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ORIGINAL RESEARCH

Serum YKL-40 Levels as a Prognostic Factor in Patients with Locally Advanced Breast Cancer

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ABSTRACT

Introduction: YKL-40 is a growth factor for connective tissue cells; it also stimulates the migration of endothelial cells. YKL-40 is secreted by cancer cells, and elevated serum levels have been associated with poorer prognosis

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in metastatic breast cancer. In the present study we evaluated the prognostic role of serum YKL-40 levels in patients with locally advanced breast cancer.
Methods: YKL-40 levels were measured using ELISA in serum samples obtained from 45 breast cancer patients prior to surgery and chemotherapy. The median follow-up time was 46 months (range, 10–96 months). All patients underwent surgery after chemotherapy. During the follow-up period, 21 patients relapsed and there were 17 deaths.
Results: The median serum YKL-40 concentration in patients with locally advanced breast cancer was 149.5 μg/l (range, 25.0–1021.3 μg/l). This was higher than levels observed in healthy female controls but the difference was not significant (P=0.44). Serum YKL-40 levels were also higher in patients with tumour size >2 cm and node-positive disease but again the differences were not significant (P>0.05). Tumour volume was correlated with serum YKL-40 levels (r=0.308, P=0.039). High serum YKL-40 levels were associated with shorter disease-free and overall survival although this trend failed to reach significance (P>0.05). Multivariate analysis including tumour size, lymph node status, oestrogen and progesterone receptor status, tumour grade, and serum YKL-40 levels indicated that serum YKL-40 levels were an independent prognostic variable for overall survival (hazard ratio, 1.004; 95% confidence intervals: 1.00, 1.07; P=0.027). Tumour size, lymph node status and oestrogen receptor status were also independent prognostic variables for overall survival (P<0.05).
Conclusion: Our results show that serum levels of the growth factor YKL-40 may be a useful prognostic indicator of outcome for patients with locally advanced breast cancer. Further studies are required to fully elucidate the biological function of YKL-40 in breast cancer.

Keywords: breast cancer; locally advanced; prognosis; YKL-40

INTRODUCTION

YKL-40, a member of the mammalian chitinase-like proteins, was first identified in vitro in significant quantities in the human osteosarcoma cell line MG63.[1–3] The molecular weight of YKL-40 is 40 kd and it contains a single polypeptide chain of 383 amino acids.[2] It belongs to the glycosyl hydrolase family 18. YKL-40 is expressed and secreted by several types of solid tumours including breast, colon, kidney, lung, thyroid, osteosarcoma, ovarian, prostate, uterine, oligodendroglioma, glioblastoma and germ cell tumours.[1,4–12]

The biological function of YKL-40 is still unknown. Several studies have shown that YKL-40 is a differentiation marker in macrophages and foetal chondrocytes.[13–16] It is not produced by fibroblasts, but it is a

growth factor for connective tissue cells.[17–19] Furthermore, it may play a role in angiogenesis by stimulating the migration and reorganisation of vascular endothelial cells, and it is also an adhesion and migration factor for vascular smooth muscle cells.[20,21] YKL-40 also has an anticatabolic effect on the extracellular matrix during tissue destruction and remodelling.[19]

Several studies have evaluated the prognostic value of YKL-40 levels in patients with cancer. High serum YKL-40 levels have been associated with shortened survival in patients with breast cancer, small cell lung cancer, colorectal carcinoma, metastatic melanoma, prostate carcinoma, glioblastoma multiforme and ovarian carcinoma.[4–6,8–12]

The prognostic value of YKL-40 in patients with breast cancer has been investigated in only a limited number of studies. In our study, we evaluated the prognostic role of serum levels of YKL-40 in patients with locally advanced breast cancer.

