Cancer Growth and Metastasis

ORIGINAL RESEARCH

Serum YKL-40 Levels in Patients with Esophageal Squamous Cell Carcinoma

Ozkan Yilmaz[1], Ozgur Kemik[1], Ahu Kemik[2], Aziz Sumer[1], A. Cumhur Dülger[3], Ismail Hasirci[1], Nejat Almali[1], Sevim Purisa[4] and Çetin Kotan[1]

[1]Department of General Surgery, Medical Faculty, University of Yüzüncü Yıl,Van, Turkey. [2]Department of Biochemistry, Cerrahpasa Medical Faculty, University of Istanbul, Istanbul, Turkey. [3]Department of Gastroenterology, Medical Faculty, University of Yüzüncü Yıl,Van, Turkey. [4]Department of Biostatistics, Istanbul Medical Faculty, University of Istanbul, Istanbul, Turkey. Corresponding author email: ozgurkemik@hotmail.com

Abstract:

Aims and background: YKL-40 is a glycoprotein secreted by macrophages, neutrophils and malignant tumor cells. YKL-40 is expressed and secreted by several types of tumors. The aim of this study examined the clinical usefulness of YKL-40 for detection in esophageal squamous cell carcinoma (ESCC).

Methods: Using ELISA kits, we measured the concentration of YKL-40 in serum from 100 patients with ESCC and compared this concentration with healthy population.

Results: We found significantly higher serum levels of YKL-40 in patients with ESCC compared to the healthy population ($P < 0.0001$).

Conclusions: These results suggested that regarding serum YKL-40 as a tumor marker could be benefical in the early clinical diagnosis.

Keywords: esophageal squamous cell carcinoma, YKL-40

Cancer Growth and Metastasis 2011:4 1–5

doi: 10.4137/CGM.S7046

This article is available from http://www.la-press.com.

Introduction

Esophageal squamous cell carcinoma (ESCC) is one of the most aggressive malignant tumors associated with poor prognosis, rapid clinical progression, and a high rate of metastases.[1,2] Degradation or breakdown of the extracellular matrix is the main structural change during the invasion and metastases of esophageal squamous cell carcinoma.[3]

YKL-40 is a heparin- and chitin-binding lectin[4–6] secreted by activated neutrophils,[7] macrophages during later stages of differentiation,[8,9] arthritic chondrocytes,[1] differentiated vascular smooth cells,[6] and fibroblast-like synovial cells.[10,11]

YKL-40 is a mammalian member of the chitinase protein family which has no chitinase activity.[4,11] Although the physiological function of YKL-40 is unknown, the pattern of its expression in normal and disease stage suggests a function in remodeling or the degradation of extracellular matrix.

YKL-40 is a new biomarker with an important value in cancers.[13] A number of studies in patients with solid tumors and hematological malignancies[14–21] have shown that an elevated serum YKL-40 is an independent biomarker of many malignant cells.[22]

In vivo, YKL-40 mRNA has been demonstrated in tumor-associated macrophages[23] and in normal and malignant epithelial cells of the breast.[20,24] The human YKL-40 gene (CHI3LI)[8,25] and the crystal structure of the protein[26,27] have been characterized.

The serum levels of YKL-40 are unknown in the esophageal squamous cell carcinoma. We hypothesized that YKL-40 serum levels in patients with ESCC might be associated with prognosis. Thus, in our study we investigated the value of serum YKL-40 in ESCC.

Patients and Methods

The study included 100 patients with ESCC (53 women and 47 men, aged 40–60 years) diagnosed by gastroenterologists and operated at the Department of General Surgery at the Yüzüncü Yıl University Medical Faculty, Van. The control group comprised 40 healthy volunteer subjects (10 women and 30 men, aged 39–58 years). The absence of disease such as infection and asymptomatic early adenocarcinoma and adenoma was assessed by clinical history, physical examination, routine laboratory tests, including liver and renal function tests, and colonoscopy.

The study was approved by the locals Ethics Committee. All patients gave their informed consent.

