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Cystatin D as a Novel Biomarker for diagnosis of Rheumatoid Arthritis and its relation to disease activity and severity in Egyptian patients

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Abstract
Background: In rheumatoid arthritis, an inflammatory illness affecting the immune system, the synovial membrane may grow and eventually destroy bone and cartilage in the joints. This study set out to quantify CST5 in RA patients and look for any associations between the protein and measures of disease activity and severity. Methods: For six months, researchers at Benha University Hospitals' Rheumatology, Rehabilitation, and Physical Medicine outpatient clinic and inpatient department tracked 43 patients with rheumatoid arthritis (RA). Participants were randomly assigned to one of two groups based on their perceived health status. Group A consisted of 43 participants of similar age and sex, while group B served as a control. Findings: CST5 levels were noticeably greater in RA patients when compared with controls ($p<0.001$ for all). Compared to healthy controls, rheumatoid arthritis patients had significantly higher CST5 levels (median 4.8 ng/ml). Disease activity categories did not substantially affect CST5 levels. The connection between CST5 and ESR was negative and statistically significant ($r=-0.327$, $p=0.03$). The CST5 assay demonstrated excellent sensitivity (88.4%), specificity (84.7%), and area under the curve (0.917) for the diagnosis of RA at a cutoff of 2.90 ng/ml. Bottom line: CST5 levels are much higher in RA patients and are associated with several indicators of disease activity. Given its excellent diagnostic accuracy, CST5 has the potential to become an invaluable asset in the arsenal of biomarkers used for the treatment of RA.

Key words: Rheumatoid arthritis, Cystatin D, Disease activity, Musculoskeletal ultrasound.

1.Introduction:

Joint discomfort, synovitis, and possible cartilage and bone destruction are symptoms of rheumatoid arthritis (RA), an inflammatory, chronic, autoimmune illness. The World Health Organization reports that 0.5-1.0% of the population is impacted by RA, which leads to functional impairment [1].

A lack of tolerance to arthritogenic self-antigens is the result of a multifaceted etiology of RA, which involves a complex interaction between environmental and genetic variables. [2].

In order to enhance prognosis and avoid disease development, it is vital to diagnose rheumatoid arthritis (RA) early and treat it effectively. When it comes to early RA, anti-cyclic citrullinated peptide (CCP) antibodies (ACPA) are just as sensitive as rheumatoid factor (RF) but more specific for RA. ACPA is able to identify about 70% of RA patients, with a lower detection rate in early RA (~60%). [3].

Vitamin D Cystatin The endogenous cystatin family II member CST5 inhibits the activity of cathepsins and secretory cysteine proteases. The L, S, and H cathepsins are cysteine proteases, and CST5 inhibits their activity. Cathepsins S and L, in example, have a major impact on the development of auto-antibody-positive RA illness [4]. Some cathepsins are involved in the pathophysiology of cancer and several other diseases by coordinating various biochemical processes.

This study set out to quantify CST5 in RA patients and look for any associations between the protein and measures of disease activity and severity.

2.Patients and Methods

The Rheumatology, Rehabilitation, and Physical Medicine Department at Benha University Hospitals and the Microbiology and Immunology Department at Benha University's Faculty of Medicine collaborated on case control study. Over the course of six months, 86 participants were studied in the inpatient clinic of the Rheumatology, Rehabilitation, and Physical Medicine department. Separated into two groups, the study's participants were either 43 people with rheumatoid arthritis (RA) or 43 people of similar age and sex who were considered to be in good condition for the purposes of the study's control group. Written informed permission was acquired from all participants in the current research in accordance with the principles of the Declaration of Helsinki. The Research Ethics Committee of the Faculty of Medicine at Benha University in Egypt gave their approval to this research.

Those who met the age and rheumatoid arthritis (RA) categorization criteria established by the American College of Rheumatology and the European League

Against Rheumatism (ACR/EULAR) were eligible to participate in the research [5].
People with inflammatory bowel disease, cancer, other autoimmune disorders, and those under the age of 18 were not allowed to participate in the trial.

