YKL-40 IN HEALTHY SUBJECTS

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ABSTRACT

YKL-40 is a plasma protein, belonging to the chitinase protein family, but has no chitinase activity. It is expressed and secreted by macrophages, chondrocytes, activated neutrophils, differentiated monocytes, vascular smooth muscle cell and cancer cells. The objective of the present study was to determine serum YKL-40 levels in healthy subjects and to develop a valid reproducible enzyme-linked immunosorbent assay. Serum YKL-40 concentrations were determined by a two-site, sandwich-type, enzyme-linked immunosorbent assay (ELISA) in 10 healthy female volunteers aged 18-50. Our investigation show a mean value 41.11 (20-59) ng/ml of serum YKL-40 in healthy women. We determined that the correlation between protein level and age is feeble, but positive. Our study is the first in Bulgaria to measure serum YKL-40 level in healthy subjects. Elucidation of YKL-40 functions in normal and pathologic processes is an important objective of future analyses.

Keywords: YKL-40, biomarker, ELISA

Introduction

YKL-40, also known as a human cartilage glycoprotein 39 (6), chitinase-3 like-1 (32) and breast regressing protein 39kd (27) is a glycoprotein belonging to the family 18 of glycosyl hydrolases that lack chitinolytic activity but retain chitin-binding ability. The name is based on the three NH2 terminal amino acids – tyrosine, lysine, and leucine and its molecular mass of 40 kDa (12).

The YKL-40 gene is located on 1q32.1 and consists of 10 exons. The complete amino acid sequence contains 383 amino acids in a single polypeptide chain (7).

The protein is expressed and secreted by macrophages (33), chondrocytes (39, 14), activated neutrophils (38), differentiated monocytes (33), vascular smooth muscle cells (25, 28) and cancer cells (5, 8, 22, 30, 35).

Specific receptors for YKL-40 have not been identified and the complete biological function of this glycoprotein in normal and pathologic processes is not clear yet.

The aim of the present study is to determine serum YKL-40 levels in healthy female subjects and to introduce a valid reproducible enzyme-linked immunosorbent assay.

Materials and Methods

Serum YKL-40 concentrations in 10 healthy female volunteers aged 18-50 was measured. Clinical and routine hematological, biochemical and coagulation tests were performed to assess their health status. The study was approved by the University Ethics Committee. Informed content was asked and achieved from all examined individuals according to the Helsinki Declaration.

Venous blood samples were collected in the morning (4.5 mmol/l blood, Monovette, Sarstedt) asatraumatically as possible after a 12-hour fasting and 30-minute rest immediately prior to testing. The samples were centrifuged at 2500 rpm for 10 minutes. The serum was kept at -20°C for no more than a month before analysis.

Serum YKL-40 concentrations were determined by a two-site, sandwich-type, enzyme-linked immunosorbent assay (ELISA) (Quidel Corporation, Cat. № 8020) according to the manufacturer's instructions. The validation of the method was performed in compliance with the international standard of quality and competence of medical laboratories (BDS/EN/ISO 15189). The method showed high precision. The results were consistent with the recommended minimal non-reproducibility (intra-assay CV<10%; inter-assay CV<12%) for ELISA in studying YKL-40 as given by the
manufacturer. The detection limit of the YKL-40 assay was 20.0 ng/ml. All samples were tested in duplicates.

Microsoft Excel 2000 and SPS 12.0 (Windows XP) were used to analyze the results. The level of statistical significance of null hypothesis was P<0.05. The effect of the various factors was considered using correlation and regression analyses simultaneously determining the size and direction of correlation.

Results and Discussion

Our investigation showed a mean value 41.11 (20-59) ng/ml of serum YKL-40 in healthy women. Other authors revealed that in healthy adults (aged 18-79), the median level of serum YKL-40 determined by ELISA was 43 μg/l (range 20-184 μg/l) (21).

YKL-40 protein expression appears at specific time in early developing human musculoskeletal system. These tissues are characterized by rapid proliferation, differentiation and undergo morphogenetic changes. (20). Recent studies show that the expression of YKL-40 in normal adult tissues is feeble and related to metabolic activity (34). The possible biological functions of YKL-40 in healthy subjects are presented on Fig. 1.

According to Johansen et al. (2008), serum YKL-40 increases with age but in the normal reference interval. Aging is associated with enhanced inflammatory activity reflected by increased circulating levels of TNF-α, IL-6, cytokine antagonists and acute phase proteins (2). We detected also that serum YKL-40 increased with age (r_{xy}=0.46). The correlation between protein level and age was feeble, but positive.

YKL-40 is a secreted protein and although its exact function is unknown yet, several possibilities have been suggested. It may act as a growth factor for normal cells, fibroblasts and chondrocytes, working synergistically with insulin growth factor-1 (IGF-1) (24). It is assumed that YKL-40 is a cellular survival factor in responses to a variety of adverse environments, inflammation, hypoxia and nutrient absence (22, 31). Another study shows that the protein determines which cells to survive during mammary involution (26).

YKL-40 probably has a function in embryonic development, promoting cell migration and adhesion (28). It could not be excluded that YKL-40 might facilitate cell spreading and reorganization for cancer cells, too. It has been hypothesized that YKL-40 could be a differentiation marker for monocytes (33), mesothelial cells (36) and chondrocytes (11).

Elevated values are found in processes characterized by acute or chronic inflammation and intensive extracellular tissue remodeling- rheumatoid arthritis (19, 39), osteoarthritis (23, 40), inflammatory bowel disease (37), giant cell arteritis (13), pulmonary sarcoidosis (17), liver fibrosis (29). YKL-40 could function in proliferation, differentiation, apoptosis and angiogenesis in cancer cells (18). It have been reported that YKL-40 might be used as a novel tumor marker for ovarian cancer (4, 8), small cell lung cancer (16), metastatic breast cancer (15), and metastatic prostate cancer (1). Recent investigation identified that YKL-40 (CHI3L1) played a unique role during the development of intestinal inflammation (5). The expression of YKL-40 in inflammatory and tumor processes is presented on Fig. 2.

The protein is not specific for all types of cancer disease. It is considered that serum YKL-40 could be more useful for monitoring disease and progression in cancer patients after treatment, providing independent information of survival (3, 9, 10, 17).

The clinical significance of YKL-40 as a biomarker is discussed in different aspects. Several biological questions regarding this protein are searching their answers.
Conclusions
Our study is the first in Bulgaria to measure serum YKL-40 level in healthy female subjects. Elucidation of YKL-40 functions in normal and pathologic processes is an important objective of future analyses.

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REFERENCES