

## SERUM YKL-40 LEVELS IN CHRONIC HEART FAILURE

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### SUMMARY

Inflammatory processes are involved in the pathogenesis and development of chronic heart disease. The promising novel inflammatory biomarker YKL-40 is related to the degree of inflammation and pathological tissue remodeling.

The aim of this study was to determine serum YKL-40 levels in patients with chronic heart failure and to evaluate the potential relationship with ultrasonography findings.

Forty-three individuals were enrolled in the study – 24 patients (10 females and 14 males) with chronic heart failure, aged 70±11 (mean ± standard deviation) and 16 healthy people as age-matched controls (above 50 years). The serum YKL-40 levels were assessed by ELISA. Sonographic measurements such as two-dimensional, Power wave, Continuous Wave, Colour mode and M-Mode were performed using a diagnostic ultrasound system (PHILIPS Ultrasound, Washington, US) with a L11-3 probe of 3-11 MHz. The six minute walk test was used to assess functional capability of patients.

Our study revealed significantly higher serum YKL-40 levels in patients compared to the control group (P=0.010). No relation was found between the glycoprotein and the results from the ultrasonographic and functional examination.

We suppose that increased serum YKL-40 levels in patients with chronic heart failure might reflect the inflammatory route in the development of the disease.

**Key words:** YKL-40, chronic heart failure, biomarker

### INTRODUCTION

Despite advances in the prevention and treatment of cardiovascular diseases heart failure is still a leading cause of mortality and disability. It represents a global public health problem. Chronic heart failure (CHF) is a heterogeneous disease with diverse etiologies and clinical phenotypes (1).

In CHF, activated inflammatory processes are observed. Several studies demonstrate that the increased serum levels of inflammatory cytokines and chemokines are significantly associated with altered functional cardiac performance (2).

YKL-40, known as chitinase-3 like-1 protein (CHI3L1), is a glycoprotein secreted by activated macrophages and neutrophils in different tissues with inflammation, arthritic chondrocytes, vascular smooth muscle cells and cancer cells (3, 4).

In patients, elevated YKL-40 levels are associated with pathological processes characterized by inflammation, extracellular remodeling and tissue fibrosis (5). The exact function of YKL-40 remains unknown, but it was suggested that the glycoprotein participated in cell proliferation and differentiation, endothelial dysfunction, atherosclerosis and neoangiogenesis (6, 7, 8).

The purpose of the current study was to determine serum YKL-40 levels in patients with CHF and to evaluate the potential relationship with ultrasonography and functional tests.

### SUBJECTS AND METHODS

#### Subjects

The target group consisted of 24 patients with CHF with mean age 70 years (55-89; min-max), of whom 58% were males. Levels of hemoglobin, creatinine, uric acid and aminotransferases were measured with standard laboratory techniques and all of them were within the reference range. The controls were 16 age-comparable volunteers who attended a health screening.

The study was approved by the University Ethics Committee. All participants provided informed consent, according to the Helsinki Declaration.

#### ELISA

Blood samples were taken in the morning and serum was isolated by centrifugation at 2500 rpm for 10 minutes. The sera were stored at -80 °C until analysis. Serum YKL-40 levels were determined by ELISA (Quidel, CA, USA). The intra-assay and inter-assay coefficients of variation were 5.8 and 6.0%, respectively. All samples were analysed in duplicates.

#### Echocardiography

Left ventricular end-diastolic (EDD) and end-systolic dimension (ESD) were measured by echocardiography (Envision, Philips). Two-dimensional parasternal short and long axis images were obtained, Power wave, Continuous

Wave and Colour mode, and targeted M-mode tracings at the level of the papillary muscles were recorded. Ejection fraction (EF) was calculated using the formula  $(SV/EDV \times 100)$ . Sonographic measurements were performed by a diagnostic ultrasound system (PHILIPS Ultrasound, Washington, US) with a L11-3 probe of 3-11 MHz.

*Six minute walk test (6MWT)*

The patients had to walk the longest distance possible in interval of 6 min, through a walking course (corridor) 30 m long, according to the guidelines of American Thoracic Society.

*Statistical methods, graphics and applied software*

The Mann-Whitney U test was used to compare the values of a continuous variable in two independent groups. Boxplot diagrams were used for graphical visualization of a continuous variable as well as to distinguish outliers in the data series (the criteria 1.5 of the interquartile range have been accepted). The correlations between two continuous variables were estimated by the Kendall's tau-b correlation coefficient. All calculations have been made with MS Excel 2010.