MATERIALS AND METHODS

Patients

Forty-five patients with locally advanced breast cancer were included in this study. All patients underwent a physical examination and ultrasonography/mammography of the breast. None of the patients had evident distant metastasis. Patients had a good performance (performance status ≤2) and had adequate renal, hepatic and bone marrow function. Serum samples were obtained from the patients before surgery and chemotherapy. All patients underwent modified radical mastectomy and axillary dissection after three courses of chemotherapy (cyclophosphamide, doxorubicin, fluorouracil). Patients were assessed every 3 months during follow-up. The median follow-up time was 46 months (range, 10–96 months). Serum levels of YKL-40 were compared with those of 20 healthy Turkish females (median age, 45 years; range, 35–55 years) who attended the blood bank at Gazi University Hospital, Ankara, Turkey. The local ethics committee approved the research protocol.

Biochemical Analysis

Patients' blood samples were taken in the morning 1–2 weeks before surgery and chemotherapy. Blood samples from the patients and controls were collected into dry tubes and sera separated from cellular elements by centrifugation within half an hour after blood sampling. The sera were stored at −80°C until analysis. Serum YKL-40 was determined by ELISA (Quidel Corporation, San Diego, CA, USA). The YKL-40 assay is a sandwich enzyme immunoassay in a microtitre strip-well format. The Fab fragment of a monoclonal anti-YKL-40 antibody conjugated to biotin binds to streptavidin on the strip and captures YKL-40 in a standard, control, or sample. A polyclonal anti-YKL-40 antibody conjugated to alkaline phosphatase binds to captured YKL-40. Bound enzyme activity is detected with p-nitrophenyl phosphate as a substrate. The sensitivity of ELISA is 20 μg/l. The intra- and interassay coefficients of variation were 6.6% and 6.8%, respectively.

Statistical Analysis

Statistical analyses were performed using SPSS statistical software. Linear regression was used to estimate the correlation with serum YKL-40 levels and age. Median values were compared using the Mann–Whitney unpaired test with two-tailed significance. Significance of odds ratios was estimated with the $\chi 2$ test with two-tailed significance. The endpoint for survival analysis was breast cancer-related death. Kaplan–Meier analysis was used to calculate survival curves. The Cox proportional hazards model was used for analysis of covariates.

RESULTS

The median age of patients was 47 years, and the majority of patients were at disease stage IIb, IIIa or IIIc (Table 1). The median follow-up time was 46 months (range, 10–96 months). During the study period, 21 patients relapsed and there were 17 deaths.

The median serum YKL-40 concentration was 149.5 μg/l (range, 25.0–1021.3 μg/l) in patients with locally advanced breast cancer. This was higher than values observed in healthy females (mean, 102.4±78.9 μg/l) but the difference was not significant (P=0.44).

Serum YKL-40 levels were higher in patients with tumour size >2 cm when compared with tumours ≤2 cm. Patients with node-positive disease also had higher serum YKL-40 levels when compared to patients with node-negative disease. Serum levels

Table 1. Demographic and baseline information of the study population (locally advanced breast cancer patients).

	Patients, *n* (%)
Median age, years	47 (range, 27–72)
Female gender	45 (100)
Pathological stage	
I	4 (9)
IIa	4 (9)
IIb	9 (20)
IIIa	10 (22)
IIIb	2 (4)
IIIc	16 (35)
Tumour size	
≤2 cm	6 (13)
>2 cm	39 (87)
Number of metastatic lymph nodes	
0	9 (20)
1–3	11 (24)
4–9	13 (29)
≥10	12 (27)
Oestrogen receptor status*	
(+)	23 (52)
(–)	21 (48)
Progesterone receptor status*	
(+)	16 (36)
(–)	28 (64)
Tumour grade	
I	0 (0)
II	23 (51)
III	22 (49)
Patients undergoing surgery	45 (100)

*n=44. For one patient, receptor status data was not correctly obtained.

Table 2. The relationship between clinical characteristics and serum levels of YKL-40 in the study population (locally advanced breast cancer patients).

