DETERMINATION OF NEURON SPECIFIC ENOLASE (NSE) IN SMALL CELL LUNG CARCINOMA (SCLC) AND NON-SMALL CELL LUNG CARCINOMA (NSCLC) PATIENTS

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ABSTRACT:
Neuron specific enolase (NSE) is routinely used as tumor marker in Small cell lung carcinoma (SCLC), and to some extent in non-small cell lung carcinoma (NSCLC). In Pakistan, tumor marker technology is not a new one. It is however mostly directed towards uses in hepatic, breast, ovarian, uterine and colorectal cancers, whereas availability and general practice of its use for diagnosis of respiratory metastasizing disease such as lung cancer is seldom and rare, especially the SCLC/NSCLC specific NSE. The aim of present study is to determine the potential usefulness of NSE in diagnosis and prognosis of SCLC and NSCLC patients in our setting. Fifty-eight patients of lung cancer were identified and selected, between January 2004 to December 2007, and divided into various groups depending upon their clinical stage of disease. NSE level was determined in all patients and clinical history data and related pathphysiological components of all selected patients were carefully assessed and compulsorily followed to avoid any bias. Cancer status of patients were evaluated by data available from multiple bronchoscopies, X rays, cytology and histopathology examinations and grouped as SCLC with all five stages (I, II, IIIA, IIIB and IV) and NSCLC with only stage IV. NSE level was also determined in Healthy subjects and patients with non-malignant lung diseases (NMLD) for comparison. We observed significant elevation in levels of NSE for different stages of SCLC and NSCLC in comparison with healthy and NMLD groups. Most significant increase was noted in SCLC stage IV not only in comparison with healthy (P < 0.001) and NMLD groups (P < 0.001) but also with stage I (P < 0.001) within the group. Elevated difference in NSE levels was also correlated with stage II, IIIA and IIIB. However, no significant difference in levels was established among Stage II, III A and IIIB of SCLC group. As regard NSCLC, where patients belonged only to stage IV of disease, significant difference was observed with healthy (P < 0.001) and NMLD (P < 0.001) groups. Comparison among SCLC and NSCLC groups revealed significant difference of NSE levels in stage I (P < 0.01) and stage IV (P < 0.01) when compared with NSCLC, whereas non-significant difference in NSE levels was noted in group-SCLC stage II, IIIA and IIIB. In comparison, all stage IV patients (n = 7) of SCLC exhibited higher levels of NSE with a range of 136.19 ng/ml to 175.01 ng/ml, higher than detected in patients of stage IV in NSCLC. The result of our study suggests that NSE appears to be a useful tumor marker for SCLC and to some extent, NSCLC. Moreover, NSE exhibits higher levels in some stages of SCLC suggesting, its specificity, not only for advanced stage of SCLC but also for SCLC in general as compared to NSCLC. Its

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determination, therefore, is beneficial in the diagnosis, treatment and a possible follow-up for patients’ survival.

**Keywords**: Neuron Specific enolase (NSE), Small cell lung carcinoma (SCLC), non-small cell lung carcinoma (NSCLC), tumor markers.

**INTRODUCTION**

It is a known fact that lung cancer represents one of the most fatal metastatic diseases and is one of the commonest malignancies in causing deaths after heart disease (Chen et al., 2008; Kulpa et al., 2002; Molina et al., 2008). It this regard, the patients suffering from non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) are frequent with occurrence rate of 40% and 20-30% respectively. Rising mortality from lung cancer has leveled off in men but it continues to rise in women in western world accounting for one out of eight deaths from all malignant disease in women (Kulpa et al., 2002). Unfortunately, despite advancement and development of diagnostic methods, a large majority of lung cancer patients are diagnosed in advanced stages (Kulpa et al., 2002), causing hardships in treatment and recovery. It is reported that a considerable number of patients undergone surgical treatment for lung cancer, especially NSCLC, relapse and result into local growths and distant metastases (Hespanhol et al., 1995; Kulpa et al., 2002). Such problems are faced by clinicians, oncolgists and diagnostic specialists on daily basis and re-enforce the idea of developing new diagnostic tools such as specific tumor markers to evaluate and assess diseases, its treatment and prognosis. Several tumor markers such as carcino-embryonic antigen (CEA), squamous cell carcinoma antigen (SCC-Ag) and cytokeratin-21 (Cytofra-21) have been used as indicators of lung cancer, but none was found as an independent factor in biochemical diagnosis of the disease (Ando et al., 2004; Barlesi et al., 1994; Holdenrieder et al., 2008; Hillas et al., 2008; Mino et al., 1988; Molina et al., 2008; Ochino et al., 1984; Schneider, 2006; Schneider et al., 2003a,b). However, it is reported that neuron specific enolase (NSE) is a specific tumor marker in SCLC patients as well as in NSCLC, where NSE levels were often found elevated (Ferrigno et al., 2003; Fischbach and Jany, 1986; Fizazi et al., 1998; Chen et al., 2007; Karnak et al., 2005; Petrovic et al., 2008; Muley et al., 2003; Satoh et al., 2002; Wei et al., 2006; Zych et al., 2002).