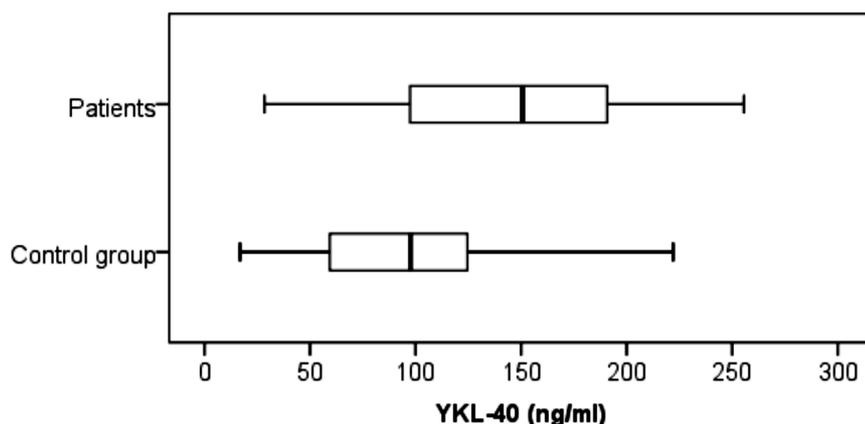
**RESULTS**

Our study determined significantly higher YKL-40 levels in serum of CHF patients compared to controls ( $P=0.010$ ). The median serum concentration of YKL-40 in the target group was  $151 \pm 64$  ng/ml vs  $95 \pm 51$  ng/ml (median  $\pm$  standard deviation) in healthy people. The results are presented on Fig. 1.

Echocardiography showed that EDD and ESD levels were in upper limit values in patients. The data from EF and 6MWT decreased compared to reference range. Table 1 indicates the results from the laboratory and clinical measurements performed in the patients' group. These results revealed weakness of the heart muscle, ventricular hypertrophy with impaired diastolic filling and function. The damaged pumping function of the heart muscle leads to heart failure.

Next, we evaluated the correlation between serum YKL-40 levels, echocardiography examination and the 6MWT. We did not detect any relationship between the investigated parameters and YKL-40.

**Fig. 1.** YKL-40 levels in serum of CHF patients and healthy subjects.



**Legend:** The median level of YKL-40 in CHF patients was  $151 \pm 64$  ng/ml while in the control group it was  $95 \pm 51$  ng/ml. YKL-40 was increased in patients compared to healthy subjects ( $P=0.010$ ).

**Table 1.** Laboratory and clinical characteristic of CHF patients.

Characteristics	Number of patients	Mean $\pm$ SD	Reference range
Age (years)	24	70 $\pm$ 11	age-matched
YKL-40 (ng/ml)	24	146 $\pm$ 64	68-122 ng/ml*
EDD	24	5,3 $\pm$ 0,6	5.6
ESD	24	3,8 $\pm$ 0,9	4.2
Ejection fraction (T) %	12	44,4 $\pm$ 13,6	75
Walking test (min)	11	18 $\pm$ 7	30

\*Confidence interval at 95% probability (n=16)

## DISCUSSION

New therapies and biomarkers are needed to follow up the progression of CHF. Inflammatory mediators could provide important prognostic information in CHF patients (9). Although YKL-40 was supposed to be a potential biomarker in cardiovascular diseases, there is a controversy in the findings of different groups (10, 11).

In our study, we demonstrated significantly higher serum YKL-40 levels in CHF patients compared to healthy subjects. These findings were in accordance with a large study of 717 patients with CHF followed up for 7 years, suggesting that YKL-40 was a new biomarker for all-cause mortality. On the other hand, some researchers claimed that the concentration of the glycoprotein could not serve as a prognostic indicator in cardiovascular pathologies (7).

The functional role of YKL-40 in CHF is not clearly defined yet. *In vivo*, macrophages in atherosclerotic plaques express the glycoprotein and the highest YKL-40 mRNA was determined in macrophages in early atherosclerotic lesions. The researchers concluded that the protein could be used as a marker for atherosclerosis at the onset of the disease (12).

*In vitro*, YKL-40 induced lymphocyte and endothelial cells adherence, migration and proliferation, leading to extracellular matrix remodeling and abnormal angiogenesis (7). Protein expression was detected by immunohistochemistry in developing rat hearts (13).

Recent investigations revealed that glycoprotein levels increased in patients after an acute myocardial infarction correlating with the number of damaged vessels (10, 14).

The impact of echocardiography and the 6MWT is to provide objective evidence of structural and functional cardiac disorder (15). In our study there was no relationship between YKL-40 levels and the results from these functional tests. Weakness of the heart muscle and ventricular hypertrophy were observed. We detected impaired pumping function of the heart muscle, leading to heart failure. This fact was in agreement with a study of patients with CHF where YKL-40 was elevated compared to healthy age-matched people, but was not associated with other clinical characteristics or prognosis (7). Further investigations are needed to clarify the biological role of this glycoprotein in CHF.

The ultrasonographic and functional examinations detected evidence-based pathological changes in heart structure and function. We suppose that YKL-40 might reflect the inflammatory route in the development of CHF.

## CONCLUSION

The increased serum YKL-40 levels in patients with chronic heart failure highlight the possibility this novel inflammatory biomarker to be associated with disease pathogenesis and progression.

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