primary OA is more common in females and their ages ranged from 40 to 70 years. Another 50 apparently healthy

individuals with the same age and sex were included for comparison.

**Inclusion criteria** were knee OA patients aged 40 to 70 years old who met the 2010 EULAR Criteria for diagnosis of knee osteoarthritis<sup>[9]</sup>.

**Exclusion criteria** were secondary OA as infective arthritis, crystal deposition arthritis, inflammatory arthritis, malignancy, liver diseases, acromegaly, pancreatic diseases, hypothyroidism, hyperthyroidism, and hyperparathyroidism.

**Ethical consideration:** A written informed consent has been taken from all participants after an explanation of our study. The study was approved from the medical ethical committee of the Faculty of Medicine for Girls(Cairo), Al-Azhar University, Egypt.

The following procedures were performed to all of the patients; Full History taking and clinical examination including general and musculoskeletal examination. Assessment of knee osteoarthritis according to WOMAC index including: Pain, stiffness, limitation of physical activity<sup>[10]</sup>, Lequesen index<sup>[11]</sup> and Kellgren Lawrence classification including 4 grades,( 4 patients were grade I, 12 patients were grade II, 14 patients were grade III and 20 patients were grade IV. Changes in x rays in OA patients as regards KL are: grade I (osteophyte only), grade II (osteophyte and Joint Space narrowing), grade III (as grade II plus minimal sclerosis) and grade IV (as grade II plus severe sclerosis and deformity of bone ends)<sup>[12]</sup>.

Laboratory investigations; general tests: -complete blood count (CBC) and erythrocyte sedimentation rate (ESR). -c-reactive protein (CRP) and rheumatoid factor (RF) using latex agglutination, and antinuclear antibody (ANA) using indirect immunofluorescence technique.

Special test; estimation of serum decorin using the ELISA technique. From bioassay technology laboratory with cat. No E3702Hu according to instructions of the manufacturer. The assay has a standard curve range of 0.2-60ng/ml and a sensitivity of 0.105ng/ml. ELISA reader 1851 Das; Italy and 16041412 Bio Tek; USA washer were used.it was done at Al-Zhrra University Hospital.

### Statistical analysis

The data was statistically analyzed using SPSS version 21. Independent-samples t-test was to compare two means. Mann-Whitney U test was utilized when comparing two groups of non-parametric data. ANOVA was used for comparisons between more than two means, while Kruskal-Wallis test is used to compare multiple groups in non-parametric data. The Chi-square ( $\chi^2$ ) test of significance has been utilized to compare the proportions between qualitative parameters. Scatter plot is a graph in which the values of two variables are plotted along two axes, the pattern of the resulting points revealing correlation present. If one or both of the variables were skewed,

Spearman's rank correlation coefficient ( $r_s$ ) has been utilized for evaluating the degree of association between them. The overall predictability of the parameter and the best cut-off value, as well as the specificity, sensitivity positive predictive value (PPV), negative predictive value (NPV) and accuracy at this cut-off value, were ascertained by employing receiver operating characteristic (ROC curve) analysis. The margin of error accepted has been set to 5% and the confidence interval has been set to 95%. As a result, a P-value of  $\leq 0.05$  has been considered significant while a p-value of  $> 0.05$  has been considered insignificant.

## RESULTS

Regarding demographic data, there has been a highly statistically significant difference in BMI between the patient group with a mean of  $33.26 \pm 5.39$  and the control group with a mean of  $26.30 \pm 3.56$ , with  $p < 0.05$ , and an insignificant difference in age and sex with  $p > 0.05$ , as displayed in table (1). According to WBC, there have been highly statistically significant differences between patients and controls with  $P < 0.05$ , also according to Hb and CRP, there have been statistically significant differences between patients and controls with  $p < 0.05$ ; however, ESR was insignificant with  $p > 0.05$ , as displayed in Table (2).

As regards decorin level, there have been highly statistically significant differences between the patient group with a median of 52.4 (9.8-66.8) compared with the control group with a median of 8.6 (6.6-9.13) with a  $p < 0.05$ , as displayed in table (3). There was a positive significant correlation between serum decorin level and WOMAC ( $p < 0.05$ ), as displayed in figure (1) and table (4).

