RESEARCH ARTICLE

For Which Cancer Types can Neuron-Specific Enolase be Clinically Helpful in Turkish Patients?

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Abstract

Background: The aim of the present study was to evaluate the serum neuron-specific enolase (NSE) levels in patients with prostate cancer, Hodgkin lymphoma, lung cancer and peripheral nerve tumors. **Materials and Methods:** NSE levels were determined by ELISA in the sera of 100 prostate cancer, 47 Hodgkin lymphoma, 35 lung cancer and 35 peripheral nerve tumor patients and also in 132 healthy controls. **Results:** The median levels of serum NSE were elevated in patients with lung cancer (p=0.018) and peripheral nerve tumors (p=0.008). NSE levels in prostate cancer and Hodgkin lymphoma patients were higher than the controls but there was no statistically significant difference (p>0.05). **Conclusions:** We conclude that NSE may be applied in routine to gain insight about the clinical statuses of various cancer patients, but more studies are needed to determine the organ specificity.

Keywords: NSE - cancer - tumor marker - clinical status - Turkey

Introduction

Serum tumor markers play an important role in diagnosis, determining of the pathology, staging, monitoring the response to the therapy and predicting the prognosis (Zhao and Wang, 2011). They are often used in conjunction with other clinical parameters such as biopsy and radiological evidences.

Enolase, also known as phosphopyruvate hydratase is an enzyme responsible for the catalysis of the conversion of 2-phospho-D-glycerate (2-PG) to phosphoenolpyruvate (PEP) in the glycolytic pathway (Peshavaria and Day, 1991). It also catalyses the reaction of PEP to 2-PG in gluconeogenesis. The enzyme uses magnesium ion as a cofactor.

There are three subunits of enolase, α, β and γ enolase. That can combine five different isoenzymes, three of these isoenzymes are homodimers and are commonly found in human cells. α-enolase, known as non-neuronal enolase (NNE) or enolase-1 is expressed in various tissues like brain, kidney, liver. β-enolase or enolase-3 is mainly present in skeletal muscle cells (Peshavaria and Day, 1991). Neuron-specific enolase (NSE), the γ homodimer of enolase, was first found in extracts of brain tissue, and was later shown to be present in APUD (amine precursor uptake and decarboxylation) cells. It is produced in central and peripheral neurons (Greenberg and Lee, 2007). It has a high stability in biological fluids and can easily diffuse

to the extracellular medium and cerebrospinal fluid (CSF) when neuronal membranes are injured. NSE is frequently used clinically as a sensitive and useful marker of neuronal damage in several neurological disorders including stroke, hypoxic brain damage, status epilepticus, Creutzfeldt-Jakob disease, and herpetic encephalitis as well as a marker of tumors of neuroendocrine origin and small cell lung carcinoma (SCLC) (Song et al., 2012). Measurement of NSE levels in patients with these diseases can provide information about the patients' prognosis and the response to treatment (Sung and Cho, 2008).

In this study the serum concentrations of NSE in patients with cancer and in healthy controls were determined in order to clarify the role of NSE in various cancer types.

Materials and Methods

217 (100 prostate cancer, 47 Hodgkin lymphoma, 35 lung cancer, 35 children with peripheral nerve tumor) patients with histopathologically verified carcinoma, consecutively admitted to the Istanbul University, Oncology Institute during a five – months period, January 2012 to May 2012 were investigated. Serum samples were obtained on first admission before any type of treatment was given. Staging was performed on a pathological basis according to the American Joint Committee on Cancer (AJCC). The median age of patients was 69 (47-83) years

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for prostate cancer patients, 48 (1-74) years for Hodgkin lymphoma patients, 53 (4-71) years for lung cancer patients and 5 (1-18) years for children with peripheral nerve tumor.

Normal healthy subjects (n=132; 55 male, 77 female) with median age of 51 (17-68) years were included as the control group. Controls were blood donors undergoing regular physical and laboratory examinations. Our study on human materials has been approved by the relevant institutional committee (Local Ethics Committee, Number: 15052009/1-2). Written informed consent was obtained from all patients included in the study. The protocol was consistent with the Declaration of Helsinki (2000).

Serum samples were obtained from each patient in the morning. Blood samples were obtained by venipuncture and clotted at room temperature. Serum NSE analyses were performed the same day.

