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Serum tumor markers in small cell lung carcinoma patients treated with cyclophosphamide, epirubicin and vincristine combination

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ABSTRACT

Tumor markers are frequently used in the management of cancer patients especially for determination of the extent of the disease at diagnosis and monitorization of response to therapy. Neuron specific enolase (NSE), carcinoembryogenic antigen (CEA), lactic dehydrogenase (LDH) and ferritin were analyzed in 84 patients with small cell lung cancer who were treated with cyclophosphamide, epirubicin, and vincristine (CEV) combination. Sixty-six patients were male and 18 were female. Median age was 58 years (34-74). NSE was significantly higher in extensive disease than limited disease. There was no significant difference in other markers between stages. Although there was no statistically significant difference in CEA, ferritin, LDH before and after treatment, NSE levels decreased significantly in all patients and limited stage disease after treatment. In extensive stage, changing of NSE levels before and after treatment was not significant. We obtained higher response rate in patients with normal NSE levels. Progression free and overall survival was higher in patients with normal NSE levels (p=0.05 and p=0.09 respectively). NSE is in concordance with stage and may reflect response to therapy and early survival in patients who were treated with cyclophosphamide, epirubicin and vincristine.[Turk J Cancer 2005;35(2):81-87].

KEY WORDS:

Small cell lung cancer, NSE, tumor markers, chemotherapy

INTRODUCTION

Tumor markers are substances usually of peptide nature secreted by tumor cells. These substances are normally absent in the serum, since they are not secreted by normal cells or are secreted in very small amounts. While several serum components have been defined as biomarkers of small cell lung carcinoma (SCLC) as indicators of the extent of disease at diagnosis and monitors of response to therapy. Any biomarker of SCLC has not been seemed either sensitive or specific enough to mandate their widespread use either in the management of the patients or in screening for early detection. Several tumor markers have been evaluated in patients with SCLC. Enolase is a glycolytic enzyme and neuron specific enolase (NSE) is the YY dimer (izoenzyme) of enolase, which was first found in the extract of brain tissue and was later shown to be present in neuroendocrine cells and tumors (1,2). Carcinoembryogenic antigen (CEA), lactic dehydrogenase (LDH) and ferritin have been studied in patients with SCLC (3-7). NSE has been widespreadly used in management of patients with SCLC and CEA, ferritin and LDH are valuable, easily and routinely used than many tumor markers.

Many factors affect serum tumor markers. Some tumor markers can be elevated in normal patients or non-malignant disease. Stage and tumor burden are the most important factors that affect the tumor markers at diagnosis. Cisplatin and etoposide combination is one of the most favorable

Table 1
Patients' characteristics

	n
Number of Patients	84
Age (median and range)	58 (34-74)
Sex	
Female	66
Male	18
Stage	
Limited disease	48
Extensive disease	36
History of smoking	
Smoker	69
Nonsmoker	15
Performance status	
ECOG* 0	8
ECOG 1	50
ECOG 2	26

**ECOG: Eastern Cooperative Oncology Group*

choices in the treatment of SCLC. Most studies, which analyzed the association between levels of serum tumor markers and response to therapy in patients with SCLC, were performed in patients who were treated with cisplatin and etoposide combination (8). Trials of serum tumor markers with other chemotherapeutic drugs were inadequate. We studied tumor markers in SCLC patients who were treated with combination of cyclophosphamide, epirubicin and vincristine.

PATIENTS AND METHODS

Patients were eligible for the study if they had histologically proven SCLC with any stage of disease. Inclusion criteria were as follows: Histologically confirmed small cell lung cancer; age between 18 and 75; performance status (PS) favorable than 3 according to Eastern Cooperative Oncology Group (ECOG); normal hematological, renal and hepatic functional parameters. Patients were excluded if they had any significant other medical illness; previous or concomitant cancer except basal cell carcinoma of skin and cervical carcinoma in situ; had received chemotherapy and radiotherapy previously.