In Pakistan, tumor marker technology is not a new one, but mostly directed towards uses in hepatic, breast, ovarian, uterine and CRC cancers. Furthermore, availability and general practice of using tumor marker for diagnosis of respiratory metastases disease such as lung cancer is seldom and rare, especially for SCLC/NSCLC specific NSE. The aim of present study is to determine the potential usefulness of NSE for diagnosis, prognosis and treatment of lung cancer in our setting.

**MATERIALS AND METHODS**

**Patients and Controls**: Fifty-eight patients (54 males and 4 females) of lung cancers were identified and selected from Pulmonology and Chest medicine wards and out patients departments at Liaquat National Hospital, Baqai Medical University and Government Lyari General Hospital, Karachi. They were divided into different groups depending upon their clinical stage of the disease according to Kulpa et al (2002). The study covered the period of January 2004 to December 2007. Clinical history data and related components of all lung cancer patients were carefully noted and compulsorily followed to avoid any bias. Cancer status of patients were evaluated by data available from multiple bronchoscopies, X rays, cytology and hispathology examinations and grouped as SCLC and
NSCLC. It was also ensured that all selected patients were in pre-treatment period. Those lung cancer patients with ongoing chemotherapy, radiotherapy or post-treated and post-operated were included in another set of study that shall be completed in Dec 2010 (unpublished data). For simplification, both types of cancer groups were assessed and divided into five subgroups as per stage I, II, IIIA, IIIB and IV as per description provided earlier (Kulpa et al., 2002). Group (or stages) I-IIIA, are those in which lymph node may have involved and cancer was localized to a part or single lung. Stage IIIB and IV consisted of those group of patients in which cancer had spread to other lung or chest cavity and also metastasized to other sites and organs. Final categorization was such that SCLC has patients in all five groups (I to IV) whereas NSCLC has patients in only the advanced stage IV. All patients had confirmed diagnosis of lung cancer and were in pre-treatment period with specification of 48 with SCLC and 10 with NSCLC. Further investigations revealed that out of 48 SCLC cases, 7 had slight manifestations of metastasis in upper abdomen and breast (in case of two females) and therefore were placed in stage IV. 10 patients with NSCLC were all stage IV and have marked metastasis towards stomach (n = 2), breast (n = 1), stomach and small intestine (n = 2) upper GIT (n = 2), and liver (n = 3). Out of 58 patients, there were only a total of four female patients, 3 in SCLC and 1 in NSCLC group. All patients were in age range of 49-79 years, with female ranging of 56-74 yrs and males between 49-79 yrs. 23 were smokers but with varying degree of smoking habits. The remaining were non-smokers including three females. In smoker’s category, 10 were heavy smokers (20 cigarettes per day), 5 with moderate smoking habits (6-8 cigarettes per day) and remaining were occasional smokers including one female. The clinical diagnosis of SCLC and NSCLC was confirmed by cytological/and or histopathological examinations. For comparison, 50 healthy subjects (25 males and 25 females) as well as 63 (40 males and 13 females) patients with non-malignant lung disease (NMLD) were also included in the study. The age, sex and smoking status in both groups were comparable. NMLD includes pneumonia (n = 22), tuberculosis (n = 6) asthma (n = 20), benign tumors (n = 6) and fibrosis (n = 9).

