

Association of Serum YKL-40 Level with Tumor Burden and Metastatic Stage of Prostate Cancer

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Purpose: To investigate the relationship between serum level of YKL-40 and Gleason score, grade and stage of the disease, and for the first time, with tumor burden in patients with prostate cancer (PCa).

Materials and Methods: Serum levels of YKL-40 and prostate-specific antigen were measured in 34 men (mean age: 66 years) with newly diagnosed and untreated PCa, in 34 men (mean age: 65 years) with biopsy proven benign prostatic hyperplasia, and in 29 healthy young men (mean age: 24 years).

Results: Serum YKL-40 concentration in men with PCa and benign prostatic hyperplasia, and in controls were 165.67 ± 107.84 ng/mL, 137.38 ± 82.04 ng/mL, and 69.69 ± 18.46 ng/mL, respectively. Serum level of YKL-40 was correlated with tumor burden in 30.4% of the patients with PCa ($P = .04$). A cut-off serum YKL-40 value of 92.696 ng/mL produced 70.6% sensitivity and 93.1% specificity. Elevated serum level of YKL-40 was strongly associated only with metastatic stage of the PCa. No association was observed between elevated level of YKL-40 and Gleason score groups or Gleason grade.

Conclusion: Our results suggest that elevated serum level of YKL-40 may be a useful indicator of tumor burden and metastatic stage of PCa. Further studies are warranted to better elucidate the meaning of YKL-40 in tumor burden and invasiveness.

Keywords: prostatic neoplasms, tumor marker, tumor burden, prostate-specific antigen

INTRODUCTION

YKL-40 (chitinase-3-like-1), also called human cartilage glycoprotein-39 (HC gp-39), is a member of family 18 glycosyl hydrolases and plays a significant role in cancer cell proliferation, survival, and invasiveness, and has a regulating role in cell-matrix interactions and in the production of the altered extracellular matrix surrounding the cancer cells.⁽¹⁾ Previous studies have reported elevated serum levels of YKL-40 in patients with primary and metastatic carcinoma of the breast,⁽²⁾ colorectal,⁽³⁾ ovary,⁽⁴⁾ lung,⁽⁵⁾ and prostate.⁽⁶⁾ The relationship of elevated serum level of YKL-40 with the prostate cancer (PCa) is not yet fully established; however, possible association of YKL-40 elevation with only high Gleason score was suggested.⁽⁶⁾ Elevated serum level of YKL-40 in patients with PCa, especially re-elevation after androgen resistance stage of the disease, was reportedly associated with short survival, and serum YKL-40 concentration was independent of other prognostic factors.⁽⁷⁾

A number of prognostic factors, including prostate-specific antigen (PSA), Gleason grade and score, and the stage of disease, have been suggested as predictors of the outcome of primary treatment and prognosis of PCa.⁽⁸⁾ Serum PSA concentrations significantly affect treatment modalities in man with PCa. Nonetheless, over-diagnosis and as a result over-treatment of occult cancer have occurred significantly. Prostate-specific antigen test suffers from both limited sensitivity and specificity. Furthermore, the significance of PSA declines in later stages of the disease.⁽⁹⁾ Identification of additional and/or better biomarkers is of utmost importance to better predict clinical behavior of PCa and define the need for additional therapies. Several new biomarkers have shown promise, and are still being evaluated in studies to investigate the role of these markers in the early detection, staging, and prognosis of PCa.

In this prospective study, we investigated the association of serum level of YKL-40 with serum level of PSA, Gleason grade and score, and pathologic stage in newly diagnosed and untreated patients with PCa, and compared to that of the patients with benign prostatic hyperplasia (BPH) and healthy controls. Moreover, for the first time, the correlation of serum level of YKL-40 with tumor percentile in the pathologic

specimens was also investigated.

MATERIALS AND METHODS

Patients

Blood samples were obtained from 34 men with newly diagnosed and untreated PCa, 34 men with BPH, and 29 healthy volunteers from June 1, 2008 to December 31, 2009. Mean age of the patients with PCa and BPH was 66 years (range, 54 to 82 years) and 65 years (range, 38 to 79 years), respectively, and that of healthy controls was 24 years (range, 23 to 25 years).