We researched stage extensively before treatment. We performed stage evaluation with clinical examination, direct radiography, computerized tomography (CT), brain and thoracic scan, unilateral iliac crest bone marrow aspiration, and biopsy, radionuclide bone scan and abdominal ultrasonography and/or computerized tomography. Diagnosis usually was established on biopsy specimen by fiber optic bronchoscope. Liver biopsy or CT guided transthoracal lung biopsy were used if bronchoscopic biopsy was not satisfactory. Serum analysis including complete blood count, electrolyte concentrations, creatine, calcium, alkaline phosphatase, transaminase levels and prothrombin time were performed before treatment. Complete blood count and chemistry analysis was performed before every chemotherapy course.

All patients received cyclophoshamide 750 mg/m^2 iv day 1, epirubicin 90 mg/m^2 iv day 1, and vincristine 1 mg/m^2 (maximum 2 mg) iv day 1 every three week as first line. Twenty-seven patients received Dalteparin 5000 U/day during first line chemotherapy. Dose reduction was performed at the next course as 25% rate of total dose in patients who had grade 4 myelotoxicity. Patients with limited disease underwent local thoracic radiotherapy if they had partial response and stable disease after six-course chemotherapy. Patients with complete remission underwent prophylactic cranial irradiation. All patients who had progression were treated with second line chemotherapy.

Table 2
Serum levels of tumor markers at diagnosis according to stage

	Limited disease	Extensive disease	Total
CEA (ng/ml) (Median and range)	2.2 (0.1-100)	5.2 (0.5-550)	2.5 (0.1-550)
Ferritin (ng/ml) (Median and range)	215 (27.7-989)	228 (5.2-1865)	222 (5.2-1865)
LDH (U/L) (Median and range)	426 (278-1660)	902 (267-2355)	472.5 (267-2355)
NSE (ng/ml) * (Median and range)	19.2 (5.0-189.4)	45.6 (11.2-616)	24.4 (5.0-616)

**Limited disease versus extensive disease, p=0.001*

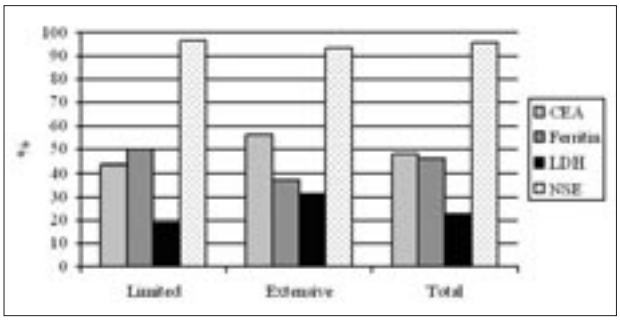

Fig 1. Rate of patients with elevated serum levels of tumor markers at diagnosis

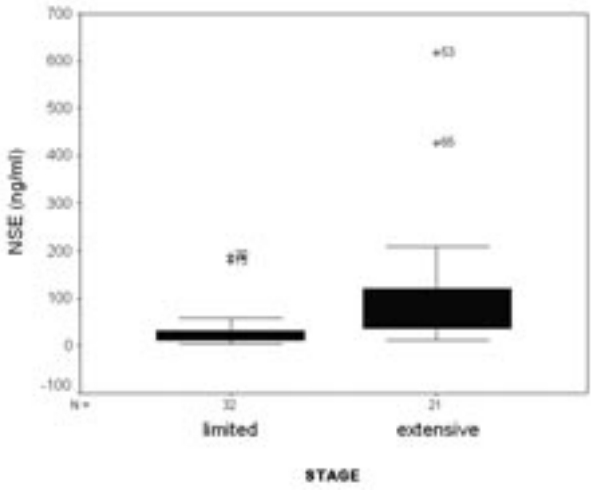

Fig 2. Distributions of NSE levels according to stage groups

We studied the incidence and levels of NSE, LDH, CEA and ferritin in patients' sera. Tumor markers were determined at diagnosis and after chemotherapy. Marker levels were classified as normal and elevated. NSE and ferritin were analyzed by radioimmunoassay (RIA) (Cis kit for NSE, Icn kit for ferritin), CEA by automated chemiluminescence's system (Chiron kit), LDH by auto analyzer (Konelap 60) (Dialab kit). Reference ranges were 4.7-14.7 ng/ml for NSE, less than 3.5 ng/ml for CEA, 0-450 U/L for LDH, and 17-390 ng/ml in male, 10-90 ng/ml in female for ferritin.