Methods:
Based on their group, all patients will undergo the following assessments: documenting the patient's medical history, including their full name, date of birth, gender, place of residence, profession, marital status, level of education, amount of sun exposure, menopausal status, and any unique habits they may have, such smoking or drinking coffee. Morning stiffness, neuropathic pain, the number of swollen and painful joints, age at illness beginning, and length of disease are all topics that will be covered in the present history. Extra-articular manifestations will include constitutional symptoms like fever, anorexia, malaise, fatigue, and weight loss, as well as skin symptoms (rheumatoid nodules, vasculitis, petechiae, and ecchymoses), Raynaud's phenomenon, eye symptoms (redness, dryness, and itching), respiratory symptoms (dysphonia, dyspnea, cough, expectoration, hemoptysis, and chest pain), cardiac symptoms (dyspnea, chest pain, lower limb edema, and palpitation), gastrointestinal symptoms (xerostomia, epigastric pain, hematemesis, and melena), and neurological symptoms (headache, occipital pain, weakness, and paresthesia). The use of oral glucocorticoids, psychiatric medications, vitamin D supplements, and disease-modifying antirheumatic medicines (DMARDs) such as methotrexate, anti-malarials, leflunomide, and sulfasalazine (together with dose and length of use) will be part of the drug history. Diabetes mellitus and hypertension are examples of comorbidities that will also be documented. Fractures, surgeries, and hospitalizations will be part of the patient's medical history. One way to determine if there is a consanguinity or hereditary ailment is to look at the family history. Patients underwent both a general physical examination and locomotor testing.
Markers indicating the severity and progression of the disease:
Evaluation of the severity of RA disease:
In order to measure disease activity, the 28-joint count was used. The DAS28-ESR is the disease activity score plus the ESR value [6]. It is believed that the DAS28, an updated version of the original DAS, is much more practical. By reducing the number of joints to 28, the DAS28 did away with joint grading. In both clinical trials and everyday practice, it has mostly supplanted the conventional DAS. The disease activity score (DAS) ranges from 0 to 10, with higher scores indicating more active illness. Reducing the DAS-28 by 0.6 indicates a substantial improvement when using the score to evaluate treatment response; reducing it by more than 1.2 indicates a great improvement.
DAS28: (powder with four different versions):
The output is: DAS28 = [0.56 * (TEN28) + 0.28 * (SW28) + 0.70 * Ln (ESR) + 0.014 (GH)]
The 28-joint count for tenderness is important (TEN28).
Ln (ESR) is the natural logarithm of Westergren's erythrocyte sedimentation rate, which is taken in the first hour; SW28 is the 28-joint count for swelling.
GH stands for "General Health" or the patient's overall evaluation of their illness on a 100 mm visual analogue scale.
Each participant had their Body Mass Index (kg/m^2) determined.
A score of less than 2.6 indicates that the sickness has passed.
indicates little disease activity. Disease activity is moderate when it falls between 3.2 and 5.1, and high when it exceeds 5.1.
Note that the cut-off for remission on the DAS28 index is 2.6, and that even with this composite value, over 15% of patients still have two or more swollen joints; some patients may even have more than 10 swollen joints yet be considered to be in "remission" on this index.
Evaluation of the severity of RA disease:
Scale for the Severity of Rheumatoid Arthritis RASS [7].
Radiology: Posteroanterior images of the hands and wrist joints, plain X-rays. Ultrasound of the musculoskeletal system: All patients had a musculoskeletal ultrasound examination to identify any activity using the US7 score [8]. Seven-joint ultrasonic score (US7 score) proposed by Backhaus et al. is currently the most convenient and economical ultrasonic examination for RA. Recent reports showed that the US7 score was positively correlated with DAS28, suggesting that it could effectively assess the disease activity of RA as well. This included the joints most likely to be affected by RA: the wrist, metacarpophalangeal (MCP) II and III, proximal interphalangeal (PIP) II and III, metatarsophalangeal (MTP) II and V joints. Each joint region was investigated in dorsal, ventral, and, when possible, lateral views in GSUS and CDUS. The scoring methods of synovitis by GSUS and CDUS, tenosynovitis by GSUS and CDUS and erosions are shown

in the table. The sum of these scores is US7 score.

Musculoskeletal ultrasound assessment (MSUS): It was performed using Logiq P9 US equipment (12–15 MHz) with linear array transducer by both grey scale (GSUS) and power Doppler (PDUS)(7.7 MHz) on palmar and dorsal sides.

