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YKL-40 and cytokines - a New Diagnostic Constellation in Rheumatoid Arthritis?

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Background: Rheumatoid arthritis (RA) causes chronic inflammation and alteration of articular tissue and joints. The pathogenesis of the disease remains unclear although it is known that proinflammatory cytokines play a major role in its induction.

YKL-40 is a chitinase-like glycoprotein produced by activated macrophages, neutrophils, arthritic chondrocytes and cancer cells. It has been shown that YKL-40 is implicated in tissue remodeling, angiogenesis and inflammation.

Aim: to investigate serum and synovial YKL-40 levels in relation to IL-1β, TNF-α, and IL-6 in RA patients.

Materials and methods: Serum and synovial concentrations of YKL-40, TNF-α, IL-6, and IL-1β were determined by ELISA in 39 patients (mean age 53.18 ± 16.54 yrs) with active RA.

Results: Serum YKL-40 levels were increased in all patients. The highest levels were found in synovial fluid ($P<0.01$). Our study showed a strong association between serum and synovial levels of YKL-40 and serum TNF-α and IL-1 β ($P<0.05$).

Conclusion: This is the first study finding a significant correlation between serum TNF-α and IL-1β and YKL-40 in active RA. We suggest that these molecules together might play a dominant role in the pathogenesis and disease activity and could possibly serve as a new diagnostic constellation in rheumatoid arthritis.

BACKGROUND

Rheumatoid arthritis (RA) is a chronic inflammatory disease leading to functional disability caused by joint destruction.[1] The pathogenesis of RA is multifactorial, susceptibility and severity is connected with HLA class II alleles.[2] Several antigens of potential interest have been investigated, such as type II collagen and heat shock proteins[3], but despite the extensive research, the particular antigens involved in the activation of disease have not been found yet.

YKL-40 is a 40-kDa mammalian glycoprotein related to the chitinase protein family but without enzymatic activity. The name derives from the one letter code for the three terminal amino acids - tyrosine (Y), lysine (K) and leucine (L) and its molecular mass of 40 kDa.[4] It is secreted by activated macrophages and neutrophils, arthritic chondrocytes and synovial cells.[5,6] The YKL-40 mRNA expression is found in inflamed articular cartilage from patients with RA and osteoarthritis whereas in normal joints YKL-40 is not detected.[7]

The glycoprotein is considered to be a novel biomarker of disease activity and an indicator of poor prognosis in patients with disorders characterized by inflammation and tissue remodeling.[8] For some of the diseases, assessment of serum YKL-40 has proven to be of both diagnostic and prognostic value.[9]

Despite the growing number of studies in recent years, the complete biological function and specific receptor of YKL-40 are still unknown. Circulating

YKL-40 levels were shown to be higher in RA patients compared to healthy controls.[8] Correlation with conventional parameters such as CRP and ESR was determined.[10] Our previous investigations revealed an association of YKL-40 serum and synovial levels with data from ultrasonographic examinations.[11]

Cytokines are known to serve as mediators of cellular differentiation, inflammation and immunity. The balance between pro-inflammatory and anti-inflammatory cytokines is essential for the development of a well-regulated effector immune response. It is known that the increased production of pro-inflammatory cytokines or the deficiency of anti-inflammatory cytokines leads to immune pathology.[12-14]

However, the interaction between YKL-40 and the major cytokines involved in RA pathogenesis remains unrevealed yet.

AIM

The aim of the present study was to determine serum and synovial YKL-40 levels in relation to proinflammatory cytokines such as IL-1β, TNF-α, and IL-6 in RA patients.

