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Research Article

Serum Cathepsin K Levels of Patients with Rheumatoid Arthri-tis Correlation with Radiological Destruction

Omar AS[1*], Elsawy MM[1], Maksoud MAHA[1], Zawawy AIE[2] and Nekiedy AAME[3]

[1]Clinical and chemical pathology department, Faculty of Medicine, University of Alexandria, Egypt
[2]Internal Medicine department, Faculty of Medicine, University of Alexandria, Egypt
[3]Diagnostic radiology department, Faculty of Medicine University of Alexandria, Egypt

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2. Keywords

Cathepsin k; Rose waaler

1. Abstract

The aim of the work was to investigate the correlation of cathepsin K with radiological destruction in bone and cartilage. We studied 40 rheumatoid arthritis patients varied in disease severity and 10 healthy blood- donners of matched age and sex as control. All subjects in the study were subjected to the following: Full history taking: particularly for age, sex, disease duration and extra articular manifestations, through clinical examination including joint examination with stress on Disease Activity Score (DAS-28). Routine laboratory investigations, including com-plete blood count, liver function tests, Renal function test, ESR, CRP and serological investiga-tions, including Rheumatoid Factor (RF), Rose Waaler (RW), cathepsin K level were performed. We find that Serum cathepsin K levels in patients with RA compared with the healthy control group were significantly elevated. Modified Larsen score ranged from 0 to 110 (removed part). The highest level of cathepsin K was found to be present in patients with highest modified Lars-en score. There was significant correlation between cathepsin K level, modified Larsen score and DAS-28 in patients with rheumatoid arthritis. There was also significant correlation between cathepsin K level and: Erythrocyte sedimentation rate (removed part). Cathepsin K was found to be elevated more in patients complaining of limitation of movement than those had no limi-tation of movement; also it was correlated significantly with morning stiffness. Conclusion: The study demonstrates increased cathepsin K level in serum of patients with rheumatoid arthritis and significantly correlated with the joint destruction. Cathepsin K is a valuable biomarker for the assessment of bone metabolism in patients with established rheumatoid arthritis, its mea-surement will probably contribute to developing targeted therapies for the prevention of further bone destruction.

3. Introduction

RA is traditionally considered a chronic, inflammatory joint au-toimmune disorder affecting extra-articular tissues throughout the body including the skin, blood vessels, heart, lungs, and mus-cles [1] (removed part).

Activated macrophages over express Major Histo-Compitability (MHC) class II molecules and produce Pro-Antigen activat-ed CD_{4+} T cells stimulate monocyte, macrophages and syno-vial fibroblasts to produce the cytokines. Activated CD_{+4}T cells stimulate B cells through cell surface Contact and through αIb2 integrin, CD154 (CD40 Legend) and CD28 to produce immuno-globulins, including rheumatoid factor.

Activated CD4+T cells also express RANKL (Receptor-Activator of Nuclear Factor Kappa B Legend). The interaction of RANKL with RANK (Receptor Activator of Nuclear Factor Kappa B), was found on osteoclast precursor cells and mature osteoclasts, is es-sential for osteoclastogenesis and osteoclast activation. Pannus is considered the most destructive element affecting the joint it can attack the articular cartilage and destroy it [2].

Conventional radiography is the recommended method for monitoring progression of structural joint changes in the routine management of RA patients as well as in clinical trials. It has a

***Corresponding Author (s):** Aida Saleh Omar, Department of Clinical and Chemical Pathology, University of Alexandria, Egypt, E-mail: ashrafzawawy@hotmail.com*

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lower sensitivity than MRI for bone erosions.

3.1. Grading system of rheumatoid arthritis

Larsen system for grading RA and related conditions by using standard reference film radiograph**[3]**

Grade 0: No radiographic changes (Abnormalities not related to arthritis may be present)

Grade 1: slight abnormality (Peri articular swelling, paraarticular demineralization, slight joint space narrowing) **Grade 2:** definite early abnormality(Definite presence of erosions except in weight-bearing joints, joint space narrowing)

Grade 3: Medium destructive abnormality (Definite presence of erosion in all types of joints, joint space narrowing.)

Grade 4: Severe destructive abnormality (Erosions, joint space narrowing, bone deformation in weight-bearing joints.)

Grade 5: Mutilating abnormality (Disappearance of the original articular forces, gross bone deformities in weight-bearing joints.)

Note: A set of standard reference film radiographs has to be available for comparative purpose. Dislocation and bony ankylo-sis should not be considered in the grading.