Solid phase- enzyme linked immunosorbent assay (ELISA) was used to determine the serum values of NSE as ng/mL (DRG International Inc., New Jersey, USA). The amount of NSE was quantitated by an automated ELISA reader (Rayto, RT-1904C Chemistry Analyzer).

SPSS software (version 16; SPSS, Chicago, IL) was used for statistical analysis. The data did not show a normal distribution, therefore non-parametric Mann-Whitney U test was used to evaluate differences between patients and normal controls. A two-tailed p value <0.05 was considered statistically significant. The report design was adopted from the standarts for reporting diagnostic accuracy (STARD) group (Bossuyt et al., 2004). The sensitivity and specificity of the tests were calculated by using receiver operating characteristics curves (ROC).

Results

Descriptive statistics and the serum NSE levels of patients and the control group are shown in Table 1. The mean serum NSE levels were higher in lung cancer (p=0.018) and peripheral nerve tumor (p=0.008) patients compared with healthy controls (Table 1). The median and minimum-maximum ranges of serum NSE values in the groups are shown in Figure 1.

To determine the cut-off values and sensitivity and specificity of serum NSE tests in the patients, we used receiver operating characteristic (ROC) curves (Figure 2-5). The cut-off values were chosen according to the ROC curve coordinate points and cut-off point for serum NSE was equal to its mean value. The cut-off levels (x±2sd) of NSE were 3.5-22.3 ng/mL. The sensitivities and specificities determined from the ROC curves are
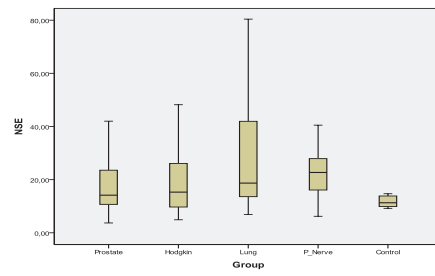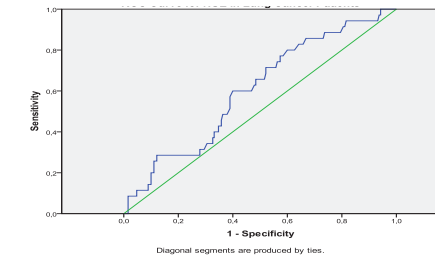

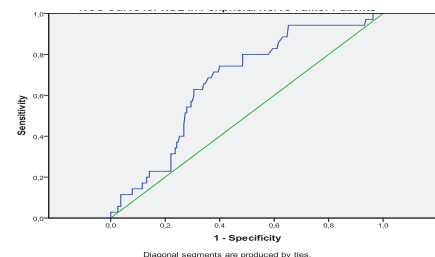**Figure 1. Median NSE Values of the Groups**
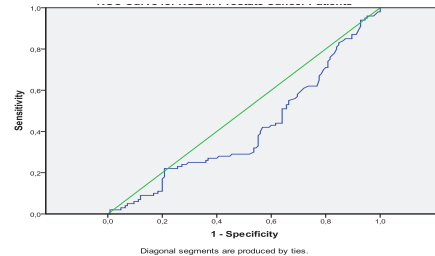
Figure 2. ROC Curve for NSE in Prostate Cancer Patients
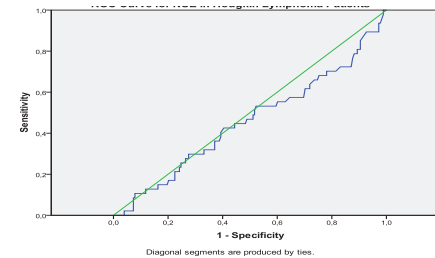
Figure 3. ROC Curve for NSE in Hodgkin Lymphoma Patients

Figure 4. ROC Curve for NSE in Lung Cancer Patients

Figure 5. ROC Curve for NSE in Peripheral Nerve Tumor Patients

Table 1. The Levels of NSE according to the Groups

	Prostate Ca. n=100 x±sd; median; min-max	Hodgkin Lymphoma n=47 x±sd; median; min-max	Lung Ca. n=35 x±sd; median; min-max	Peripheral Nerve Tm. n=35 x±sd; median; min-max	Control n=132 x±sd; median; min-max
Serum NSE (ng/mL)	24.4±26.5; 14.3; 3.7-149.2	23.8±22.3; 16; 4.9-104.8	32.2±28.7; 20.3; 6.9-120.6	29.9±27.3; 22.2; 6.2-106.9	12.9±4.7; 11.3; 9.2-23.7
p-value	0.174	0.228	0.012	0.003	

*x±sd: mean±standard deviation; m: median

Table 2. The Sensitivities and Specificities of NSE According to the Groups

	Prostate Ca.	Hodgkin Lymphoma	Lung Ca.	Peripheral Nerve Tm.
Sensitivity	48%	47%	43%	54%
Specificity	36%	50%	65%	72%

shown in Table 2.