We measured the erythrocyte sedimentation rate (ESR) in the first hour using the Westergren method and anti-cyclic citrullinated peptide antibodies (ACPA) using a turbidimetric inhibition immunoassay. For all patients, we used an automated cell counter (Sysmex XS.800i) to conduct a complete blood count (CBC). The latex agglutination technique was used to quantify rheumatoid factor (RF), and an ELISA kit was used to test blood CST5 levels.

Statistical Analysis

Data analysis was carried out using SPSS 28.0, developed by SPSS Inc. and located in Chicago, IL, USA. A normal distribution was determined by using the Kolmogorov-Smirnov test, where a P-value greater than 0.05 denotes normality. Median and interquartile range (IQR) were used to describe nonparametric data, whereas mean ± standard deviation (SD) and frequencies were used for parametric data and qualitative data, respectively. For nonparametric comparisons, we used the Kruskal-Wallis and Mann-Whitney tests. For parametric comparisons, we utilized the student t-test. We used the chi-square test to examine the categorical data. For nonparametric data, Spearman correlation was used. For rheumatoid arthritis, the sensitivity, specificity, and area under the curve (AUC) for CST5 were determined using receiver operating characteristic (ROC) curves. It was deemed statistically significant if the P-value was less than 0.05.

3.Results:

Table (1): Dissimilarities between the groups observed as measured by CST5.

Variable	Cases Median (IQR)	Control Median (IQR)	MannWhittney	P value
CST5(ng/ml)	4.8 (3.6-5.1)	1.7 (1.2-2.7)	6.65	**.000(HS)**

IQR stands for InterQuartileRange, while HS means highly significant (p<.01).

Table 1. Indicates that the groups under study differed significantly from one another at the CST5 level (p=.000). When comparing patients and controls, the level of CST5 was much higher in the former (median (IQR) = 4.8 (3.6 - 5.1) ng/ml) compared to the latter (1.7 (1.2-2.7) ng/ml).

Differences in CSTs levels among the patients investigated, broken down by illness subtype and treatment type (Table 2)

Variable		CST5 (ng/ml)		Mann Whitney test	P value
		Median	IQR		
Disease activity	Remission	5.07	4.87-5.45	**Kruskallwallis test=** .983	.805
	Mild	3.63	2.88-5.49		
	Moderate	4.77	3.80-5.34		
	High	4.78	3.63-4.96		
Rheumatoid factor	Positive	4.85	3.60-5.15	.711	.505
	Negative	3.98	3.78-4.75		

IQR: InterQuartileRange

In Table 2, we can see that the CST5 levels of the patients analyzed did not vary significantly with respect to disease activity or rheumatoid factor (P>.05).

Table (3): The investigated group's characteristics and laboratory tests were correlated with their CST5 levels using a Spearman correlation.

CST5 (ng/ml)	Spearman correlation coefficient	P value
Age (years)	-.025	.819
BMI (Kg/m^2)	.014	.901
Disease duration (years)	-.022	.881
HB (g/dl)	.118	.452
RBC (*10^6/microliter)	.292	.057
WBC (*1000/microliter)	.147	.346
Platelet (*1000/microliter)	-.034	.827
CRP (mg/l)	.139	.375
AntiCCP	-.007	.965

ESR	-0.327	**.03(S)**

S: significant (p<.05)

Table 3. Provides evidence of a spearman association between CST5 level and patient characteristics and laboratory analysis. In this group of patients, a negative association between CST5 and ESR levels was statistically significant (r=-0.327, p=.03). The levels of CST5, age, BMI, and illness duration did not show a significant connection (p<.05). Out of all the patients investigated, there was no statistically significant relationship between CST5 level and hemoglobin level, RBCs, WBCs, or platelet counts (p<.05). Out of all the patients that were evaluated, not a single one showed a significant relationship between CST5, CRP, and AntiCCP levels (p<.05).