Magnetic Resonance Imaging (MRI) is the best imaging modality for RA, a multiplanar approach is useful for the distinction between erosions and pre-erosive changes and for the assessment of pannus. Up to 47% of patients may develop erosions within 1 year after onset of RA.

In advanced stages, the inflammatory process may lead to massive bone erosions. Use of scored radiographs as an outcome measure can help estimating the progression of rheumatoid arthritis.

The most commonly used methods:

1. Larsen methods: produce an overall score for erosion and joint space narrowing [3].

2. Sharp methods: provide separate scores for erosion and joint space narrowing [4].

3. Simple erosion narrowing score (SENS): Provide scores for erosion and joint space narrowing that are summed thereafter to give a figure comparable to the sharp total score[5].

Cathepsin is a protease, a type of protein that breaks apart other proteins, found in many types of cells. There are approximately a dozen members of this family, which are distinguished by their structure and which proteins they cleave. Most of the members become activated at the low pH found in lysosomes. Thus, the activity of this family lies almost entirely within those organelles.

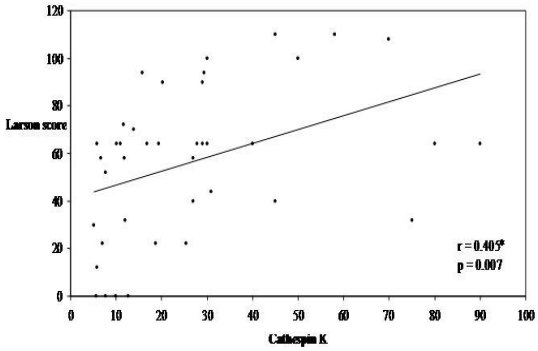

Figure 1: Correlation between cathepsin K and modified Larsen score(SENS) in patient group.

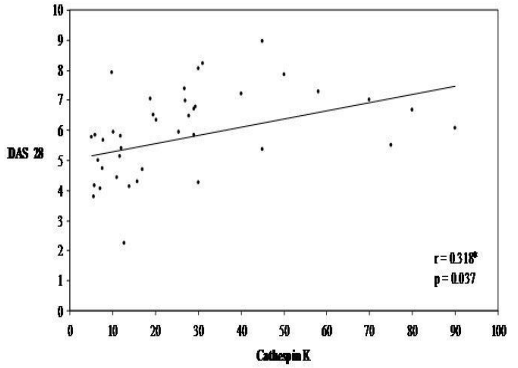

Figure 2: Correlation between cathepsin K and DAS 28 in patient group.

Cathepsin B, K, L, S, V, F, are classes of lysosomal enzymes which are implicated various disorders including inflammation, rheuma-toid arthritis, osteoarthritis, osteoporosis, tumors, coronary disease atherosclerosis, autoimmune diseases and infectious diseases.

Cathepsin K is a cysteine protease that plays an essential role in osteoclast function and in the degradation of protein components of the bone matrix by cleaving proteins such as collagen type I, collagen type II and osteonectin. Cathepsin K therefore plays a role in bone remodeling and resorption in diseases such as osteoporosis, osteolytic bone metastasis and rheumatoid arthritis [6].

Although the degradation of the extracellular matrix in joints is clearly mediated by proteolytic activities, the nature of the individual proteases remains unknown in most cases. To date, two protease families have been implicated in cartilage degradation: Matrix Metalloproteinase (MMP) and cysteine proteases [7].

Traditionally, metalloproteinase have been favored as potential culprit enzymes over cysteine proteases, but inhibitors of both protease classes have proved to be equally, effective in reducing inflammation and cartilage erosion in animal models of RA. Ca-thepsins are potential drug targets to treat tissue degenerative and inflammatory processes [8].

Cathepsin K has been identified as the predominant osteoclastic protease with a unique and potent collagenolytic activity [9].

Two types of cathepsin K-containing cells appear to be present in the synovial lining: $CD68_+$and $CD68_-$ cells. However, the dense

population of cathepsin K-positive cells in the lining of the inflamed synovium suggests a role of the enzyme in cartilage- and bone-invasive processes. This role is further supported by the observation that cathepsin K- positive SFs accumulate at sites of bone and cartilage erosion [10].

Cathepsin K-positive SFs were highly enriched not only at sites of vascularization and angiogenesis but also around necrotic vessels. It can be hypothesized that the protease plays a role in the loosen-ing of the surrounding matrix to facilitate angiogenic growth on one side and contributes to the degradation of expired vessels on the other side [10].