Due to the wide range of serum decorin level among patients group as it was highly elevated in patients with KL grade I, II, III and slightly elevated in patients with KL grade IV when compared with control group. So they have been further subclassified into: Group (A): Patients with higher serum decorin, they were 30 patients, their radiological assessment according to KL classification were grade (1,2,3), Group (B): Patients with lower serum decorin level and slightly elevated when compared with control group, they were 20 patients with grade (4) on KL There have been highly significant differences between group A, group B and the control group, with a higher level in group A with a median of 66.5 (55.2-70.3) followed by group B with a median of 9.55 (8.4-10.2) and the lowest level in the control group with a median of 8.61 (6.6-9.13) with  $p < 0.05$ , as displayed in table (5).

Receiver operator characteristics (ROC) curves were indices of decorin as predictors of prediction of knee osteoarthritis in included patients, As shown in Figure (1), Table (6), there were significantly predictive as indicated by significantly

large areas under the curves (AUCs) that were used to delineate the best cut off value of decorin, that was > 9.5, with sensitivity of 80%, specificity of 84%, PPV of 83.3%,

NPV of 80.8%, diagnostic AUC of 0.888, and accuracy of 82%.

**Table (1): Baseline characteristics of patients and controls**

Baseline characteristics	Patients Group (n=50)	Control Group (n=50)	Stat. test	p-value
<b>Age (years)</b>				
Mean ± SD	52.54±9.11	49.22±8.89	t=1.844	0.068
Range	40-70	40-70		
<b>Sex</b>				
Female	47 (94.0%)	46 (92.0%)	χ <sup>2</sup> =0.154	0.695
Male	3 (6.0%)	4 (8.0%)		
<b>BMI [wt/(ht)^2]</b>				
Mean ± SD	33.26±5.39	26.30±3.56	t=7.610	0.001*
Range	20.36-46.6	20-39.54		

t: Independent sample t-test, χ<sup>2</sup>: Chi-square test, \*:Significant p-value (<0.05)

**Table (2): Comparison of the patients and controls based on laboratory data**

Laboratory data	Patients Group (n=50)	Control Group (n=50)	Stat. test	p-value
<b>Hb.</b>				
Mean ±SD	11.64±0.90	12.03±0.50	t=-2.708	0.008*
Range	9.2-13	11.2-13		
<b>WBCs</b>				
Median (IQR)	6.3 (5.2-7.7)	4.9 (4.6-5.2)	U=-5.456	<0.05*
Range	4.3-11.8	4.2-6		
<b>ESR</b>				
Median (IQR)	12 (10-14.3)	12 (12-13)	U=-1.402	0.161
Range	5-42	10-15		
<b>CRP</b>				
Median (IQR)	2.5 (2-3)	2 (2-2)	U=-3.160	0.002*
Range	1-13.7	2-4		

Using: t-Independent Sample t-test; U-Mann-Whitney test; IQR: Interquartile range, \*: Significant p-value (<0.05)

**Table (3): Decorin biomarker comparison between patient and control groups**

Biomarker Decorin (ng/ml)	Patients Group (n=50)	Control Group (n=50)	Stat. test	p-value
Median (IQR)	52.4 (9.8-66.8)	8.6 (6.6-9.13)	U=-6.681	<0.05*
Range	5.8-105.4	3.14-13.9		

Using: U-Mann-Whitney test, IQR: Interquartile range, \*: Significant p-value (<0.05)

**Table (4): Correlation between biomarker decorin with BMI and WOMAC, using Spearman's rank correlation coefficient (rs) in patients group**

Parameters	Biomarker Decorin	
	Rs	p-value
<b>BMI [wt/(ht)^2]</b>	-0.014	0.921
<b>WOMAC</b>	0.314	0.022*

Using: Spearman's rank correlation coefficient (rs), \*: Significant p-value (<0.05)

