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KLİNİK ÇALIŞMA
RESEARCH ARTICLE

The place of YKL-40 in non-small cell lung cancer

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SUMMARY

The place of YKL-40 in non-small cell lung cancer

Introduction: *Despite varies treatment options, the survival rate of non-small cell lung cancer (NSCLC) is less than 1%. If biological behaviour of any cancer could be known, the information would potentially tailor the clinical work-up, assesment and treatment. The prognostic value of serum YKL-40 level has been investigated in different types of cancer and showed poor prognostic indication with more aggressive clinical course. We studied the role of serum YKL-40 levels in patients with NSCLC to determine the stage.*

Materials and Methods: *Serum YKL-40 levels in venous blood samples of 55 patients was studied. 49 (89.1%) male and 6 (10.9%) female newly diagnosed NSCLC patients whom received neither cancer specific or symptom relieving treatment are enrolled. TNM staging was determined based on the findings of computerized tomography (CT), positron emission tomography (PET), cranial magnetic resonance imaging (MRI), bronchoscopy and mediastinoscopy. The patients with NSCLC were divided into two groups; Group A: stage Ia, Ib, IIa, IIb, Group B: stage IIIa, IIIb and IV.*

Results: *There was a statistical difference in YKL-40 serum levels between groups (Group A, Group B) when compared ($p< 0.05$). A medium and statistical correlation was found ($r= 0.48$; $p< 0.01$) between YKL-40 levels and age.*

Conclusion: *YKL-40 levels in advanced stage NSCLC (stage III, IV) was found to be significantly high compared to early stage. It should be kept in mind that when YKL levels are high, higher stages of the disease should be suspected and future tests should be performed.*

Key words: *Biomarkers, lung cancer*

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ÖZET

Küçük hücreli dışı akciğer kanserinde YKL-40'ın yeri

Giriş: *Primer akciğer kanserinin %80'ini oluşturan küçük hücreli dışı akciğer kanseri (KHDAK)'nin sağkalım oranı, tedaviye rağmen %15'ten az olup hala düşük seyretmektedir. Kanserin biyolojik davranışı tedaviye başlamadan önce belirlenebilirse, bu bilgiler tedaviyi yönlendirme, değerlendirme ve klinik çalışmaları planlamada kullanılabilir. Serum YKL-40 seviyelerinin prognostik değerleri birçok kanser tiplerinde incelenmiş ve daha çok agresif klinik gidişi yansıttığı gözlenmiştir. Biz de KHDAK'lı hastalarda serum YKL-40 seviyelerinin kanser evresini belirlemedeki rolünü araştırdık.*

Materyal ve Metod: *Yeni tanı almış ve daha önce cerrahi, kemoterapi, radyoterapi, semptomatik tedavi almamış 49 (%89.1)'u erkek ve 6 (%10.9)'sı kadın toplam 55 hastanın venöz kan örneklerinde YKL-40 düzeylerine bakıldı. Bilgisayarlı tomografi (BT), pozitron emisyon tomografi (PET), kraniyal manyetik rezonans görüntüleme (MRG), bronkoskopi, mediastinoskopi bulgularına dayanılarak TNM evrelendirilmesi yapıldı. KHDAK'lı hastalar erken evre ve ileri evre olarak iki gruba ayrılarak incelendi. Grup A: Evre Ia, Ib, IIa, IIb, Grup B: Evre IIIa, IIIb, IV.*

Bulgular: *Evreler açısından erken evre (Grup A) ve ileri evre (Grup B)'ye ayrılmış olan hasta grubumuzda YKL-40 düzeylerini karşılaştırdığımızda aralarındaki fark istatistiksel olarak anlamlı bulundu ($p< 0.05$). YKL-40 düzeyleri ile yaş arasında orta derecede ($r= 0.48$; $p< 0.001$) korelasyon saptandı.*

Sonuç: *YKL-40 düzeylerini ileri evre KHDAK (Evre III-IV) grubunda erken evre (Evre I-II) hasta grubuna göre anlamlı olarak yüksek bulduk. YKL-40 düzeylerinin yüksek bulunması durumunda hastalığın ileri evrede olabileceği akla getirilmeli ve bu konuda ileri tetkikler yapılmalıdır.*

Anahtar kelimeler: *Biyobelirteçler, akciğer kanseri*

INTRODUCTION

Lung cancer is now the most common fatal cancer in both men and women. Through all stages, the 5-year survival rate is only about 16% for males and 19% for females. Also, only 2% of the 40% of patients that are diagnosed in stage IV are still alive after 5 years (1).