Synovial fibroblast-derived cathepsin K have also been directly linked to the degradation of articular cartilage and bone. Bone and cartilage erosions are considered an irreversible degenerative process leading to the loss of joint function [11].

The inhibition of cathepsin K in RA-derived SFs results in a lysosomal accumulation of undigested type II collagen fibrils whereas the inhibition of cathepsins L, B, and S has no effect. The specific activation of the protease activity toward types I and II collagen by cathepsin K-digested aggrecan contribute to the potential role of cathepsin K in RA- associated joint destruction [12].

Osteoclastic bone resorption requires two processes: demineralization of the inorganic bone components and degradation of the organic bone matrix. These two processes occur in the osteoclast in a coordinated fashion by two separate mechanisms. The first phase involves acid secretion by the osteoclast into the resorption lacunae, and the second phase is the organic matrix degradation by cysteine proteases. An acidic microenvironment is required for bone resorption, both to dissolve the mineral component of bone and to aid protein matrix digestion. This unique metabolic milieu is achieved by lowering the pH in the resorption lacunae via acid secretion by the osteoclast. Cathepsin K inhibitors repre-sent a novel target for developing agents to treat osteoporosis and other disorders characterized by increased bone resorption Taken as a whole, these findings identified cathepsin K as a potential target for anti-resorptive drugs in RA [13,14].

The aim of the work is to measure serum level of cathepsin K in patients with rheumatoid arthritis, compare it to age matched control group and correlate the results with radiological progression.

4. Methods

The study carried out on 40 patients suffering from rheumatoid arthritis according to the criteria of the American Rheumatism Association.

- 10 samples are taken from healthy blood donners of matched age and sex.

- All the subjects signed an informed consent.

- Other cause of increase cathepsin K was excluded such as osteoporosis and osteolytic bone metastasis.

All subjects in this study (rheumatoid arthritis patient and control group) were subjected to the following:

1-Full history taking, particularly for age, sex, duration of the disease articular and extra-articular manifestations.

2- Through clinical examination joint examination and clinical assessment with.

3- Laboratory investigation

4.1. Diseases activity score-28 (DAS 28) [15]

10ml venous blood was withdrawn from the patients and controls in the morning after 8 h fasting, blood samples were withdrawn from the antecubital vein with minimal stasis. They were divided as follows:

•2 ml delivered into EDTA tubes for Complete Blood Count (CBC).

•1.6 ml delivered into tubes containing sodium citrate forEryth-rocyte Sedimentation Rate (ESR).

•Serum was used for routine chemical tests

Serum was used also for determination of the following serologi-cal test: [16]

- Rose Waaler test.

- Latex rheumatoid factor (RF)

- C - Reactive Protein (CRP)

4.2. Determination of cathepsin K level using ELISA

It is an enzyme-linked immunosorbent assay for the detection of cathepsin K. in serum of rheumatoid arthritis patients.

4.2.1. Sample collection

The assay was performed with a serum samples; grossly haemolysed or turbid samples were not used. Thoroughly thawed samples were mixed before assay, repeat freeze, thawing has been avoided. Samples were stored at -20ºC till assay.

4.2.2. Contents of the kit

1-Plate: Polyclonal sheep anti cathepsin K antibody coated microtiterstrips in stripholder, packed in alubag with desiccant.

2-Washbuf:Washing buffer 20x concentrate

3- Asybuf: Assay buffer, ready to use

4- STD: Standards, (0; 11; 33; 100; 300 pmol / l), lyophilized with blue dye

5- CTRL: Control, lyophilized, concentration after reconstitution see label

6- CONJ: Conjugate, (polyclonal anti cathepsin K-HRPO), ready to use

7- SUB: Substrate (TMB solution), ready to use

8- Stop: Stop solution, ready to use.

4.3. Assay protocol

•All reagents and samples must be at room temperature (18-26°C) before use in the assay.

•We marked position for BLANK/STD/SAMPLE/CTRL (Blank/ Standard/ Sample/ Control) on the protocol sheet.

•We Added 50 µl STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective wells, except blank.

•200 µl CONJ (anti Cathepsin K-HRPO) was added into each well, except blank, and swirled gently.

•The plate was covered tightly and incubated overnight (20-24h) at room temperature (18-26°C) in the dark.

•Wells was aspirated and washed 5x with 300 µl diluted WASH-BUF (Wash buffer), removal of remaining WASHBUF by hitting plate against paper towel after the latest wash.

•200 µl SUB (Substrate) was added into each well.