Pretreatment serum level of PSA, stage, Gleason grade, Gleason score, and percent tumor burden were recorded. According to the stage, patients with PCa were divided as localized (T1a-T2b), local-advanced (T3a-T4), and metastatic (Tx, N+, M+). Staging was based on histological and/or clinical parameters, such as digital rectal examination, plain X-ray, and radio-isotopic bone imaging and tomography. According to Gleason score, they were divided into low (2–4), medium [5–(3+4)], and high [(4+3)–10].

The research protocol was approved by the Medical Ethics Committee. Informed consents were obtained from all the patients. Patients under medications with possibility to influence serum YKL-40 and PSA levels, such as 5 α -reductase inhibitors, luteinizing hormone-releasing hormone analogs, androgen receptor inhibitors, and testosterone replacement, were excluded.

Procedure and Evaluations

The biopsy was performed using transrectal ultrasound guidance in patients with abnormal digital rectal examination with even PSA values within normal range or above 4 ng/mL. For prophylaxis of postprocedural sepsis, patients were given 500 mg ciprofloxacin twice daily starting 12 hours before and following five days. Twelve-quadrant transrectal ultrasound-guided prostate biopsies were performed under local anesthesia at department of radiology using an ultrasound probe (BK Medical, Herlev, Denmark; 2101 Falcon; 7.5 MHz). PRO-MAG biopsy gun and 18 gauge 20-cm needle were preferred.

Blood samples were collected after pathological confirmation of the diagnosis, and left on the clot, and serum was

separated by centrifugation at 3000 rpm for 10 minutes (Heraeus Biofuge Stratos; Kendo Laboratory Products, Osterode-Germany). All serum samples were stored at -80 °C until examination.

Serum samples were melted and YKL-40 concentrations were determined by MicroVue YKL-40 EIA Kit (Quidel Corporation: 10165 McKellar Court, San Diego, CA 92121 USA), and by manual Enzyme-Linked Immunosorbent Assay using washer and reader (Washer-ELX, Reader-ELX Bio-Tek, USA).

Serum levels of PSA were determined by using Chemiluminescent Immunometric Assay kit working on Immulite 2000 hormone autoanalyzer (Siemens Healthcare Diagnostics Inc. Flanders, NJ, 07836, USA).

Statistical Analysis

The statistical analysis was performed using MedCalc® Version 10.1.6.0. All values were expressed as mean ± standard deviation. Non-parametric Kruskal-Wallis test was used for multiple comparisons, non-parametric Mann-Whitney *U* test for analyzing the differences among groupings, and Pearson correlation coefficient test for the relationships of parametric data. Receiver operator characteristic (ROC) curves were plotted for relevant parameters. Statistically significance was set at $P < .05$ level.

RESULTS

The number, age, and serum levels of PSA and YKL-40 of the patients with PCa and BPH, and those of healthy controls are summarized in Table. Serum levels of YKL-40 of

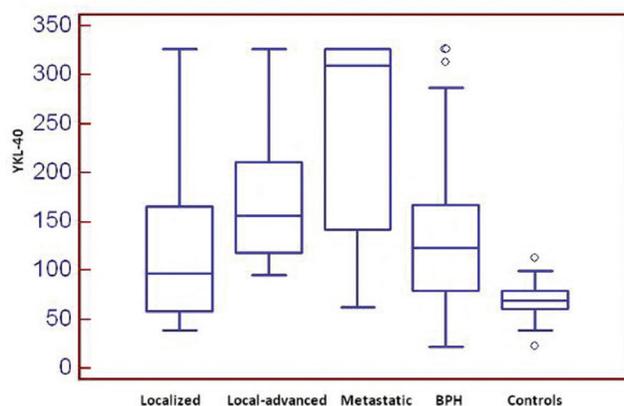


Figure 1. Box-and-Whisker plots for serum levels of YKL-40. The box represents the 25th and 75th percentiles and the median is shown by a horizontal line. The multiple comparison by Kruskal-Wallis test is significant ($P < .001$).

controls, patients with BPH, and those with PCa were 69.69 ± 18.46 ng/mL, 137.38 ± 82.04 ng/mL, and 165.67 ± 107.84 ng/mL, respectively.