Statistics

Serum levels of tumor markers at diagnosis and after treatment were compared by Wilcoxon test. The comparison of tumor marker levels at diagnosis between stage groups were performed by Mann Whitney U test. Disease free and overall survival time and survival rate were estimated by using the method of Kaplan Meier and compared by using log rank test.

Table 3
Changes in serum tumor markers after treatment

	Before treatment	After treatment	p value
CEA (ng/ml) (Median and range)	2.5 (0.1-550.0)	4.3 (0.4-100.0)	0.79
Ferritin (ng/ml) (Median and range)	222 (5.2-1865)	206 (15.1-721)	0.29
LDH (U/L) (Median and range)	472.5 (267-2355)	450 (311-1786)	0.31
NSE (ng/ml) * (Median and range)	24.4 (5.0-616.0)	13.0 (4.8-244.0)	0.0036

Table 4
NSE levels at diagnosis and after treatment according to stage group

	Limited disease	Extensive disease	Total
Before treatment (ng/ml) (Median and range)	19.2 (5.0-189.4)	45.6 (11.2-616.0)	24.4 (5.0-616.0)
After treatment (ng/ml) (Median and range)	11.79 (4.82-65.0)	25.42 (8.1-244.0)	13.01 (4.8-244.0)
P value	0.007	0.715	0.036

RESULTS

We entered 84 patients into the study. Median age was 58 years (34-74). The patients' characteristics are described in table 1. NSE was elevated in 77.6% of all patients (45 patients) at diagnosis and its level was significantly higher in extensive disease than limited disease (with medians of 45.6 (11.2-616.0) and 19.2 (5.0-189.4), respectively; p=0.001) Levels of CEA, ferritin and LDH were elevated in 47.9%, 45.8%, and 22.9% of patients, respectively. Levels of CEA, ferritin and LDH did not significantly differ between patients with limited and extensive disease. Rate of patients with elevated serum tumor markers is shown in table 2 and figure 1. Levels of tumor markers at diagnosis according to stage were summarized in table 3. Figure 2 demonstrated distributions of NSE levels in limited and extensive stage.

There was no statistically significant difference in CEA, ferritin, and LDH before and after treatment (Table 4). Median level of NSE at diagnosis was 24.4 ng/ml (range 5.0-616.0) and was 13.0 ng/ml (range 4.8-244.0) after chemotherapy in all patients (p=0.0036). Level of NSE decreased significantly in all patients and limited stage subgroups after first line treatment (Table 5, Figure 3). There was no significant difference in extensive stage patients.

We obtained higher response rate for overall objective response without statistically significant difference in patients with normal NSE levels than patients with elevated NSE levels (76.9 versus 52.4, respectively; p=0.58). Median progression-free and overall survival times were higher in patients with normal NSE levels. We calculated median progression-free survival as 11.1±1.47 months (95% CI 8.23-14.01) in patients with normal NSE levels and 8.11±0.96 months (95% CI 6.29-9.99) in patients with elevated NSE levels (p=0.05). Overall survival was median 14.0±1.20 months (95% CI 11.64-16.36) in patients with normal NSE levels and 8.0±0.85 months (95% CI 6.34-9.66) in patients with elevated NSE levels (p=0.09). One-year progression-free and overall survival rates were higher in patients with normal NSE levels but 2-year progression-free and overall survival rates were not different between two groups (Figures 4 and 5). Survival results were summarized in table 6. No correlation between response to therapy, survival and levels of CEA, Ferritin and LDH were found.