•The plate was incubated for 30 min at room temperature (18-26°C) in the dark**.**

•50 µl STOP (Stop solution) was added into each well.

• Absorbance was measured immediately at 450 nm with refer-ence 620 nm.

(removed part)

4.5. Radiological examination of the affected joint

Plain x-ray was done for each affected joint to estimate the progression of rheumatoid arthritis.

4.6 Rau and Herbon modification of Larsen scoring method for rheumatoid arthritis (SENS) (5)

•32 Joints are evaluated

•Eight PIP, Two IP of the thumb, Ten MCP, Two wrists and Ten MTP of the foot.

•6 Stages are defined or follow

0 =Normal, 1=Soft tissue swelling and/or joint space narrowing / subchondral osteoporosis.

2=Erosion with destruction of the joint surface (DJS) ≤25%,3=DJS26 – 50%.4 =DJS51 – 75%.

5 =DJS ≥ 75%.

•The score range from 0 to 160.

4.7. Method of Statistical Analysis

The collected data were coded, tabulated and statistically analyzed using a Microsoft, statistical package of social science version 15 (SPSS-15).

Mean-median and standard deviation with used to express quan-titative data.

• Comparison between means of both groups was done by ap-plication of student t-test to compare between cases and controls.

•Comparison between qualitative data of each group was as-sessed by Fisher Exact test

•Correlation between quantitative data of each group was as-sessed by calculation of Pearson's correlation coefficient

•P value less than 0.05 was adopted as a level of significance.

5. Results

The present study was carried on 50 subjects admitted to "Alex-andria Main University Hospital" divided into 2 groups:

Group I: 40 rheumatoid arthritis patients fulfilling ACR criteria for diagnosis of RA

Group II: 10. healthy blood donners of matched age and sex **5.1. Age and Sex**

In the present study. Group I included 31 female patients (77.5%) and 9 male patients (22.5%), their age ranged from 19-50 years with mean age value 37.30±7.58.

Group II included 6 females (60%) and 4 males (40%), their age ranged from 34-42 years with mean age value 38±2.94 (**Table 1**).

Table 1: Distribution of DAS-28, modified Larsen score(SENS)and serological factors (CRP-RF-Rose Waaler) among patient group.

Variables	Mean ±SD	
DAS-28		
Range	2.25-8.96	
Mean ± SD	5.94 ± 1.43	
Modified Larsen score (SENE)		
Range	0.00 -110.00	
Mean ± SD	56.60 ± 31.71	
CRP	**No.**	**%**
-ve	8	20.0
+ve	32	80.0
RF	**No.**	**%**
-ve	2	5.0
+ve	38	95.0
Rose Waaler test	**No.**	**%**
-ve	11	27.5
+ve	29	72.5

Table 2: Comparison between the two studied groups according to cathepsin K level.

	Control	Patient	T(p)
Range(pmol/L)	4.00-12.00	5.10-90.00	5.309 (<0.001)
Mean ± SD	7.94 ± 2.39	26.87 ± 22.05	

t: Student t-test
*: Statistically significant at $p \leq 0.05$

5.2. Renal function test (blood urea nitrogen creatinine level) Serum uric acid

These were no significant differences between 2 groups.

5.3. ESR (1st hour)

It ranged from 15-100 mm/h with mean value of (40.9±22.19) mm/h in group I, while ranged from (8-20) mm/h with mean value of (11.0±3.92) mm/h in group II with significant difference between the 2 groups (p<0.001).

5.4. Cathepsin K

It ranged from (5.10-90.00) pmol/l group I with mean value of (26.87±22.05) pmol/l, while it ranged from (4.00-12.00) pmol/l with mean value (7.94 ± 2.39) in group II with significant differ-ence between the two groups (p<0.001).

There was statistically significant correlation between cathep-sin K readings and morning stiffness, DAS 28, Larsen score (p 0.040,O.037, 0.007) respectively.

There was significant correlation between erythrocyte sedimen-tation rate in the first hour with the result of cathepsin K (p equal 0.007).

23 patients had no limitation of movement; cathepsin K mean value was (20.22±16.85), while 17 patientswere complaining of limitation of movement, the mean value of cathepsin K was (35.88±25.39), that show statistically significant difference. (P = 0.024).

6. Discussion

Our aim of the present work was to study cathepsin K levels and its association with radiological destruction in patients with rheumatoid arthritis (31 females and 9 males) their age ranged from 19-50 years, with mean value 37.3 ± 7.58. In addition, 10 healthy blood donners with matched age and sex were included as control group.Serum Cathepsin K levels in patient group was significantly higher than control group (P < 0.001).