The identification of prognostic factors in lung cancer is very useful for assessing the individual patients' prognosis, selecting the best treatment, defining new criteria to classify patients according to risk groups and helping to design and direct the future research (3). There is some prognostic factors, such as sex, performance status, pretreatment weight loss, and biomarkers such as carcinoembryonic antigen (CEA) and neuron specific enolase (NSE). But these factors or biomarkers have low specificity and can be elevated in non-malignant conditions as well (2,4).

YKL-40, also called as human cartilage glycoprotein-39, is extracelluar matrix glycoprotein with a molecular weight of 40 kDa and is named after amino-terminal aminoacids, tyrosin (Y), lysin (K) and losin (L) (5).

The biological function of YKL-40 in cancer is not known. However, it is known that YKL-40 may play a part in proliferation and differentiation of cancer cells, and also it has been claimed to have an effect on the protection of cancer cells subjected to apoptosis, stimulation of angiogenesis, renewal of extracellular tissue, and on the stimulation of fibroblasts adjacent the tumor (6). YKL-40 is not produced by fibrolasts, yet, fibroblasts are growth factors for synovial cells and chondrocytes (7). They are also growth factor for bond tissue cells, and migration factor for endothelial and vascular flat muscle cells (6,7).

Various studies have evaluated YKL-40 levels for its prognostic value in patients with cancer. High serum YKL-40 levels have been found to be related to frequent recurrence rate and short survival in many cancer patients including colorectal carcinoma, metastatic melanoma, prostate carcinoma, glioblastoma multiform and ovary carcinoma, and suggested that serum YKL-40 can be beneficial as a prognostic factor in patients with CA (4-22).

We investigated the role of serum YKL-40 levels in determining the stage of patients with NSCLC.

MATERIALS and METHODS

Between February 2009 and September 2009, 55 patients with non-small cell lung carcinoma cases who were newly diagnosed, not undergone any surgical, chemotherapy, radiotherapy and symptomatic treatment previously and didn't have any additional diseases were enrolled to the study. The local ethics committee approved the study protocol. Informed consent was obtained from all patients before the study enrolment.

Depending on the findings of computerized tomography (CT), positron emission tomography (PET), cranial magnetic resonance imaging (MRI), bronchoscopy and mediastinoscopy, TNM staging of the patients

was determined according to the changes suggested by IASLC international staging commitee in 2007 (23).

The patients with non-small cell lung cancers were divided into two groups as early stage (Group A) and advanced stage (Group B) based on pathological staging. Group A included; stage Ia, Ib, IIa and IIb and Group B was composed of stage IIIa, IIIb and IV.

The serum YKL-40 levels were taken from the 55 patients included in the study were divided into two groups as low and high levels, and the difference between two groups was evaluated.

Biochemical Analysis

Venous blood samples were taken from the patients newly diagnosed with non-small cell lung cancer before beginning to therapy, and were centrifuged for 15 minutes at 2500 cycle in maximum 2 hours. For YKL-40 test, not studied in the same day, serum samples were kept in deep-freezer at -80^{o}C in portions.

Serum YKL-40 levels were determined by using ELISA kyte by Micro Vue (Enzym-linked immonosorbent assay method). In this method, standards, controls and serum samples were pipetted into wells covered with avidin.

In order to bind YKL-40, biotinized anticore was added. After incubation, undound anticore was removed by washing in ELx50 instrument. Following this, alkaline phosphatase (AP) enzyme-conjugate was added to realize conjugation to biotinized anticores. After incubation unbound anticore enzyme conjugate was removed by washing. P-nitrophenly phoshate (PNPP) substrate which is chromogen was added. After the third incubation, the intensity of color formed as a result of enzymatic altivity was measured at ELx800 reading instrument as spectrophometric at 405 nm wavelength.

Statistical Analysis

Statistical analysis was carried out by using SPSS 11.5 (SPSS, Inc. Chicago, IL, USA) and Excel (Microsoft Corporation, Redmond, WA, USA) programs. Persisting variances were shown as medium (25 percentile-75 percentile). Mann-Whitney U test was used for double group comparisons. The comparison of anticipated and absorved values were made through Yates chi-square of Fisher's exact test. In the evaluation of the relations between variances, Pearson correlation coefficient (r) was used. By means of the receiver operating characteristic (ROC) analysis realized for the evaluation of the diagnostic proficiency of YKL-40, a cut-off value for the most suitable sensitivity and specitivity value was determined. Statistical significance was evaluated as $p< 0.05$ (two-talied).