Table 5
Response to therapy according to NSE levels*

	Normal		Elevated		Total	
	n	%	n	%	n	%
Complete remission	4	30.7	5	11.9	9	16.4
Partial remission	6	46.2	17	40.5	23	41.8
Stable disease	1	7.7	8	19.0	9	16.4
Progressive disease	2	15.4	12	28.6	14	25.4
Total	13	100.0	42	100.0	55	100.0

** p=0.58*

Table 6
Estimated survival time by Kaplan Meier test according to NSE levels

	Normal	Elevated
Progression-free survival*		
1-year survival rate (%)	53.0	16.3
2-year survival rate (%)	0.0	2.7
Survival time (Median ± SD (95% CI))	11.1±1.47 (8.23-14.01)	8.11±0.96 (6.29-9.99)
Overall survival **		
1-year survival rate (%)	62.0	23.8
2-year survival rate (%)	8.3	11.5
Survival time (Median ± SD (95% CI))	14±1.20 (11.64-16.36)	8±0.85 (6.34-9.66)

** p=0.05, ** p=0.09*

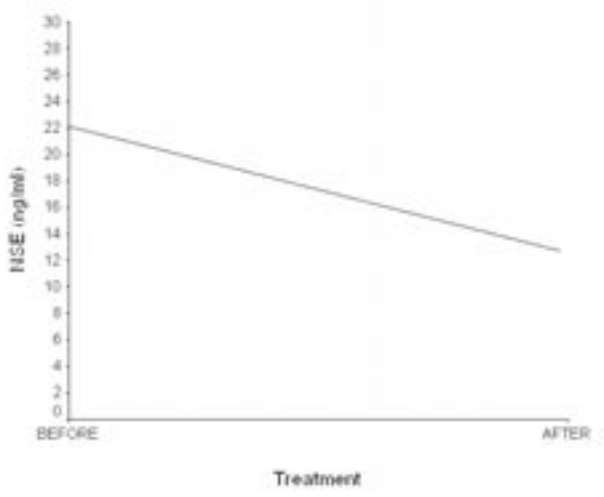

Fig 3. Changes in NSE levels after treatment with cyclophosphamide, epirubicin, vincristine

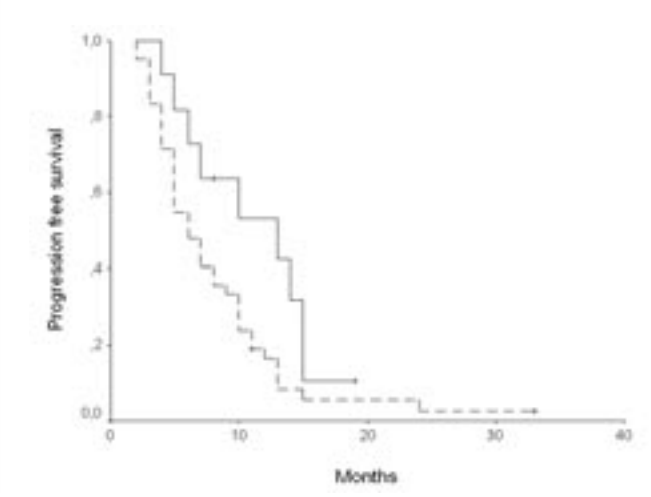

Fig 4. Progression-free survival according to NSE levels by Kaplan Meier test (p=0.05), (— Normal NSE levels, --- Elevated NSE levels)

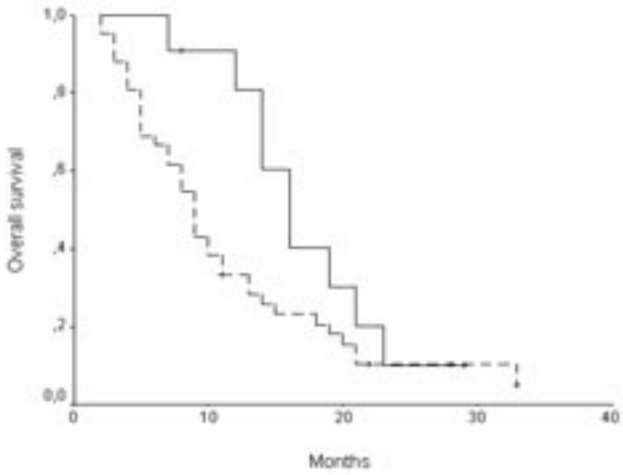

Fig 5. Overall survival according to NSE levels by Kaplan Meier test (p=0.09), (— Normal NSE levels, --- Elevated NSE levels)