	No. of patients	Percentage	Serum YKL-40 levels, μg/l, mean±SD	*P* value
Total	45		147±168	
Tumour size				
≤2 cm	6	13	85.35±64	0.243
>2 cm	39	87	157.37±177	
Node status				
Node-negative	9	20	123.96±77	0.709
Node-positive	36	80	155.88±36	
Receptor status*				
ER(+)	23	52	141±205	0.127
ER(–)	21	48	159±123	
PR(+)	16	36	180±238	0.566
PR(–)	28	64	132±115	

*n=44. For one patient, receptor status data was not correctly obtained.
ER=oestrogen receptor; PR=progesterone receptor; SD=standard deviation.

were lower in patients with oestrogen receptor-positive (ER[+]) disease compared to patients with ER(–) disease, whereas patients having progesterone receptor-positive (PR[+]) disease had higher serum YKL-40 levels compared with PR(–) disease. However, none of these comparisons reached statistical significance (Table 2). For one patient, receptor status data was not correctly obtained.

Tumour volume, measured by ultrasonography, was correlated with serum YKL-40 levels (r=0.308, P=0.039). High serum levels of YKL-40 were associated with shortened disease-free survival and overall survival although this trend did not reach statistical significance (P>0.05). The 3-year disease-free and overall survival rates were 53% and 66%, respectively (Figures 1 and 2). Median overall survival time had not been reached.

Multivariate analysis including tumour size, lymph node status, oestrogen receptor status, progesterone receptor status, tumour grade and serum levels of YKL-40 showed that serum YKL-40 was an independent prognostic variable for overall survival (hazard ratio, 1.004; 95% confidence intervals: 1.00, 1.07; P=0.027). Tumour size, lymph node status and oestrogen receptor status were also independent prognostic variables for overall survival (P<0.05).

Figure 1. Disease-free survival for all patients in the study population (locally advanced breast cancer patients, *n*=45).

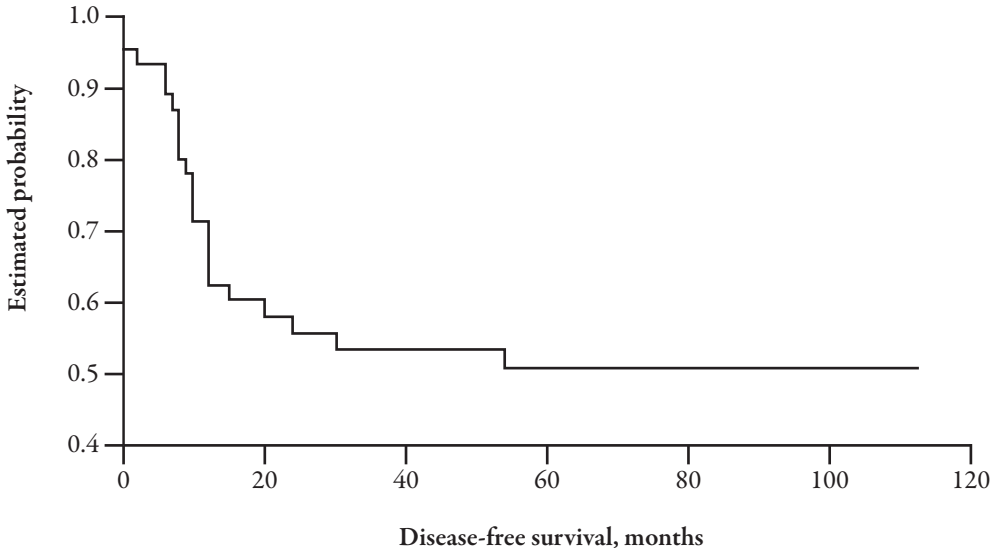
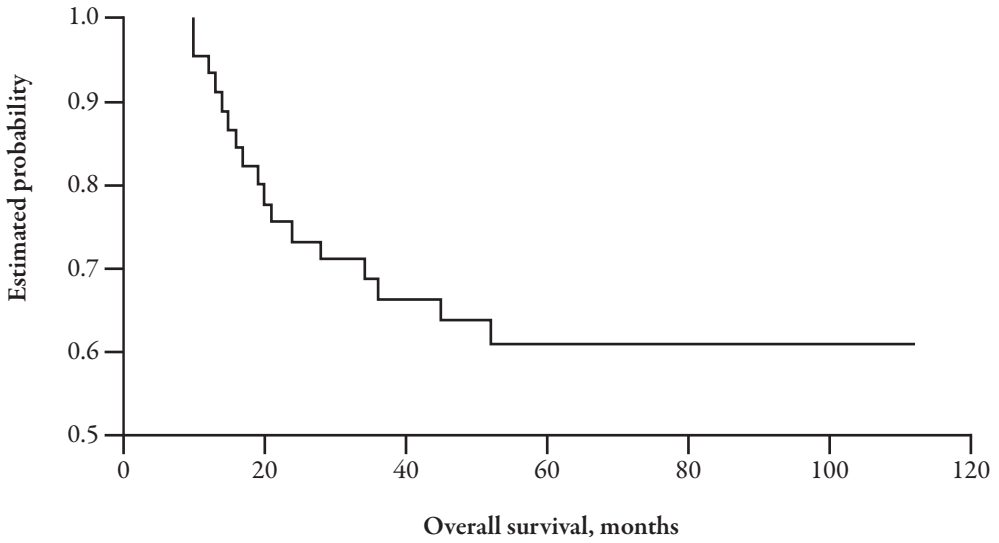