As shown in Figure 1, multiple comparison of serum levels of YKL-40 in patients with localized (T1a-T2b; $n = 20$), local-advanced (T3a-T4; $n = 5$), and metastatic (Tx, N+, M+; $n = 9$) stage PCa and that of patients with BPH yielded significant difference (Kruskal-Wallis test, $P < .0001$). There was a significant difference for the serum levels of YKL-40 between the BPH and metastatic stage in patients with PCa in Mann-Whitney *U* test ($P = .02$). Serum levels of YKL-40 also differed significantly between the patients with localized stage and metastatic stage PCa ($P = .01$), but not between localized and local-advanced, and between local advanced and metastatic stage of PCa. Comparisons of serum levels of YKL-40 between the patients with BPH and those with

Clinicopathological profiles of the patients.[‡]

Characteristics	¹ Localized PCa (T1a-T2b) (n = 20)	² Local advanced PCa (T3a-T4) (n = 5)	³ Metastatic PCa (pTx, N+, M+) (n = 9)	⁴ BPH (n = 34)	⁵ Controls (n = 29)	<i>P</i> *
Mean age (range), y	64 (54 to 75)	70 (61 to 75)	70 (60 to 82)	65 (38 to 79)	24 (23 to 25)	< .0001
PSA (mean ± SD), ng/mL	1174 ± 8.01	28.80 ± 12.10	314.38 ± 388.56	11.52 ± 10.75	0.42 ± 0.27	< .0001
Serum YKL (mean ± SD), ng/mL	126.45 ± 92.48	175.11 ± 89.71	247.57 ± 110.39	137.38 ± 82.04	69.69 ± 18.46	< .0001

[‡]PCa indicates prostate cancer; BPH, benign prostatic hyperplasia; PSA, prostate-specific antigen; and SD, standard deviation.

*Non-parametric Kruskal-Wallis test, Post-hoc analysis among groupings yielded significant differences for serum YKL-40 levels ($P < .05$) between ⁽¹⁾ and ^(2,3,5), between ⁽²⁾ and ^(1,5), between ⁽³⁾ and ^(1,5), between ⁽⁴⁾ and ⁽⁵⁾, and between ⁽⁵⁾ and ^(1,2,3,4).

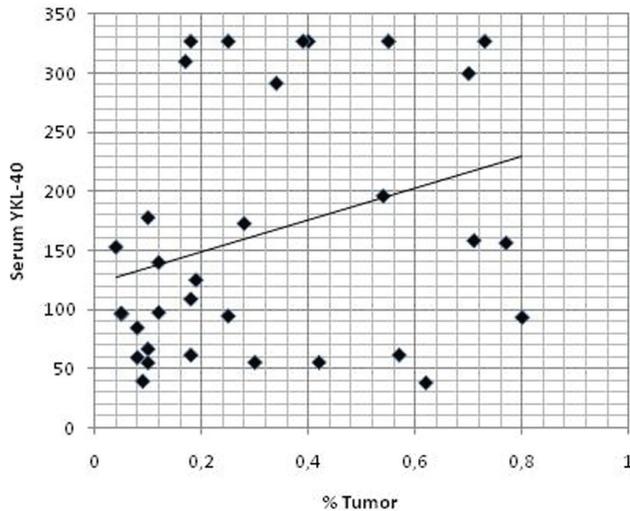


Figure 2. Correlation of % tumor burden in the pathologic specimen with serum levels of YKL-40 in patients with prostate cancer. Pearson's correlation = 0.304; $P = .04$ (1-tailed).

localized or local-advanced stage PCa did not show any significant difference.

Serum levels of YKL-40 were strongly higher in patients with PCa than that of subjects with BPH ($P < .001$). In patients with BPH, serum levels of PSA and YKL-40 were not correlated ($P = .31$). As expected, serum levels of PSA and YKL-40 in the patients with PCa were significantly correlated ($P = .02$).

We evaluated the %tumor volume in the pathologic specimen and compared it with serum levels of YKL-40 and PSA. While there was no correlation between %tumor volume and serum levels of PSA, there was a correlation of 30.4% between serum levels of YKL-40 and %tumor burden in the specimen ($P = .04$) as shown in Figure 2.