From every patient, 5 ml of blood were withdrawn from cubital vein in the operating-room before the start of the operation. Blood samples were processed into serum aliquots within 3 hr, stored at −70 °C and analyzed blinded to clinical parameters and study endpoints at the end of the study. Serum levels of YKL-40 were determined in duplicates by ELISA (Quidel Corporation, San Diego, CA).[26]

Using streptavidin-coated microplate wells, a biotinylated-Fab monoclonal mouse antibody against human YKL-40 (capture antibody), and an alkaline phosphatase-labeled polyclonal rabbit antibody against human YKL-40 (detection antibody) serum samples were evaluated.

Bound enzyme activity is detected with *p*-nitrophenyl phosphate as substrate. The detection limit of the ELISA is 20 ng/ml and the intraassay coefficient of variation (during an 11-day period) is <3.7%, and the long-term interassay coefficient of variation (during a 5-year period) is <8.6%. The YKL-40 ELISA is useful for the measurement of serum concentrations of YKL-40 in humans.

Statistical Analysis

Comparisons between two groups were performed by the Mann-Whitney U-test. The differences were considered statistically significant at $P < 0.05$. The median average is given as mean ± standard deviation.

Results

There was an amnestically no other tumor disease, or any acute or chronic inflammation (including autoimmune diseases). Average values of serum levels of YKL-40 are shown in Table 1. The scatters of age and serum YKL-40 levels are given in Figures 1 and 2.

Table 1. Mean values of YKL-40 levels in patients ESCC and in healthy subjects (Data are means ± SD).

	ESCC	Healthy	*P*
Age	43.7 ± 10.9	45.2 ± 9.7	$P > 0.05$
Female YKL-40 (µg/l)	239.5 ± 43.0	42.3 ± 18.1	<0.0001
Male YKL-40 (µg/l)	241.4 ± 27.7	49.4 ± 10.9	<0.0001
Total YKL-40 (µg/L)	244.8 ± 46.1	45.9 ± 12.5	<0.0001

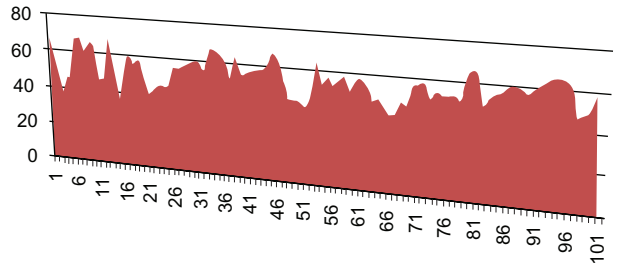

Figure 1. The age scatter of patients with esophageal squamous cell carcinoma.

We found significantly higher in the concentration of YKL-40 in patients with ESCC compared to the healthy population ($P < 0.0001$). But, there are no differences between female and male levels of serum YKL-40 in patients with esophageal squamous cell carcinoma ($P > 0.05$).

Discussion

Various factors influence the biology of esophageal squamous cell carcinoma.[2] This is to our knowledge the first report on the novel serum biomarker YKL-40 in patients with ESCC. In all patients had elevated serum concentration of YKL-40, which were significantly higher than healthy population.

The YKL-40 ELISA is useful for the measurement of serum (or EDTA plasma) concentrations of YKL-40 in humans,[29] but not in other species like bovine, swine, rabbit, mouse, and rabbit. The detection limit of the ELISA is 20 ng/ml.[22]

The median serum concentration of YKL-40 in healthy adults is 43 µg/l (90% percentile = 95 µg/l; 95 percentile = 124 µg/l).[22,28]

In 1995, we reported increased serum levels of YKL-40 in some patients with metastatic breast cancer.[29] Recent studies have found elevated serum levels of YKL-40 in patients with several types of localized or advanced solid cancer.[14–20] These cancer

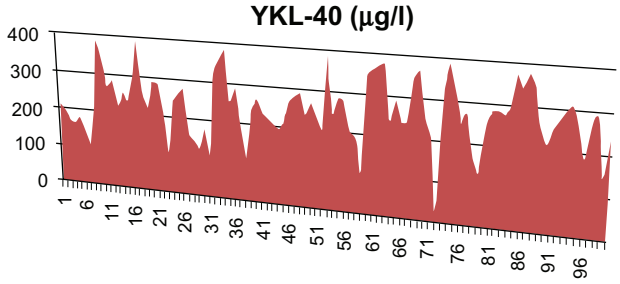

Figure 2. The scatter of the serum concentrations of YKL-40 levels of patients with esophageal squamous cell carcinoma.