Toren BR (2004) found that, bone resorption and formation is a well-balanced system and is mediated by osteoclast. Cathepsin k is essential for bone resorption, which depends on the produc-tion of cathepsin K by osteoclast and its secretion into the extra cellular department. This leads to degradation of the organic ma-trix between the osteoclasts and the bone surface [17].

Dodds RA and his colleagues (2001) found that, in vivo the ac-tivation of cathepsin K occurs intracellular before secretion into the resorbing lacunae and the onset of resorption, where by local factors may regulate the processing of pro-cathepsin K to mature cathepsin K[18].

Hou and his colleagues (2003) found that, cathepsin K has a po-tent aggreacan-degrading activity; where by the aggrecan cleav-age products increase the collagenolytic effects of this protease on collagen type I and type II. There were able to show that ca-thepsin K is also a critical protease in cartilage degradation by synovial fibroblast[19].

Increased expression of cathepsin K around lymphocytic in-filtrates in synovial tissue seems to facilitate the movement of mononuclear cells through the perivascular matrix[20].

Proinflammatory cytokines such as IL-IB and tumor necrosis factor alpha facilitate the expression of cathepsin K, its over ex-pression in rheumatoid synovium, induced by IL-IB and tumor necrosis factor alpha due to increase of cathepsin K expressing cells, proves this protease to be available tool for bone research, and cathepsin K also may become a new and highly specific bio-marker for RA[21].

Votta and his colleagues demonstrated high levels of cathepsin K expression in osteoclast at sites of extensive bone loss. According to this, they developed a peptide aldhyde inhibitor of cathepsin K that inhibits osteoclast. Mediated bone resorption in fetal rat long bone organ cultures and even in a human osteoclast. This inhibitor leads to significantly reduced bone loss[22].

In the synovium of RA, the cathepsin K protein was localized in synovial fibroblast, CD68+ macrophage like synoviocytes, stromal multinucleated giant cells. Highly interesting is the ex-pression of cathepsin K by fibroblast and giant cells at sites of cartilage erosion. This was two to five times higher compared with osteoarthritic synovium. In normal synovium, cathepsin K expression was restricted to fibroblast like cells[23].

The Larsen score ranged from (0 -110) in our study with mean value (56.60 ± 31.71) with statistically significant correlations be-tween cathepsin K and Larsen score (P = 0.007) the highest lev-els of cathepsin K were observed in patient with highest Larsen scores. The radiological destruction correlates significantly with cathepsin K[26].

DAS-28 ranged from (2.25-8.96) with mean value (5.94 ± 1.43) in patient group which show statistically significant correlation between it and cathepsin K.

Skoumal M and his colleagues (2005) studied prospectively se-rum cathepsin K in rheumatoid arthritis patient and found that

cathepsin K seems to be independent or weakly correlated with laboratory inflammation parameters. It was not associated with CRP (P = 0.27), but weak correlations were found with erythrocyte sedimentation rate (P = 0.03) and the disease activity score (P = 0.04). There was significant correlation with Larsen score, Cathepsin K levels showed an increase with the augmentation of radiological destruction (P = 0.035)[27].

Hou WS and his colleagues (2001), Found that there was significant correlation between cathepsin K and disease severity which determined by the selective and the critical role of cathepsin K in articular cartilage and articular bone erosion. It was further corroborated by the finding that cathepsin K has a potent aggrecan degrading activity and cathepsin K-generated aggrecan cleavage products specifically potentiate the collagenolytic activity of cathepsin K towards type I and type II collagens. But, no correla-tion was found between cathepsin K, erythrocyte sedimentation rate and CRP.Hou WS and his colleagues (2001), Found that there was significant correlation between cathepsin K level and joint incapacitation which determined by the Health Assessment Questionnaire disability index (HAQ), where bone and cartilage erosion derived by cathepsin K is irreversible degenerative process leading to loss of joint function[28].

This study demonstrates increased cathepsin K level in serum of patients with rheumatoid arthritis. The elevated serum levels of this protease are significantly correlated with the joint destruc-tion, which in this study was assessed by Larsen score. Cathepsin K is a valuable biomarker for the assessment of bone metabolism in patients with established rheumatoid arthritis, its measure-ment will probably contribute to developing targeted therapies for the prevention of further bone destruction.

So we could recommend more studies to be performed to verify the presence of cathepsin K in patients with early RA and its val-ue as a prognostic factor for bone destruction in RA.

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