While there was no significant difference between Gleason Grade (1 – 5) and serum levels of YKL-40 in patients with PCa, Gleason grading was strongly associated with serum levels of PSA ($P = .009$). Accordingly, Gleason score groupings as low (2 – 4), medium [5 – (3 + 4)], and high [(4 + 3) – 10] were also not associated with serum levels of YKL-40. While the association of Gleason score groupings with serum levels of PSA was strong ($P = .006$). Furthermore, both Gleason grade and Gleason scoring groups were strongly associated with %tumor burden in the specimens ($P < .001$).

The ROC curve for serum levels of YKL-40 in 34 patients with PCa and in 29 healthy controls is shown in Figure 3. Re-

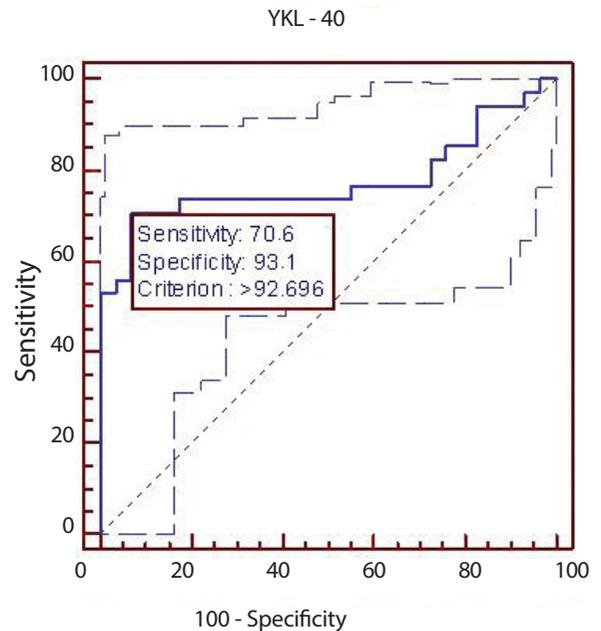


Figure 3. Receiver operation characteristic curve of serum level of YKL-40 in patients with prostate cancer and in controls. The area under the curve is 0.774.

ceiver operator characteristic analysis suggested that a serum YKL-40 level cut-off value of 92.696 may have predictive value (Area under curve: 0.774; sensitivity: 70.6%; specificity: 93.1%) for PCa ($P < .0001$; 95% Confidence Interval: 0.651 to 0.870).

Initial therapy was radical prostatectomy in 22 (64.7%) patients, and pathologic stage was localized in 20 and local advanced in 2 patients. Three patients with clinically local advanced stage disease and 9 patients with metastatic stage disease underwent medical castration. None of our patients were treated with radiotherapy.

DISCUSSION

Serum YKL-40 elevations observed in patients with PCa and its correlation with tumor burden suggest a possible role of YKL-40 as a marker in PCa. The present study is the first report showing the relationships of elevated serum level of YKL-40 with %tumor volume in the pathological specimen. We found a significant correlation of 30.4% between serum levels of YKL-40 and %tumor burden in the pathologic specimen, contrasting to no correlation of %tumor volume with serum levels of PSA in our patients with PCa. Partin and col-

leagues evaluated usefulness of PSA in pre-operative staging of PCa, and reported that serum level of PSA is not reflecting the tumor burden.⁽⁹⁾ They suggested two reasons for this; the first one is unpredictable contribution of BPH region within the gland and the other one is decreased production of PSA by higher grade lesions as tumor volume increases. Furthermore, our finding of strong association of serum YKL-40 elevations solely with metastatic stage of the patients with PCa suggests that increased serum level of YKL-40 may be a marker of increased tumor burden and metastasis in patients with PCa.

Our results also confirmed the previous finding of significantly elevated serum levels of YKL-40 in patients with PCa at the initial diagnosis than that of patients with histological BPH.⁽⁶⁾ On the other hand, we found no association of elevated serum levels of YKL-40 with medium, low, and high Gleason score groups and also Gleason grades by using nonparametric multivariate analysis, contrasting to the findings of previous researchers.⁽⁹⁾ Furthermore, we found strong differences among these groupings for the serum levels of PSA.