patients were scored as having elevated serum YKL-40 if it was higher than the upper 95th percentile confidence limit of serum YKL-40 in healthy subjects adjusted for age.[28–30] Preoperative serum levels of YKL-40 were elevated in 19% of patients with primary breast cancer, and patients with metastases to axillary lymph nodes had higher serum YKL-40 compared with lymph node-negative patients.[31] Preoperative serum levels of YKL-40 from patients with colorectal cancer were elevated in 26%, and there was an association between serum YKL-40 and Dukes stage: 16% of the patients with Dukes' A, 265 with Dukes' B, 19% with Dukes' C, and 39% with Dukes' D had elevated preoperative serum YKL-40.[31,32] Serum YKL-40 decreased significantly after curative operation for colorectal cancer in patients with high preoperative YKL-40[17] indicating that serum YKL-40 reflects tumor burden. In patients with small cell lung cancer, 22% with local disease and 40% of the patients with extended disease had elevated serum YKL-40.[15] Forty-three percent of patients with metastatic prostate cancer,[14] 83% of patients with metastatic renal cell cancer[33–35] and 45% of patients with metastatic malignant melanoma[12] had elevated serum YKL-40. In patients with glioblastoma, the serum YKL-40 level was related to tumor grade and burden: 72% of patients with glioblastoma multiforme and 57% of patients with lower grade gliomas had high serum YKL-40.[21] Also, we found higher serum levels of YKL-40 in all patients with ESCC.

The biological function of YKL-40 in cancer is not yet known. It has been suggested that YKL-40 may play a role in the proliferation and differentiation of malignant cells, protects the cancer cells form undergoing apoptosis, stimulates angiogenesis, has an effect on extracellular tissue remodeling, and stimulates fibroblasts surrounding the tumor, although in vivo proof of these hypotheses are yet to be obtained.[35] YKL-40 is not produced by fibroblasts, but it is a growth factor for fibroblasts, synovial cells, and chondrcytes.[9] Some studies demonstrated that increased serum YKL-40 levels found in various cancer patients reflect YKL-40 secretion from a subset of tumors with a more aggressive phenotype, especially esophageal squamous cell carcinoma.

Recently, an in vitro study has shown that ectopic expression of YKL-40 in breast and colon cancer cells respectively led to tumor formation with an extensive

angiogenic phenotype and that recombination YKL-40 protein promoted vascular endothelial cell angiogenesis both in vitro and in vivo.[36] Furthermore, immunohistochemical analysis of human cancer showed a correlation between YKL-40 expression and blood vessel density.[36] Therefore, the occurrence of high YKL-40 levels in highly differentiated and advanced cancers and recurrent cancer states could be explained by the role of YKL-40 in both angiogenesis and fibrogenesis, since highly differentiated tumors are characterized by high vascularization and a high turnover of extracellular matrix.

YKL-40, initiates mitogen-activated protein kinase (MAP) and PI-3K signaling cascades in fibroblasts leading to phosphorylation of both the extracellular signal-regulated kinase (ERK)-1/2 MAP kinase and protein kinase B (AKT)-mediated signaling cascades,[36] which are associated with the control of mitogenesis. This suggests a role of YKL-40 as an anti-apoptotic protein.

Not all patients with cancer had elevated serum YKL-40 levels compared with healthy age-matched controls, suggesting that not all tumors secrete YKL-40 or that the protein is secreted at a low level. Cancer cells that secrete YKL-40 may have a different phenotype than cancer cells that do not express and secrete YKL-40, and the protein may therefore reflect differences in the biology of various cancer types. Serum concentrations of YKL-40 were independent of serum carcinoembryonic antigen in patients with colorectal cancer,[20] of serum CA-125 and CA15-3 in patients with ovarian cancer,[19] of serum HER2 in patients with metastatic breast cancer,[14] of serum prostate-specific antigen in patients with metastatic prostate cancer,[17] and of serum lactate dehydrogenase in patients with small cell lung cancer,[18] indicating that serum YKL-40 reflects other aspects of tumor growth and metastasis than these tumor markers.[22]

This study shows that serum concentrations of YKL-40 have a high sensitivity for esophageal squamous cancer, and that determination of serum YKL-40 can be used as a test. First of all, in this study, we investigated the effect of YKL-40 that we have obtained interesting results.