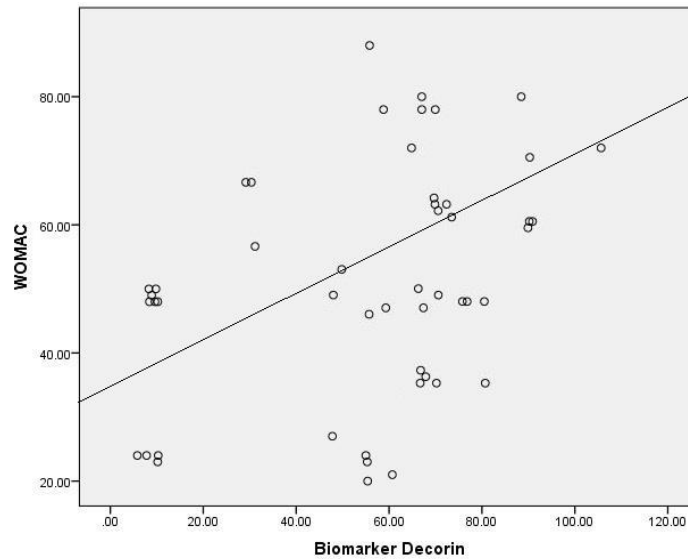


Fig. (1): Scatter plot, positive correlation between decorin and WOMAC in patients group.

Table (5): Comparison of groups (A) (B), and control based on the biomarker decorin.

Biomarker Decorin (ng/ml)	Patients Group		Control Group (n=50)	Stat. test	p-value
	Group A (n=30)	Group B (n=20)			
Median (IQR)	66.5 (55.2-70.3)A	9.55 (8.4-10.2)B	8.61 (6.6-9.13)B	H= 65.648	<0.05*
Range	44.8-105.4	5.8-55.4	3.14-13.9		

Using: H-Kruskal–Wallis was performed & Multiple comparison between groups through Mann-Whitney test, IQR: Interquartile range, Values in each row which have different letters are significantly different at (P<0.05) , \*: Significant p-value (<0.05), Group A= KL classification in (Grade I,II,II), Group B= KL classification in (Grade IV)

Table (6): Diagnostic accuracy of decorin

Cut-off	Sensitivity	Specificity	PPV	NPV	AUC [95% C.I.]
>9.5	80%	84%	83.3%	80.8%	0.888 [0.809-0.942]

PPV: positive prediction value, NPV: negative prediction value, AUC: area under the curve, CI: confidence interval

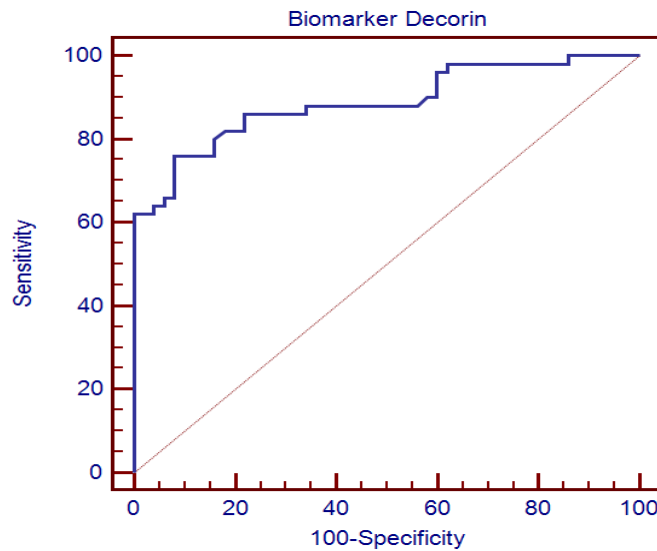


Figure (2): ROC curve of decorin for discrimination of knee OA patients and controls.

**DISCUSSION**

Knee OA is a chronic degenerative disease that causes cartilage degeneration, subchondral bone erosion and

synovitis. Patients present by clinical symptoms, like pain, stiffness, and limitation of range of motion<sup>[13]</sup>.

Cartilage degeneration is the most significant change for occurrence and progression of OA. Decorin regulates aggrecan network integration by increasing aggrecan-aggrecan and aggrecan-collagen molecular adhesion,<sup>[14]</sup> thus improving aggrecan network integration in the cartilage extracellular matrix<sup>[15]</sup>. It is particularly destructed by extracellular matrix metalloproteinases (MMP) including MMP-2, MMP-3 and MMP-7<sup>[16]</sup>. Decorin loss aggravates aggrecan depletion as well as surface fibrillation, resulting in accelerated cartilage damage<sup>[17]</sup>.

In the present study, as regard to decorin level, there were highly statistically significant differences between the patient group with a median of 52.4 (9.8-66.8) and the control group with a median of 8.6 (6.6-9.13) (p-values <0.05).