Brasso and associates measured serum level of YKL-40 in patients with metastatic PCa and compared with that of normal controls.⁽¹⁰⁾ They found meaningful serum YKL-40 elevations in 43% of the patients with metastatic PCa. Therefore, they suggested that elevated serum level of YKL-40 may be an independent prognostic factor for short survival in patients with metastatic PCa. Johansen and coworkers administered total androgen blockade or parenteral estrogen therapy for 6 months to the patients with metastatic PCa and reported meaningful decrease in the serum level of YKL-40.⁽⁷⁾ Moreover, they suggested the restart of increase in the serum level of YKL-40 in patients with androgen insensitive metastatic PCa as an important prognostic factor for deaths within the following seven months. Although decreased serum level of YKL-40 after radical prostatectomy was reported,⁽⁶⁾ it did not decrease to the normal values. This finding clarifies that YKL-40 protein production is continued by surrounding structures without evidence of cancer remnants.

Defining a proper cut-off value for markers is crucial for better detection of cancer. Despite small sample numbers in our

study, ROC curve was generated. With a cut-off serum YKL-40 value of 92.696, a predictive 70.6% sensitivity and 93.1% specificity were produced for PCa ($P < .001$). Future large scale studies are being awaited for better predicting the diagnostic value of serum YKL-40 in patients with PCa.

Immunohistochemical tissue expression of YKL-40 in solid tumors has not been published yet.⁽⁶⁾ Interesting data are expected with future studies analyzing the tissue expression level of YKL-40.

The ages of the healthy volunteers were preferentially selected less than 30 years, just before the start of the development of BPH nodules, in our study as previous research.⁽⁶⁾ Ideal similar-age control group without histological evidence of BPH is almost impossible. All of our patients with histological BPH and primary PCa were not on any medication and had no clinical signs or symptoms of other diseases, such as other cancers or joints, liver, metabolic, and hormonal diseases. Controls had no clinical signs or symptoms of illnesses or hormonal disturbances.

YKL-40 was first discovered as a 40 kDa protein secreted by the MG63 human osteosarcoma cell line.⁽¹¹⁾ YKL-40 gene is located on chromosome 1. Structurally, it is related to mammalian chitinase-like proteins; however, lacking their characteristic enzymatic activity.

Elevated levels of YKL-40 have been reported in a wide variety of diseases, such as inflammatory bowel disease, cirrhosis, rheumatoid arthritis, bacterial sepsis, and malignancies.⁽¹²⁻¹⁶⁾

Along with cancer cells, macrophages and neutrophils have been demonstrated to secrete YKL-40. Immunohistochemical reactivity of YKL-40 is cytoplasmic. YKL-40 protein is possibly associated with such processes in malignancy as cell differentiation and proliferation, inhibition of apoptosis, angiogenesis, and remodeling of extracellular matrix.⁽¹⁾ Cancer development and invasion are closely related with the interaction of surrounding structures. Stromal tissues surrounding cancer have quite a lot of unique characteristics resembling granulation tissue developed around inflammation and tissue healing processes. Tumor associated macrophages and leukocytes within this granulation tissue secrete growth factors, angiogenesis stimulating factors, cytokines, and tissue destruction enzymes, such as metalloproteinases.⁽¹²⁾ Elevated

serum levels of YKL-40 in cancerous patients may play some roles in all of these processes.

The relationship of elevated serum levels of YKL-40 with cancer cell proliferation, invasion, and development of metastasis is extensively investigated in several cancer types, including PCa.⁽⁶⁾ Furthermore, YKL-40 is regarded as a potential target in the design of anticancer therapy. Potential methods for inhibition of YKL-40 activity include inhibition of YKL-40 production, development of specific molecules or antibodies against YKL-40 and its receptors, and inhibition of signal transporting cascade.⁽¹⁾ One potential limitation of our study is the relatively small number of subjects.

CONCLUSION

Elevated serum level of YKL-40 may be a useful indicator of tumor burden and advanced stage PCa. YKL-40 is neither organ- nor cancer-specific. Meaningful serum YKL-40 elevations have been reported consistently only in advanced stage cancer, and indicates poor prognosis in different types of cancer. More studies are required to further clarify YKL-40 as a biomarker; our findings highlight the importance of YKL as a marker in PCa.

CONFLICT OF INTEREST

None declared.

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