DISCUSSION

Tumor markers have widespread use in oncology. Potential clinical applications of tumor markers are: facilitate the early diagnosis, offer a guide for the evaluation of prognosis, help in selecting patients for adjuvant chemotherapy, help in assessing the response to therapy and in diagnosis of early relapses. Moreover the serum level of an ideal marker should: increase pathologically in the presence of a neoplasm (high sensitivity), not increase in the absence of neoplasm (high specificity), relate to tumor burden and metastatic spread, change in accordance with the clinical evolution, reflecting the current status of disease. There are many efforts for reaching the ideal tumor marker in oncology. Tumor associated antigen such as carcinoembryogenic antigen (CEA), tissue polypeptide antigen (TPA), squamous carcinoma antigen (SCC-Ag), other polypeptide antigen such as ferritin, soluble interleukin 2 receptors (sIL-2r), chromogranin A, enzymes such as, neuron specific enolase (NSE), creatine phosphokinase-BB (CPK-BB), glycosyl transferases and hormones such as bombesin/gastrin releasing peptide (BN/GRP), adrenocorticotropin (ACTH), antidiuretic hormone (ADH), calcitonin (CT), insulin like growth factor (IGF I and II) have been investigated in patients with lung cancer (9-10).

NSE is the most useable tumor marker in lung cancer patients with small cell histology. NSE is the neurol isoenzyme of the intracytoplasmic enzyme enolase, which was first found in the extract of brain tissue and was later shown to be present in neuroendocrine cells and tumors. Elevated serum concentrations of NSE have been found in over 70% of patients with SCLC. In general, studies noted that NSE levels are higher in the patients with extensive stage than limited stage (11-13). In our study, tumor marker levels were classified as elevated or not elevated. NSE was elevated in 77.6%, CEA in 47.9%, ferritin in 45.8%, LDH in 22.9%. Concentration of NSE in serum was higher with statistically significant difference in extensive disease than limited disease. The serum NSE levels are associated with stage and reflect the tumor burden. After treatment NSE levels decreased significantly in all patients with limited stage disease. NSE levels in patients with extensive stage didn't decrease significantly. Patients with extensive stage had bad prognosis and combination chemotherapy was less effective than limited stage. Therefore tumor markers could not reflect the response to therapy.

We determined that patients whose NSE levels were not elevated had higher response rate than patients with elevated NSE levels but this difference was not statistically significant. Median progression-free and overall survival times in patients with normal NSE levels were superior to patients with elevated NSE levels. One-year survival rate was also superior in the patients whose NSE levels were not elevated, but 2-year survival rate was similar. Many studies examined the prognostic significance of serum NSE in patients with SCLC (5,14). Jorgersen at al. (15) showed in 770 patients that NSE was the most powerful predictor of survival followed by performance status and stage of disease. Bonner et al. (8) showed that both pretreatment NSE and treatment induced minimum NSE were independent prognostic predictors of time to progression and survival.

We studied serum tumor markers in patients who were treated with combination of cylophosphamide, epirubicin and vincristine. NSE is correlated with stage and decrement after treatment significantly. NSE levels may play a role as prognostic and predictive factor in patients who were treated with this combination. Although there are many data in the literature about CEA, ferritin, and LDH that are used as biomarkers in patients with SCLC, we did not find any correlation in our patients who were treated with this combination (6,7,16,17). Comparison of patients who did and did not receive low molecular weight heparin has not revealed further significant results for NSE, CEA, ferritin and LDH. Receiving low molecular weight heparin didn't affect results of tumor markers analysis. In conclusion; NSE was the most reliable tumor marker in small cell lung cancer patients who were treated with combination of cylophosphamide, epirubicin and vincristine. NSE was in correlation with stage and reflected the response to therapy in patients who were treated with this regimen.

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