There were also highly statistically significant differences in decorin levels between groups A, B, and the control group, with a higher level in group (A) = KL classification in (Grade I, II, III) with a median of 66.5 (55.2-70.3), followed by group (B) = KL classification in (Grade IV) with a median of 9.55 (8.4-10.2), and the lowest level in the control group with p-value <0.05, this may be explained by marked loss of cartilage in severe OA in patients group B.

A positive correlation between serum decorin levels and WOMAC was also discovered in this study, with p-value <0.05. This is in agreement with Ozlar<sup>[18]</sup> who studied 44 knee OA patients and investigated levels of serum decorin and their relationship with clinical indices. He found significantly elevated serum decorin levels in knee OA patients relative to the control group and a positive association between levels of serum decorin and WOMAC score. The study by Bock et al.<sup>[19]</sup> demonstrated that levels of SLRPs like decorin and biglycan had been significantly increased in OA patients relative to the healthy group. This study was done by taking knee cartilage samples from 10 knee OA patients.

In contrast to our study, Manfort *et al*<sup>[20]</sup>, reported that there have been no differences in serum decorin levels between early-stage OA patients and the healthy group.

In the present study, as regard to age, we found no statistically significant differences between the patient group, with a mean of 52.54±9.11, compared with the control group, with a mean of 49.22±8.89, with P value >0.05.

This is consistent with Ozlar<sup>[18]</sup>, who revealed no significant differences in their study between patients regarding age with a mean age of 63.04±5.68 and controls with a mean age of 59.94±6.50.

## CONCLUSION

Serum decorin level was significantly increased in patients with knee OA compared with controls. Also a positive correlation between serum decorin and WOMAC scale was detected. This indicates the possible role of decorin as a marker of disease grading. We recommend further studies with larger series to prove role of decorin in OA grading.

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**Conflict of interest:** No conflict of interest

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## الملخص العربي

مستوى الديكورين كبروتيوجليكان صغير غني باللوسين في مرضى خشونة مفصل الركبة شيماء منصور حجازي<sup>1</sup>، مها صلاح الدين محمد<sup>1</sup>، بسمه محمد النجار<sup>1</sup>، ساره يونس ابوزيد<sup>2</sup>  
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### ملخص البحث

**الخلفية:** خشونة مفصل الركبة هو مرض مزمن يتميز بفقدان الغضروف التدريجي للمصفوفة خارج الخلية والاضطراب اللاحق في بيولوجيا الخلايا الغضروفية. الديكورين عبارته عن بروتيوجليكان صغير غني باللوسين، يوفر ارتباطاً مادياً بين جزيئات الأجرىكان والكولاجين من النوع الثاني مما يجعله مكوناً هيكلياً مهماً لسلامة المصفوفة والوظيفة الميكانيكية الحيوية للغضروف. ويساعد مستوى الديكورين على تحديد شدة الخشونة ومن ثم العلاج.

**الهدف:** تهدف هذه الدراسة إلى قياس مستوى مصلى الديكورين في مرضى خشونة مفصل الركبة وارتباطه بأعراض وشدة المرض.

**الطرق:** في هذه الدراسة تمت مقارنة 50 مريضاً بدرجات متفاوتة من خشونة مفصل الركبة مع 50 مريضاً ممن يتطابقون مع العمر والجنس والذين يبدو أنهم يتمتعون بصحة جيدة كعناصر ضابطة. تم قياس مستوى مصلى الديكورين في كلا المجموعتين باستخدام تقنية مقايضة الممتز المناعي المرتبط بالإنزيم.

**النتائج:** كان هناك ارتفاع ذو دلالة إحصائية عالية في مستوى مصلى الديكورين في مجموعة خشونة مفصل الركبة بمتوسط 52.4 (9.8-66.8) مقارنة بـ 8.6 (6.6-9.13) في المجموعة الضابطة، وكانت هناك علاقة إيجابية بين مصلى الديكورين ومؤشر خشونه المفاصل في جامعات غرب اونتاريو وجامعة ماكماستر.

**الاستنتاجات:** تم رفع مستوى مصلى الديكورين بشكل كبير في مجموعة المرضى ويرتبط مستواه بشكل إيجابي مع مؤشر خشونه المفاصل في جامعات غرب اونتاريو وجامعة ماكماستر.

**الكلمات المفتاحية:** مؤشر خشونه المفاصل في جامعات غرب اونتاريو وجامعة ماكماستر، خشونة مفصل الركبة، مصلى الديكورين.

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