Blood samples and analysis of NSE:

Blood specimens were obtained by vein puncture. Serum was separated immediately and stored at –20°C until analyzed. NSE concentration was measured by electrochemiluminescence assay using Roche Diagnostic reagents on Elecsys 1010 and 2010 analyzers (Roche Diagnostics, Basel). The manufacturer’s (Roche Diagnostic, Basel) suggested cut-off value of < 15.2 ng/ml NSE in healthy and NMLD groups. Specificity and sensitivity were determined by methods described earlier (Fateh-Moghadam, A and Stieber, P. Sensible Use of Tumor Markers-1993; Roche, Editiones Roche, Basel, Switzerland). To assess the diagnostic utility of NSE in both SCLC (and its sub groups) and NSCLC, a maximum cutoff value of 95 percentile (Kulpa et al. 2002) was evaluated in healthy persons and NMLD groups.

Statistical Analysis:

Different groups were compared and evaluated by one-way ANOVA and students’ t-test. The values were considered significant only when P < 0.01.

RESULTS

Results are summarized in Tables and Figures. We observed significant difference in levels of NSE for different stages of SCLC and NSCLC in comparison with healthy and NMLD groups (Table I). Most significant increase was noted in SCLC stage IV not only in comparison with healthy (P < 0.001) and NMLD groups (P < 0.001) but also with stage I (P < 0.001) within the group. Difference in NSE levels was also imperative with stage II, IIIA and IIIB (Table I). However, no significant comparison in levels was established among Stage II, III A and IIIB of SCLC group. As regard NSCLC, where patients belonged only to stage IV of disease,
significant difference was observed with healthy (P<0.001) and NMLD (P<0.001) groups. Comparison among SCLC and NSCLC groups revealed significant difference of NSE levels in stage I (P<0.01) and stage IV (P<0.01), whereas non-significant difference in NSE levels was noted in group-SCLC stage II, IIIA and IIIB when compared with NSCLC.

NSE levels in healthy patients aged 43-70 yrs was in the range of 2.21-16.0 ng/ml with a median of 7.50 ng/ml, whereas that for
NMLD were in the range of 8.10-17.66 ng/ml with a median of 12.93 ng/ml. Mean NSE for total number of lung cancer patients was 81.53 ng/ml with a range of 20.21-175.01 ng/ml. In subgroups of SCLC and NSCLC, the median and ranges were; SCLC stage I (n = 9) = 45.17 (29.60 – 82.10 ng/ml); stage II (n=16)=79.87 (20.21-160.66 ng/ml); stage IIIA (n=9)=72.99 (44.21-170.11 ng/ml); stage IIIB (n=7) = 72.45 (29.41-148.74 ng/ml); stage IV (n=7)= 150.55 (136.19-175.01 ng/ml). With reference group, sensitivity and specificity of NSE was found to be 86% and 67% respectively (Table 2). Negative and positive predictive values were also calculated in

<table>
<thead>
<tr>
<th>Lung cancer</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage IIIA</th>
<th>Stage IIIB</th>
<th>Stage IV</th>
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</thead>
<tbody>
<tr>
<td>SCLC</td>
<td>45.17</td>
<td>79.87</td>
<td>72.99</td>
<td>72.45</td>
<td>150.55</td>
</tr>
<tr>
<td></td>
<td>(29.60-82.10)</td>
<td>(20.21-160.66)</td>
<td>(44.21-170.11)</td>
<td>(29.41-148.74)</td>
<td>(136.19-175.01)</td>
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<tr>
<td>NSCLC</td>
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<td>82.57</td>
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<td></td>
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<td></td>
<td>(40.76-149.66)</td>
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<tr>
<td>Healthy</td>
<td>7.50</td>
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<tr>
<td></td>
<td>(2.21-16.00)</td>
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<tr>
<td>NMLD</td>
<td>12.93</td>
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<td></td>
<td>(8.10-17.66)</td>
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</table>

Results are expressed as mean with ranges (in parenthesis). Healthy and NMLD are placed in 1st column for presentation purpose and have no relation to stages of diseases.

Comparison among various stages of SCLC (a vs b,c,d = P < 0.01; a vs e = P < 0.001; e vs b,c,d = P < 0.01)

Comparison of NSCLC stage IV with various stages of SCLC (f vs a = P < 0.01; f vs e = g = P < 0.001; f vs a-d = h = Non significant NS)

Comparison of Healthy and NMLD with SCLC (where I = healthy vs a-e = P < 0.001); II = NMLD vs a = P < 0.01 and III = NMLD vs b-e = P < 0.001), IV-V with NSCLC (where IV = healthy vs NSCLC = P < 0.001 and V = NMLD vs NSCLC = P < 0.001).