Figure 2. Overall survival for all patients in the study population (locally advanced breast cancer patients, *n*=45).



DISCUSSION

As a differentiation and migration factor, YKL-40 is a new biomarker for several cancer types including breast, colorectal and ovarian cancers. The prognostic value of serum YKL-40 levels has been investigated in many cancer types, and it has generally been shown to reflect a more aggressive clinical course.[2] In patients with metastatic prostate carcinoma or metastatic melanoma, high serum YKL-40 levels were associated with shortened survival.[10,12] In small cell lung cancer, serum YKL-40 levels were higher in patients with extensive disease and an aggressive clinical course.[8] Similarly, elevated postoperative serum YKL-40 levels were associated with a high recurrence risk and poor survival after curative resection for colorectal carcinoma.[6]

In the present study, we observed elevated serum levels of YKL-40 in patients with locally advanced breast cancer, although the difference between healthy females and cancer patients was not statistically significant. Since YKL-40 is also expressed in non-malignant human cells and inflammatory diseases, high serum levels can also be observed in healthy females. Elevated serum levels of YKL-40 have also been observed in 19%–31% of patients with primary or metastatic breast cancer.[4,5,22]

Johansen et al. showed that in patients with primary breast cancer, high serum YKL-40 levels were associated with reduced relapse-free survival and overall survival.[4] In patients with metastatic breast cancer, high serum YKL-40 levels at the time of first recurrence were correlated with shorter time to disease progression and reduced survival compared with patients having normal YKL-40 levels.[22] We also found that serum YKL-40 levels were an independent prognostic factor for overall survival in a multivariate Cox analysis including tumour size, node status, receptor status and tumour grade. The results of the present study are therefore in accordance with previous studies.

High serum YKL-40 levels have been found to be associated with an advanced stage of many cancer types including colorectal, ovarian and small cell lung cancer.[7,23,24] In this study, higher serum YKL-40 levels were observed in patients with tumours larger than 2 cm, and in node-positive patients, compared with those having smaller tumours or node-negative patients, consistent with previously published studies.[4,5] However, our results did not demonstrate statistical significance, most likely due to the small number of patients included in the study. Johansen et al. also observed higher serum levels of YKL-40 in patients with metastasis to axillary lymph nodes compared with node-negative patients.[4] Increased serum levels of YKL-40 have also been demonstrated in patients with visceral metastases, particularly liver metastases, and in patients with more than two metastatic sites.[22] These results suggest that there is an association between serum levels of YKL-40 and tumour burden. Similar findings have been observed in patients with advanced colorectal and ovarian cancer.[6,9]

In conclusion, we found that the serum levels of YKL-40 in patients with

locally advanced breast cancer is an independent prognostic factor for overall survival. Elevated serum levels of YKL-40 were associated with increased tumour size and node-positive status, and may reflect a high tumour burden and a more aggressive clinical course. YKL-40 may be a new prognostic factor in locally advanced breast cancer, in addition to other factors such as receptor status and Her2-neu amplification. However, the prognostic value of serum YKL-40 should be investigated in larger studies.

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