MATERIALS AND METHODS

Serum and synovial fluid samples were obtained from 39 patients (mean age 53.18 ± 16.54 yrs) with active RA. Disease activity was assessed by conventional biochemical parameters, such as ESR, CRP and ultrasonography (B mode and power Doppler). In addition, physician-based assessment of disease activity using the Disease Activity Scale (DAS28 /CRP) was obtained. The level of disease activity was interpreted as low (DAS ≤ 2.4), moderate (2.4 < DAS ≤ 3.7), or high (DAS > 3.7). A DAS < 1.6 corresponds to remission according to the American Rheumatism Association (ARA) criteria.[15]

Serum was collected also from the control group which consisted of 40 healthy age-matched volunteers.

The serum and synovial fluid samples were stored at -80°C until assayed. The study was approved by the Human Ethics Committee of Medical University, Plovdiv (No 1-19.05.2011) and was conducted in accordance with the Declaration of Helsinki. All participants signed an informed consent.

The concentrations of YKL-40, TNF-α, IL-6, and IL-1β were determined by ELISA assay using commercial kits (Quidel, San Diego, CA; Biolegend, Genaxxon) according to the manufacturers' instructions. All samples were analyzed in duplicates. Statistical analysis was carried out with the SPSS v 17.0 statistical software. Correlations between the different parameters were calculated by the Spearman test and p values of 0.05 were considered significant.

RESULTS

Serum YKL-40 levels in RA patients were significantly higher (239.3±29.29 ng/ml) than those of the control group (84.19±11.39 ng/ml). The concentration of YKL-40 in the synovial fluid of patients (496.97±52.9 ng/ml) was considerably elevated compared to the serum level (P<0.01) (**Fig. 1**). DAS 28 (CRP) was above 5.1 in all patients but it was unrelated to any other investigated parameters.

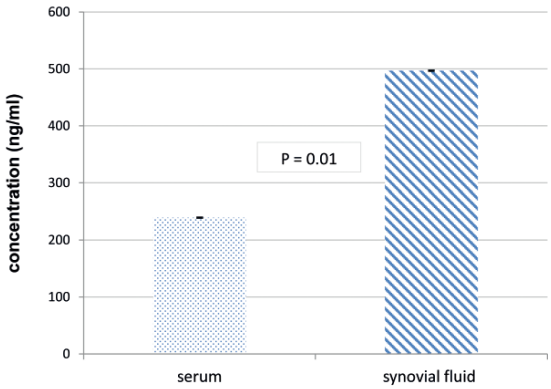

Figure 1. Serum and synovial YKL-40 levels in RA patients.

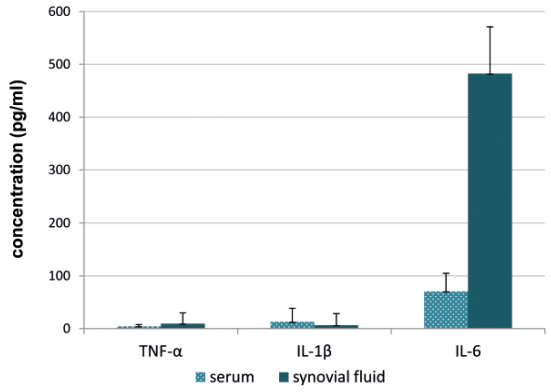

Figure 2. Serum and synovial levels of proinflammatory cytokines in RA patients.

Higher serum levels of TNF-α (4.42 pg/ml) and IL-6 (70.46 pg/ml) were found in the patients' group compared to the reference ranges - 3.011 pg/ml and 7.5 pg/ml, respectively.[16] In our study, higher serum levels of both examined cytokines were measured. The synovial fluid levels of TNF-α and IL-6 were

elevated in comparison with the serum level. Only the serum concentration of IL-1β exceeded that in the synovial fluid. IL-6 showed the highest concentration both in serum and synovial fluid (70.46±139.24 vs 482.3± 98.8) (**Fig. 2**) but no correlation with YKL-40. There were no gender differences in serum or synovial concentrations of YKL-40 and cytokines.

We found a strong association between serum and synovial levels of YKL-40 and serum TNF-α and IL-1β (P<0.05). The data is presented in **Table 1**.