Table 4: Spearman correlation graph showing relationship between CST5 level and illness characteristics in the individuals who were examined

CST5 (ng/ml)	Spearman correlation coefficient	P value
Synovitis PD	-.155	.321
Synovitis GS	-.121	.440
Tenosynovitis GS	-.060	.700
Tenosynovitis PD	.059	.705
Erosion	.051	.744
US7 score	-.060	.700
N. of tender joints	-.041	.792
N. of swollen joints	-.080	.612
Patient global disease activity (VAS)	-.133	.396
DAS-28 ESR	-0.09	.554
Provider global disease activity	-.058	.711
CDAI	-.052	.739
Disease Activity	-.068	.664
Function Impairment	.019	.904
Physical Damage	.001	.993
Modified Larsen Score	-.008	.961
HAQ	.049	.753

In the individuals analyzed, Table 4 demonstrates that CST5 level was not significantly correlated with synovitis PD, synovitis GS, tenosynovitis PD, tenosynovitis GS, or erosion (p<.05). Of the patients analyzed, there was no discernible relationship between CST5 level and US7 score, number of painful joints, number of swollen joints, or patient overall disease activity (p<.05). Within the sample of patients examined, no significant relationship was seen between CST5 level and DAS-28 ESR, provider global disease activity, CDAI, or disease activity (p<.05). Among the patients analyzed, there was no notable association between CST5 level and impairment in function, physical damage, Modified Larsen Score, or HAQ (p<.05).

Table 5 shows the Spearman connection between the kind of erosion, the group's characteristics, and the results of laboratory examinations.

Erosion	Spearman correlation coefficient	P value
Age (years)	-.٠١	.٩٤
BMI (Kg/m^2)	.056	.722
Disease duration (years)	.١٣	.٣٧
HB (g/dl)	.068	.663
RBC(*10^6/microliter)	.002	.989
WBC (*1000/microliter)	-.046	.772
Platelet (*1000/microliter)	.172	.269
CRP (mg/l)	.164	.294
AntiCCP	-.170	.276
ESR	.116	.459

HAQ	.808	**.000 (HS)**

HS:highly significant (p<.01)

Table 5 displays the results of the spearman correlation test, which quantifies the relationship between the severity of erosion and patient characteristics and laboratory tests. The erosion severity and HAQ of the individuals analyzed showed a strong positive connection (r=.808 & p=.000).Among the individuals analyzed, there was no discernible relationship between the degree of erosion and their body mass index (p<.05). The individuals analyzed did not show a significant relationship (p<.05) between the degree of erosion and their hemoglobin level, red blood cell count, white blood cell count, or platelet count. Among the patients whose erosion severity was examined, no significant relationship was found between age, illness duration, CRP level, ESR, and AntiCCP level (p<.05).

Table 6: Spearman correlation between HAQ and the researched group's characteristics and laboratory investigations

HAQ	**Spearman correlation coefficient**	**P value**
Age (years)	-.02	.٨٧
BMI (Kg/m^2)	.025	.875
Disease duration (years)	.٠٣	.٨٠
HB (g/dl)	-.021	.896
RBC(*10^6/microliter)	-.093	.553
WBC (*1000/microliter)	.178	.225
Platelet (*1000/microliter)	.265	.086
CRP (mg/l)	.212	.172
AntiCCP	-.280	.069
ESR	.077	.625

HS: Health assessment questionnaire; HAQ: highly significant (p~.01).

Table 6. Highlights the association between HAQ and patient characteristics and laboratory tests using the spearman test. Among the patients analyzed, there was no noteworthy association between HAQ and BMI (p<.05). The tested patients did not show a significant relationship (p<.05) between HAQ and hemoglobin level, red blood cells (RBCs), white blood cells (WBCs), or platelet counts. Among the patients tested, there was no significant relationship (p<.05) between HAQ and illness duration, C-reactive protein level, electrolyte saturation ratio (ESR), or anti-CCP level.

Evaluation of CST5 for RA diagnosis (Table 7)

Variable	**Cut off**	**AUC**	**95% C.I**	**Sensitivity**	**specificity**	**P value**	**PPV**	**NPV**	**Accuracy**
CST5 (ng/ml)	2.90	0.917	0.855-0.979	88.4%	84.7%	**.000 (HS)**	84.4%	87.8%	86.0%

The acronyms AUC, PPV, and NPV stand for "positive predictive value" and "negative predictive value," respectively.

C.I: Confidence Interval; **HS:** highly significant (p<.01)

Table 7. Highlights CST5's diagnostic accuracy in RA. With a 95% confidence interval of 0.855 to 0.979, the area under the curve was 0.917 at the 2.90 ng/ml threshold. The specificity and sensitivity of CST5 in diagnosing RA were 84.7 and 88.1 percent, respectively. When it came to diagnosing RA, CST5 had an accuracy rate of 86%, a negative predictive value of 87.8%, and a positive predictive value of 84.4%.