In conclusion, this study has shown that YKL-40 is expressed in esophageal squamous cell carcinoma. This may be due to contribution to the serum YKL-40 level from esophageal squamous cells, which also produce the protein. Also, serum concentrations of YKL-40 could be useful in the early diagnosis of esophageal squamous cell carcinoma.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

References

1. Kuwano H, Nakajima M, Miyazaki T, et al. Distinctive clinicopathological characteristics in esophageal squamous cell carcinoma. *Ann Thorac Cardiovasc Surg*. 2003;9:6–13.
2. Ohashi K, Nemoto T, Nakamura K, et al. Increased expression of matrix metalloproteinase 7 and 9 membrane type 1-matrix metalloproteinase in esophageal squamous cell carcinomas. *Cancer*. 2000;88:2201–9.
3. Samantaray S, Sharma R, Chattopadhyaya TK, et al. Increased expression of MMP-2 and MMP-9 in esophageal squamous cell carcinoma. *J Cancer Res Clin Oncol*. 2004;130:37–44.
4. Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem*. 1993;268:25803–10.
5. Renkema GH, Boot RG, Au FL, et al. Chitotriosidase, a chitinase, and the 39 kda human cartilage glycoprotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrophages. *Eur J Biochem*. 1998;251:504–9.
6. Shackelton LM, Mann DM, Millis AJ. Identification of a 38 kDa heparin-binding glycoprotein (gp38k) in differentiating vascular smooth muscle cells as a member of a group of proteins associated with tissue remodeling. *J Biol Chem*. 1995;270:13076–83.
7. Volck B, Price PA, Johansen JS, et al. YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. *Proc Assoc Am Physicians*. 1998;110:351–60.
8. Rehli M, Krause SW, Anderseen R. Molecular characterization of the gene for human cartilage gp-39 (CHI3LI), a member of the chitinase protein family and marker for late stages of macrophage differentiation. *Genomics*. 1997;43:221–5.
9. Boot RG, van Achterberg TA, van Aken BE, et al. Strong induction of members of the chitinase family of proteins in atherosclerosis: chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. *Arterioscler Thromb Vasc Biol*. 1999;19:687–94.
10. Nishikawa KC, Millis AJ. Gp-38k (CHI3LI) is a novel adhesion and migration factor for vascular cells. *Exp Cell Res*. 2003;287:79–87.
11. Nyirkos P, Golds EE. Human synovial cells secrete a 39 kDa protein similar to a bovine mammary protein expressed during the non-lactating period. *Biochem J*. 1990;269:265–8.
12. De Ceuninck F, Gaufillier S, Bonnaud A, Sabatini M, Lesur C, Pastoureau P. YKL-40 induces proliferative events in cultured chondrcytes and synoviocytes and increases glycosaminoglycan synthesis in chondrocytes. *Biochem Biophys Res Commun*. 2001;285:926–31.
13. Johansen JS, Jensen BV, Roslind A, Nielsen D, Price PA. Serum YKL-40, a new prognostic biomarker in cancer patients? *Cancer Epidemiol Biomarkers Prev*. 2006;15:194–202.
14. Jensen BV, Johansen JS, Price PA. High levels of serum HER-2/neu and YKL-40 independently reflect aggressiveness of metastatic breast cancer. *Clin Cancer Res*. 2003;9:4423–34.