Table-2

<table>
<thead>
<tr>
<th>Tumor marker</th>
<th>Cut-off (ng/ml)</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>*PPV</th>
<th>*NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Cutoffs for Healthy persons</td>
<td></td>
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<tr>
<td>NSE</td>
<td>7.50</td>
<td>86</td>
<td>67</td>
<td>0.72</td>
<td>0.74</td>
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<tr>
<td>B. Cutoffs for NMLD</td>
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</tr>
<tr>
<td>NSE</td>
<td>12.93</td>
<td>63</td>
<td>57</td>
<td>0.68</td>
<td>0.51</td>
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</tbody>
</table>

*NPPV = positive predictive value, *NPV = negative predictive value.

NMLD were in the range of 8.10-17.66 ng/ml with a median of 12.93 ng/ml. Mean NSE for total number of lung cancer patients was 81.53 ng/ml with a range of 20.21-175.01 ng/ml. In subgroups of SCLC and NSCLC, the median and ranges were; SCLC stage I (n = 9) = 45.17 (29.60 – 82.10 ng/ml); stage II (n=16)=79.87 (20.21-160.66 ng/ml); stage IIIA (n=9)=72.99 (44.21-170.11 ng/ml); stage IIIB (n=7) = 72.45 (29.41-148.74 ng/ml); stage IV (n=7)= 150.55 (136.19-175.01 ng/ml); NSCLC stage IV (n=10)=82.57 (40.76-149.66 ng/ml). With reference group, sensitivity and specificity of NSE was found to be 86% and 67% respectively (Table 2). Negative and positive predictive values were also calculated in
It was also observed that NSE concentration increased with advance in the stage of disease, especially in SCLC (Fig 1). Data cannot be compared with NSCLC as patients in NSCLC were of stage IV only (Fig 2). However, NSE concentration in stage IV of NSCLC is comparatively higher than stage I and IIIB of SCLC, though the increase was insignificant. In comparison, percent increase in NSE level of SCLC subgroups with respect to healthy and NMLD subjects were; 83.4% and 71.37% in stage I, 90.60% and 83.81% in stage II, Stage IIIA = 89% and 82.28%; stage IIIB = 89.64% and 82.15% and Stage IV = 95.01% and 91.40%, respectively. In NSCLC, it was 90.91% and 84.34 % when compared with healthy and NMLD subjects. Data for percent increase in NSE levels in control groups and patients subgroups revealed that, significantly higher levels was obtained in disease stages as compared to healthy and NMLD individuals, however percent increase among different stages of disease was non-significant in SCLC and NSCLC patients as well. Interestingly, assessment of NSE level in individual SCLC patients revealed that higher levels of NSE was found in patients of stage II and IIIA; 160.66 ng/ml and 170.11 ng/ml, respectively, whereas, the lowest NSE levels were also found within these cancer group. In comparison, all stage IV patients (n = 7) of SCLC exhibited higher levels of NSE with a range of 136.19 ng/ml to 175.01 ng/ml, higher than detected in patents of stage IV in NSCLC. This exhibit specificity of NSE for, not only advanced stage of SCLC but also for NSCLC as compared to NSCLC, where highest individual NSE level was 149.66 ng/ml.