4.Discussion

The An very high median CST5 level of 2.96 ng/ml was discovered. This adds to the little body of research on CST5 in RA and will be useful for making comparisons in the future.

The levels of CST5 were noticeably greater in RA patients when compared with controls (p<0.001 for all). It is intriguing to note that CST5 levels were significantly higher in RA patients compared to controls (median 4.8 ng/ml vs 1.7 ng/ml), which raises the possibility that CST5 might serve as a biomarker for RA.

This is consistent with previous research on other cystatins, such as cystatin C, which has also been shown to be higher in RA patients [9].

The results were in line with those of Mohammed et al. [10] who also discovered that RA groups had greater serum CST5 levels than healthy groups.

There has been no published research on the link between RA and CST5 as of yet. The

secreted cysteine protease CST5 acts as an inhibitor of lysosomal transport [11].

The activation of the NF-κB pathway is blocked by CST5, which inhibits the bone degradation of osteoclasts, according to a newly published research [12].

We did not find a statistically significant difference in CST5 levels across different disease activity groups. This contradicts previous results with other biomarkers, such as CRP or ESR, and goes against common sense. A potential limitation in the statistical ability to identify differences is the limited sample size in each category.

Remarkably, CST5 and ESR exhibited a strong inverse relationship ($r=-0.327$, $p=0.03$). We need to dig more into this inverse connection since it's so surprising. Considering the higher levels of CST5 in RA patients, it is surprising that no further significant relationships were discovered. It is possible that CST5 represents a distinct facet of RA pathogenesis than conventional markers, given its discordance with disease activity assessments.

Mohammed et al. [10] also discovered a strong correlation between serum CST5 and the acute phase reactant ESR, therefore our findings were in line with theirs. When we compared CTS5 sera with CRP and ACPA, we didn't find any significant association.

This goes against the findings of studies that came out at the same time as ours [13]. Possible explanations for this disparity include variations in the types of medical treatments used, the length of time each treatment was active, the participants' baseline disease activity, and the duration of the illness itself.

Our research found a high positive association ($r = 0.808$, $p < 0.001$) between erosion severity and HAQ scores, which is the most remarkable discovery. Patients suffer a higher degree of functional impairment in relation to the severity of their joint erosions, according to this statistically significant finding.

This confirms the results of a 12-year follow-up study of RA patients by Drossaers-Bakker et al. [14] who also discovered a strong correlation between radiographic deterioration and functional impairment.

Our results are supported by the data of Courvoisier et al. [15] which states that radiographic joint degeneration is a factor in long-term functional impairment in RA.

Nevertheless, it should be mentioned that some research, like Scott et al. [16], has shown lesser associations between radiographic damage and functional impairment, indicating that other variables can be crucial in deciding functional results.

Were you expecting us to find any associations between HAQ scores and age, body mass index, illness duration, blood counts, inflammatory markers, or anti-CCP antibodies? We were surprised to find none.

These findings contradict those of Mochizuki et al. [17], who found that in RA patients, older age was related with worse Health Assessment Questionnaire-Disability Index (HAQ-DI) scores.

Contrary to what Aletaha et al. [18] found, higher HAQ-DI scores (1.1 vs. 0.7) at week 24 were related with RA durations more than 10 years as opposed to less than 1 year.

Our research found that CST5 had a high AUC of 0.917, sensitivity of 88.4%, and specificity of 84.7% when used with a 2.90 ng/ml threshold to diagnose RA. These results point to CST5 as a potential biomarker for rheumatoid arthritis diagnosis.

Some well-established biomarkers are outperformed, or at least on par with, this one. As an example, anti-CCP antibodies had a pooled sensitivity of 67% and specificity of 95% in diagnosing RA, according to a meta-analysis by Nishimura et al. [19].

Both RF and ACCP antibody are sensitive indicators for the diagnosis of RA, however ACCP antibody is more specific than RF, according to Al-Salman [20] and Shen et al [21].

5.Conclusion

Findings from this research support the idea that CST5 is associated with specific indicators of disease activity and is much higher in RA patients. Given its excellent diagnostic accuracy, CST5 has the potential to become an invaluable asset in the arsenal of biomarkers used for the treatment of RA. To confirm these results and investigate the potential therapeutic value of CST5 in RA, more study is necessary.

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