RESULTS

Study was carried out on totally 55 cases, 49 (89.11%) of whom were male, and 6 (10.9%) of whom were female, ages were between 34 and 77. The mean age of the cases was 60 ± 10 years.

Cases were investigated in two groups as early stage and advanced stage. 22% (n= 12) of the patients was in Group A (stage Ia, Ib, IIa, IIb) and 82% (n= 43) was in Group B (stage IIIa, IIIb, IV).

YKL-40 median value was statistically significant in advanced stage group compared with early stage ($p= 0.034$), whereas sex, age and lactate dehydrogenase (LDH) median values were not different in these two groups ($p> 0.05$) (Table 1).

While a positive medium-well statistically significant correlation was determined between YKL-40 values and age ($r= 0.486$; $p< 0.0001$), there was no stastically significant correlation between YKL-40 values and LDH levels ($r= 0.051$; $p= 0.709$).

When cut-off value for YKL-40 was estimated to be as 152 ng/mL with ROC analysis, sensitivity was calculated as 67.4% and specifity as 75.0% and the area under the curve (EAP) was 0.702 (95% reliability: 0.518-0885; $p= 0.034$) (Figure 1).

Table 1. Comparison of YKL-40 and LDH data with demographic chareteristics in two groups divided as early stage (Group A) and advanced stage (Group B)

	Group A (n= 12)	Group B (n= 43)	p
Age (years)	56 (53-64)	63 (53-70)	= 0.275
Male/Female, n/n	11/1	38/5	= 1.000
YKL-40, ng/mL	82.28 (56.53-265.61)	240.20 (104.91-408.07)	= 0.034
LDH, U/L	176 (150-205)	177 (139-258)	= 0.669

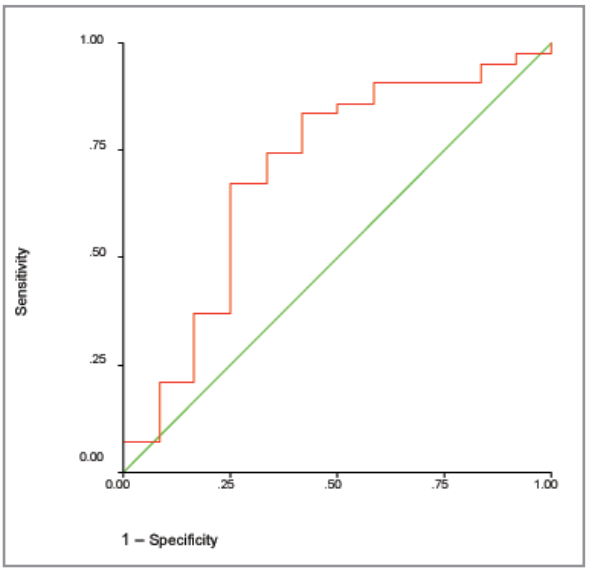


Figure 1. ROC curve drawn for the YKL-40 levels.

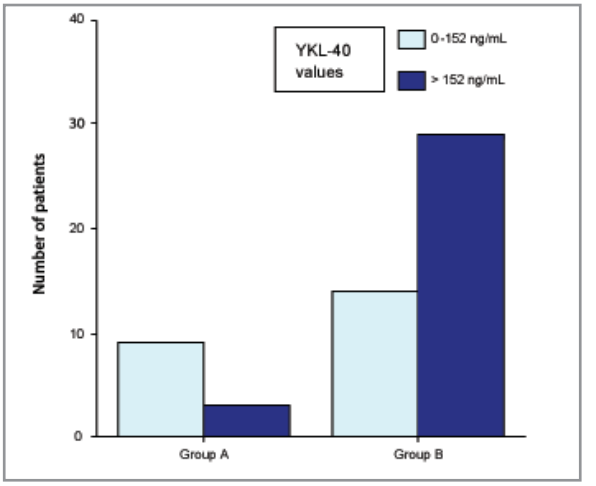


Figure 2. Number of patients (N) whose YKL-40 values in Group A and B was between 0-152 ng/mL and over 152 ng/mL.

When we used 152 ng/mL cut-off value, 3/12 (25.0%) of early stage cases had an increased YKL-40 values while advanced stage cases had a ratio of 29/43 (67.4%), (p= 0.018). Odds ratio (OR) value calculated from increased YKL-40 odds values in both early and advanced stage cases was 6.21 (95% reliability was between: 1.45-26.60).

Number of patients and their YKL-40 values can be seen at Figure 2.

DISCUSSION

In our study, YKL-40 level in advanced stage cases (III, IV) with NSCLS was found to be significantly higher than it was in early stage (I, II) cases. This situation suggests that higher YKL-40 level is indicating advanced level in NSCLC cases.