Table 1. Correlation between serum and synovial YKL-40 levels and IL-1 β and TNF-α in RA patients

Variables	YKL-40 in serum	YKL-40 in synovial fluid
IL-1 β	r=0.371	r=0.455
	P=0.05*	P=0.01*
TNF-α	r=0.336	r=0.336
	P=0.04*	P=0.000*

*Correlation is significant at the 0.05 level

DISCUSSION

A wide range of serum markers were used in the measurement of disease activity in RA patients but there is still a need of specific indicators of synovial inflammation and vascularization. No single biomarker could illustrate the complex biology of the disease and to help predict clinical course.

It is supposed that YKL-40 is a candidate autoantigen in RA, suggested to play a pathogenic role in the inflammatory process and joint destruction. It is not absolutely known whether YKL-40 directly participates in the development and progression of RA or its higher levels represent a secondary inflammation effect.

Our study revealed significantly elevated YKL-40 concentration in synovial fluid. This fact might be explained by the local secretion of the glycoprotein. Some authors found that YKL-40 functioned as a growth factor for fibroblast-like synovial cells, fibroblasts, chondrocytes, and endothelial cells.[17] It could be suggested that locally released YKL-40 has a dual role in chondrocytes and fibroblast-synovial cell proliferation rate or protect from undergoing apoptosis.

The antagonistic effect of YKL-40 on collagen fibrillogenesis was demonstrated.[16] It was supposed that the glycoprotein decreased the response of chondrocytes and synovial cells induced by pro-inflammatory cytokines. It might be due to the protective and regulative function of YKL-40 in tissue remodeling. On the other hand, increased secretion of the glycoprotein led to intensive formation and accumulation of collagen fibrils which provoke tissue fibrosis. YKL-40 was supposed to act as a protective signalling factor, which determined cells to survive during mammary involution.[8]

Our previous investigations have demonstrated that increased YKL-40 levels are associated with active inflammation in RA. A strong correlation between serum YKL-40 concentration and ESR and CRP, the gold standard for biochemical assessment of disease activity, and data from ultrasonography was determined.[11] Our results suggest potential involvement of YKL-40 in inflammation and disease activity in RA. Increased YKL-40 synthesis in the joints of RA patients may lead to enhanced YKL-40-derived peptide presentation, which in turn could amplify the local autoimmune response. This is in agreement with the suggestions of Johansen et al. that YKL-40 is a potential autoantigen in RA.[8] Recently, a multi-biomarker disease activity (MBDA) score, based on 12 serum biomarkers was estimated as a baseline predictor for 1-year radiographic progression in early RA. YKL-40 was among these[12] serum markers.[18]

We showed that DAS 28 (CRP) was higher than in all patients but was unrelated to any included in the study parameters. There is no real 'gold standard' available to determine RA disease activity. Therefore, in the development of the DAS, physician judgment of low and high disease activity was used as external standard.[19]

New markers are also needed to better stratify patients into different risk categories because current markers only account for 32% of the total variance in predicting joint destruction.[20] Some of the markers might be present as a consequence of the disease and others may be more intimately linked to the disease pathogenesis.

The role of monocyte-macrophage cells in the pathogenesis of RA remains in the research focus. These cells are concentrated in the inflamed synovial membrane and at the cartilage. The circulating monocytes are the main source of serum TNF-α and this cytokine is involved in the pathogenesis of RA and serves as a target for the therapeutic treatment.[21] It is shown that TNF-α could induce chondrocytes to secrete YKL-40.[5] Several studies reveal that YKL-40 participates in cell differentiation, tissue remodeling, angiogenesis and inflammation.[8]

Cytokines are the key mediators of inflammation in the course of autoimmune arthritis and other immune-mediated diseases. Increased secretion of pro-inflammatory cytokines such as TNFα and IL-1β could induce autoimmune processes. These soluble factors are involved in the differentiation, activation and migration of pathogenic cells into the joints, in the process of neovascularization, development and activation of osteoclasts, and the process of cartilage damage in arthritis.[22]