15. Schmidt H, Johansen JS, Gehl J, Geertsen PF, Fode K, von der Maase H. Elevated serum level of YKL-40 is an independent prognostic factor for poor survival in patients with metastatic melanoma. *Cancer*. 2006;106:1130–9.
16. Tanwar MK, Gilbert MR, Holland EC. Gene expression microarray analysis reveals YKL-40 to be a potential serum marker for malignant character in human glioma. *Cancer Res*. 2002;62:4364–8.
17. Brasso K, Christensen IJ, Johansen JS, et al. prognostic value of PINP, bone alkaline phosphotase, CTX-1, and YKL-40 in patients with metastatic prostate carcinoma. *Prostate*. 2006;66:503–13.
18. Johansen JS, Drivsholm L, Price PA, Christensen IJ. High serum YKL-40 level in patients with small cell lung cancer is related to early death. *Lung Cancer*. 2004;46:333–40.
19. Dupont J, Tanwar MK, Thaler HT, Fleisher M, et al. Early detection and prognosis of ovarian cancer using serum YKL-40. *J Clin Oncol*. 2004;22: 3330–9.
20. Cintin C, Johansen JS, Christensen IJ, Price PA, Sorensen S, Nielsen HJ. High serum YKL-40 level after surgery for colorectal carcinoma is related to short survival. *Cancer*. 2002;95:267–74.
21. Johnsen JS. High serum concentration of YKL-40 is associated with short survival in patients with acute myeloid leukemia. *Clin Cancer Res*. 2005;11: 8644–52.
22. Johansen JS, Schultz NA, Jensen BV. Plasma YKL-40: a potential new cancer biomarker? *Future Oncol*. 2009 Sep;5(7):1065–82. Review.
23. Junker JN, Johansen JS, Andersen CB, Kristjansen PE. Expression of YKL-40 by peritumoral macropahges in human small cell lung cancer. *Lung Cancer*. 2005;48:223–31.
24. Roslind A, Johansen JS, Junker JN, Nielsen DL, Dzaferi Hi Price PA, Balslev E. YKL-40 expression in benign and malignant lesions of the breast: a methodological study. *Appl Immunohistochem Mol Morphol*. In press.
25. Rehli M, Niller HH, Ammon C, et al. Transcriptional regulation of CHI3LI, a marker gene for late stages of macrophage differentiation. *J Biol Chem*. 2003;278:44058–67.
26. Houston DR, Recklies AD, Krupa JC, van Aalten DM. Structure and ligand-induced conformational change of the 39 kDa glycoprotein from human articular chondrocytes. *J Biol Chem*. 2003;278:30206–12.
27. Fusetti F, Pijining T, Kalk KH, Bos E, Dijkstra BW. Crystla structure and carbohydrate-binding properties of the human cartilage glycoprotein-39. *J Biol Chem*. 2003;278:37753–60.
28. Johansen JS, Hvolris J, Hansen M, Backer V, Lorenzen I, Price PA. Serum YKL-40 levels in healthy children and adults. Comparison with serum synovial fluid levels of YKL-40 in patients with osteoarthritis or trauma of the knee joint. *Br J Rheumatol*. 1996;35:553–9.
29. Harvey S, Weisman M, O'Dell J, et al. Chondrex: new marker of joint disease. *Clin Chem*. 1998;44:509–16.
30. Johansen JS, Cintin C, Jorgensen M, Kamby C, Price PA. Serum YKL-40 a new potential marker of prognosis and location of metastases of patients with recurrent breast cancer. *Eur J Cancer*. 1995;31A:1437–42.
31. Johansen JS, Christensen IJ, Riisbro R, et al. High serum YKL-40 levels in patients with primary breast cancer is related to short recurrence free survival. *Breast Cancer Res Treat*. 2003;80:15–21.
32. Cintin C, Johansen JS, Christensen IJ, Price PA, Sorensen S, et al. Serum YKL-40 and colorectal cancer. *Br J Cancer*. 1999;79:1494–9.
33. Geertsen P, Johansen JS, von der Maase H, Jensen BV, Price PA. High pretreatment serum level of YKL-40 is related to short survival in patients with advanced renal cell carcinoma treated with high dose continuous intravenous infusion of IL-2 meeting proceedings of American Society for Clinical Oncology ASCO. 2003;22:abstract 1603.
34. Johansen JS, Jensen BV, Roslind A, Nielsen D, Price PA. Serum YKL-40, a new prognostic biomarker in cancer patients? *Cancer Epidemiol Biomarkers Prev*. 2006;15(2):194–9.
35. Shao R, Hamel K, Petersen L, et al. YKL-40, a secreted glycoprotein, promotes tumor angipgenesis. *Oncogene*. 2009.
36. Recklies AD, White C, Ling H. The chitinase-3 like protein human cartilage glycoprotein 39 stimulates proliferation of human connective tissue cells and activates both extracellular signal-regulated kinase- and protein kinase B-mediated signaling pathways. *Biochem J*. 2002;365:119–26.