DISCUSSION

Reported cut-off values for NSE in healthy and NMLD person ranges between 12.5 ng/ml to 21.6 ng/ml (Ebert et al., 2002; Muley et al., 2003; Satoh et al., 2002; Zych et al., 2002). In our study, cut-off values were determined to be 7.50 ± 4.40 ng/ml for healthy subjects and 12.93 ± 2.87 ng/ml for patients with non malignant lung disease. Cumulative sum of NSE for all lung cancer patients, both SCLC and NSCLC was 81.53 ± 6.20 ng/ml; ranging from 20.21 ng/ml to 175.01 ng/ml. It is suggested that NSE is the tumor marker of 1st choice for SCLC, but increase serum NSE has been reported in 11.7 to 28% patients with NSCLC (Giovanella et al., 1997). Earlier studies on SCLC suggest that NSE was most sensitive and valuable biomarker in the management of SCLC (Jorgensen et al., 1989). Further more, NSE may be a useful tool in the estimation of disease extent. Moreover another study with both SCLC and NSCLC reported higher positivity of NSE, around 88%, in SCLC patients than NSCLC (20%) patients (Spinazzi et al., 1994). In present study as well, positivity of NSE was observed to be more in SCLC than in NSCLC groups of patients. Thus, NSE appears to be the tumor marker with greatest specificity and sensitivity for SCLC (Fischbach and Jany, 1986). Furthermore, post-chemotherapy level of NSE in SCLC is a strong independent predictor of both complete response to therapy and survival (Fizazi et al., 1998) as well as disease free-survival (Wojcik et al., 2008). In addition, our study also showed that NSE concentration was significantly increased in all stages of not only SCLC but stage IV of NSCLC also. However, similar to the findings of earlier study (Kulpa et al., 2002), no significant difference was found in the increased concentrations of NSE and the dependence on disease stage, especially stage II and IIIB of SCLC and stage IV of NSCLC. Nonetheless, NSE levels were found to be consistent with clinical findings in SCLC, based on imaging techniques (Ebert et al., 2002). Moreover, it was suggested that measurement of serum NSE provides discrimination between NSCLC and SCLC (Satoh et al., 2002), however such was not the case in our study, as non-significant difference was noted in NSE levels of stages II-IIIB of SCLC and stage IV of NSCLC. Supporting our findings, sensitivity of only 22% was noted for
NSE in patients with NSCLC, whereas cytokeratin 21 was suggested as tumor marker with 76% sensitivity (Molina et al., 2003). In addition, where no discrimination of SCLC and NSCLC was performed or documented, NSE level estimation becomes more un-useful (Kalomenidis et al., 2004).

Comparable sensitivity of 74.5% for NSE level was suggested in SCLC patients when tested against a new tumor marker pro GRP (Schneider et al., 2003a,b). However, pro GRP and not NSE were recommended as tumor marker of choice for discriminating SCLC and NSCLC. Moreover, in contrast, an independent study in 448 NSCLC patients concluded that serum assay of NSE is a useful marker also in NSCLC and a significant predictor of survival, independently of other prognostic factor (Ferrigno et al., 2003). Furthermore it was reported that one and two year survival rate was higher in 28.5% NSCLC patients that exhibited more than 50% of NSE positive cancer cells (Petrovic et al., 2008).

In present study, diagnostic sensitivity of NSE was 86% with 67% specificity and its concentration did not show dependence on disease stage I with comparatively low values and stage II and IIIB of SCLC and stage IV of NSCLC. Similar to our finding, it has been reported that concentration of NSE did not show dependence on disease stageI where as van-Zandwijk et al (1992) reported that high pre-treatment concentration of NSE were associated with shorter survival. However, our findings show that a very high significant difference was present in NSE level at stage IV of SCLC in comparison with all stages of SCLC and stage IV of NSCLC. It was reported that NSE seems to be an independent prognostic factor in the patients with stage IIIB-IV, which may be treated with chemotherapy (Kulpa et al., 2002). Expression of NSE in both SCLC and NSCLC, and at somewhat similar concentration especially in stages II-IIIB of SCLC and stage IV of NSCLC represent that both NSCLC and SCLC are derived from a common cell lineage and their differentiation occur at a later stage of oncogenic development (Broers et al., 1985). It was also reported that NSE expression is an unfavorable sign because squamous cell carcinoma with neuroendocrine differentiations are more aggressive than others (Kulpa et al., 2002). Several studies also suggested that samples other than serum for NSE analysis such as cytosol, sputum and pleural effusion (Kalomenidis et al., 2004; Ruibal et al., 2003; Tada et al., 2002).

CONCLUSION

The result of our study revealed that NSE exhibits higher levels in some stage of SCLC showing its specificity, not only for advanced stage of SCLC but also for SCLC in general as compared to NSCLC. This suggests that NSE is a useful tumor marker for SCLC and but to a lesser extent for NSCLC. Therefore its determination is beneficial in the diagnosis, treatment and a possible follow-up for SCLC and NSCLC patients’ survival.

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