Fibroblast-like synoviocytes play a major role in the pathogenesis of RA. At the molecular level, synovial fibroblasts are characterized by the activation of signaling cascades that result in the inhibition of apoptosis.[23] During synovial hyperplasia of the joint fibroblast-like synoviocytes proliferate and lead to cartilage destruction in the inflamed joint, release a lot of pro-inflammatory cytokines and chemokines, among which, IL-1β is the most important cytokine involved in the process of inflammation in RA.[24]

The higher concentration of cytokines in RA patients compared to the reference ranges in healthy Bulgarian population indicates the presence of immune-related inflammation.[16] Our results reveal a correlation of serum and synovial YKL-40 levels with proinflammatory TNFα and IL-1β levels. Other authors showed that these cytokines enhanced YKL-40 production by over 30% in primary chondrocytes from osteoarthritic cartilage.[25] These findings suggested that in osteoarthritis YKL-40 was produced by chondrocytes activated by inflammatory stimuli. No relationship was found between the glycoprotein and IL-6. The reason could be the pleiotropic effect of this cytokine. It is not only involved in inflammatory responses but also in the regulation of metabolic, regenerative, and neural processes. Generally, IL-6 stimulates target cells via a membrane bound interleukin-6 receptor, which, upon ligand binding, associates with the signalling receptor protein gp130 and activates in turn the MAPK pathway.[26] The characteristics of osteoarthritis includes the progressive loss of articular cartilage tissue and synovial tissue inflammation in a similar pattern as in RA.[27] Despite the recent progress, a lot of hidden potentials and features of this mysterious cytokine remain to be revealed. Future prospective research on YKL-40 as a novel inflammatory marker in combination with cytokines in clinical practice is needed.

CONCLUSION

We present the first study that finds a significant correlation between serum TNF-α and IL-1β, and YKL-40 in RA. It is suggested that these markers together might play a dominant role in the pathogenesis and disease activity and could possibly serve as reliable diagnostic markers.

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YKL-40 и цитокины - новая диагностическая констелляция при ревматоидном артрите?

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Введение: Ревматоидный артрит (РА) приводит к хроническому воспалению и изменениям в суставных тканях и в суставах. Патогенез данного состояния остаётся невыясненным, хотя известно, что противовоспалительные цитокины играют главную роль в его индуцировании.

YKL-40 является хитиназо-подобным гликопротеином, продуцированным ак-

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тивированными макрофагами, нейтрофилами, артритными хондроцитами и раковыми клетками. Установлено, что YKL-40 принимает участие в ремоделировании тканей, ангиогенезе и воспалении.

Цель: Исследовать уровни YKL-40 в сыворотке и синовиальной жидкости на предмет IL-1β, TNF-α и IL-6 у пациентов с РА.

Материалы и методы: Концентрации YKL-40, TNF-α, IL-6 и IL-1 β в сыворотке и синовиальной жидкости были установлены с применением ELISA у 39 пациентов (средний возраст которых составлял 53.18 ± 16.54 г.) с активным РА.

Результаты: Установлены повышенные уровни YKL-40 в сыворотке у всех пациентов. Наиболее высокие уровни установлены в синовиальной жидкости ($P<0.01$). Наше исследование устанавливает высокую степень взаимозависимости между уровнями YKL-40 в сыворотке и синовиальной жидкости и TNF-α и IL-1β ($P<0.05$) в сыворотке.

Заключение: В настоящем исследовании впервые устанавливается высокая степень взаимозависимости TNF-α и IL-1β и YKL-40 в сыворотке при активном РА. Нами высказывается предположение, что данные молекулы в совокупности могли бы играть ведущую роль в патогенезе и активности заболевания и могли бы быть использованы в качестве новой диагностической констелляции при ревматоидном артрите.