In a study searching relation between YKL-40 and survival in patients, YKL-40 appears to be a good candidate for a predictive survival marker in patients with advanced NSCLC. The median survival of patients with high serum YKL-40 was seven months, while patients with low serum YKL-40 had a median survival time of 18 months in this study (22).

In another study in which pretreatment serum YKL-40 levels were studied, it was indicated that mean YKL-40 value was 209 ng/mL in cases with NSCLC and metastasis (4). In that study, patients who had a pretreatment serum YKL-40 level > 209 ng/mL had significantly shorter survival than patients who had a pretreatment serum YKL-40 level ≤ 209 ng/mL (4). In our study, when we used 152 ng/mL cut-off value for YKL-40, 25.0% of early stage cases with NSCLC had an increased YKL-40 value while this ratio was 67.4 in advanced stage patients, and the difference between was statistically significant.

It was indicated that YKL-40 has expression from the macrophages related with small cell lung cancer (SCLC) and can be a prognostic factor indicating short survival for patients with SCLC (20). Besides, high serum YKL-40 level in SCLC patients is related with tumor metastasis and with weak short term prognosis (9). It was shown that YKL-40 levels before chemotherapy and during diagnosis is independent from age, performance and serum lactate dehydrogenase, and there is a weak relation between serum YKL-40 and age in patients with SCLC, but there was not a significant relation between YKL-40 level and sex (9). In another study, it was shown that YKL-40 is a potential prognostic marker of chemotherapy response in SCLC. Serum levels of YKL-40 were significantly correlated with disease stage, while there were no difference with age, gender, smoking status and performance status in this study (24).

Some studies have been carried out on YKL-40 levels in other organ cancers. High serum YKL-40 levels were associated with short survival in patients with prostate carcinoma or metastatic melanoma (12,13). In addition, it was found that high serum YKL-40 level has relation with advanced stage in ovary and cholerectal cancers (8,13). In another study, it was indicated that there was a significant relation between advanced breast cancer tumor size and YKL-40 levels, and that YKL-40 could be an independent prognostic factor for survival in advanced breast cancer

(15). It was found that in breast cancers with a positive lymphatic nodes involvement and bigger than 2 cm, serum YKL-40 level is higher than those with negative lymphatic nodes involvement and smaller (8,10). These data suggest that there can be a relation between serum YKL-40 levels and tumor size advanced stage and survival.

YKL-40 levels were found to be high in a ratio of 22% in cases with SCLC who has a local disease, and 40% in patients with metastatic (9). In another similar study, serum YKL-40 levels were found to be 43% higher in metastatic prostate cancers, 83% higher in metastatic renal cell cancer patients, and 45% higher in metastatic malign melanoma patients (12-15).

In these cancer types, serum YKL-40 levels were observed to have a relation with high recurrence and low survival, but, when serum YKL-40 levels in all cancer cases were compared with healthy control group, it was not found high. Johansen and et al., suggested that this may be due to inability of cancer cells not to secrete YKL-40 protein or express very low levels (16).

Serum YKL-40 levels were also compared with different cancer biomarkers. It was claimed that serum YKL-40 levels were independent from serum CEA in cases with colorectal cancers, from HER2 and ostrogen receptor existence in breast cancers, from serum CA-125 in ovary cancers, and from serum LDH in patients with SCLC (9,17-19). Besides, high postoperative serum YKL-40 levels in patients with colorectal cancers undergone surgery were found to be a marker independent from mortality during a six-mounth-follow up period (17). These findings show that serum YKL-40 levels reflect the tumor size and metastasis rather than the above mentioned markers, and that YKL-40 can be a beneficial prognostic factor in cancer cases including lung cancer patients.

Under the light of all these findings, it was found that although there is a significant relation between serum YKL-40 level and age in patients with NSCLC, no significant relation was found between serum YKL-40 level and gender in our study. When we compared YKL-40 levels in our patient groups divided into early stage (stage I and stage II) and advanced stage (Stage III and IV), there was a significant difference between them ($p< 0.05$).

The small number of patients in our study is the major limitation. Besides, the number of early stage patients was more limited than of advanced stage. Another important limitation of our study was not being able to carry out the survey and follow up the patients from the prognostic point of view.

As a result, YKL-40 levels in advanced stage NSCLC (stage III-IV) were significantly higher than those of early stage patient group (stage I, II). According to the studies so far, it should be kept in mind that high serum YKL-40 levels is a marker of advanced stage, and future studies are needed to support the findings.

CONFLICT of INTEREST

None declared.

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