Discussion

Neuroendocrine differentiation is the characteristic of invasive carcinomas in which there is a transformation of malignant epithelial cells into neurosecretory cells. Based on several secretory activities of neuroendocrine differentiated cells, there are many potential markers. The most important and commonly used markers of neuroendocrine differentiation are Chromogranin A (CgA) and NSE (Ramage et al., 2005).

NSE is a glycolytic enzyme found in tumors of neuroendocrine origin. Significantly elevated serum NSE values are seen in SCLC patients, and it is used in routine for monitoring the therapy. It is also used as a marker for tumors of the nervous system, especially for childhood tumors.

The NSE levels among healthy Turkish population has been found significantly higher than the normal ranges of the other European populations (Yasasever et al., 1999). The reason can be connected with the geographic and ethnical differences and the smoking rates.

The main reason of performing this study was the thought that the serum NSE levels were in rise in various cancer patients in Turkey, not only in lung cancers and neuroendocrine tumors. In 84 prostate cancer patients, Ather et al. evaluated the correlation of immunohistological detection of neuroendocrine differention marker NSE with Gleason grade. They found that NSE had a significantly higher expression with worsening Gleason grade (Ather et al., 2008). Humez et al. (2006) observed that neuroendocrine differentiation was associated with NSE expression and this was important for the prostate cancer progression and hormonal-therapy failure. Kamiya et al. (2003) also revealed that non prostate cancer patients had significantly higher serum NSE levels, although metastatic prostate cancer patients had higher NSE values than the non-metastatic patients, they claimed that pretreatment serum NSE level can predict survival of metastatic prostate cancer patients. Hvamstad et al. (2003) also claimed the same in their study with 138 prostate cancer patients, they found that NSE levels were significantly reduced after radiotherapy and that pretreatment NSE levels predicted survival, as in the study of Lilleby et al. (2001). In the study of Berruti et al. (2000) it has been found that high NSE levels were predictors for poor prognosis in patient with hormone-refractory prostate cancer.

There are limited studies on the relative significance of NSE and lymphomas. In the study of Nakatsuka et al. (2002) it is observed that serum NSE levels were elevated in non-Hodgkin lymphoma, especially in pyothorax-associated lymphoma and tended to decrease after chemotherapy. Massarelli et al. (1999) revealed in their study with 16 Hodgkin lymphoma patients that high NSE expressions were found only in subgroups expressing CD30.

A study with 63 patients with metastatic carcinomas of unknown primary site indicated that NSE levels were elevated in most of the cases. The serum levels of NSE showed good correlation with response to the chemotherapy (Pejcić et al., 2010).

NSE is widely used in routine as a diagnostic and prognostic marker in lung cancer. Serum NSE levels are highly elevated in lung cancers, especially in SCLC (Emin et al., 2010; Wójcik et al., 2010; Li et al., 2012). High NSE levels are associated with the grade of the disease (Fizazi et al., 1998; Xue et al., 2011).

As a marker to predict the prognosis and to monitor the response to the therapy, NSE is used in childhood nervous system tumors. The expression and serum levels of NSE are found highly elevated in certain studies (Schmidt et al., 1985; Kodet et al., 1991).

Our study indicates that serum NSE levels of prostate cancer and Hodgkin lymphoma patients are higher than the serum levels of healthy people, but not as much as in lung and childhood peripheral nerve tumors. Although the median serum NSE levels of prostate cancer and Hodgkin lymphoma patients are high, there is no statistically significant difference. Neuroendocrine differentiation is a common circumstance in prostate cancer, but it cannot be the key determinant, because most of the prostate carcinomas are adenocarcinomas. Hodgkin lymphoma is a solid hematologic neoplazm, its characteristics differ greatly by the other solid tumors. Therefore it can not be used as a diagnostic marker in these cancer types, but it may give an idea about the general medical condition and the prognosis of the patient. We think that more studies must be done about the correlation of NSE levels in patients of these cancer types with the clinical statuses of the patients.

Acknowledgements

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