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A comparative analysis of serum ferritin levels in advanced non-small cell lung cancer patients at IRD, SMS Medical College, Jaipur

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Abstract

Introduction: Primary lung cancer is the most common cancer in human. It is estimated that about 1 million people die of cancer every year. Non-small cell lung carcinoma (NSCLC) accounts for 80-85% of all lung carcinomas. Serum ferritin (SF) in humans act as a buffer against iron deficiency and iron overload. Iron also has a role in tumor microenvironment and in metastasis. Serum ferritin is highly expressed in tumor tissues and patients with NSCLC.

Aim and objectives: Our study aimed to compare the levels of serum Ferritin in advanced NSCLC patients and healthy subjects reporting to Institute of Respiratory disease, SMS Medical College Jaipur. We further assessed the correlation between SF expression levels with the efficacy of platinum-based therapies in advanced NSCLC patients at baseline, after 3rd and 6th cycle of chemotherapy (CT).

Materials and Methods: It was a hospital based prospective study conducted on 50 non-small cell lung cancer patients (cases) and 50 healthy subjects (controls). Electrochemiluminescence method was used to determine the expression levels of SF in the peripheral blood. Statistical analysis was performed using Student's t-test and chi-square test. A p-value <0.05 was considered statistically significant.

Results: The expression levels of SF were significantly higher in advanced NSCLC patients than those in healthy subjects ($t = 5.8217$, $p < 0.0001$). Correlation of SF levels and patient alive status upto 1 year was found to be statistically significant ($p < 0.05$). However, there was no correlation between SF expression levels and sex, age, eastern cooperative oncology group performance status, smoking history, body mass index, pathological type and metastasis (All $p > 0.05$). Out of NSCLC patients, the overall response rate (ORR) to platinum-based chemotherapy after 3rd CT and 6th CT

was 79.16% (19/24) and 64.28% (18/28) respectively in normal SF expression levels group, which was significantly higher than that was 38.46% (10/26) and 26.31% (5/19) respectively in high SF expression levels group ($\chi2$ = 8.489, p = 0.0036, $\chi2$ = 6.531, p = 0.0106 respectively).

Conclusions: SF may be a valuable blood marker for predicting the tumor prognosis and the efficacy of platinum-based therapies for advanced NSCLC patients.

Keywords: Advanced non-small cell lung cancer, efficacy, prognosis, serum ferritin

Introduction

Primary lung cancer is the most common cancer in humans.[1] The predicted incidence of lung cancer is a million new cases all over the world every year, of which most (over 80%) have a diagnosis of non small cell lung cancer (NSCLC).[1] 60% to 70% of advanced NSCLC patients are diagnosed for III-IV stage and only managed by chemotherapy or radiotherapy or both. Studies have confirmed that platinum-based chemotherapy is the current first-line standards of care for stage III-IV NSCLC, but the efficacy was only 25-30%, and 2-year survival rate is less than 15%.[1] Therefore, there is a crucial need to explore novel tumor markers for predicting the progression of advanced NSCLC as well as helping to establish treatment strategies.

Serum Ferritin (SF), a glycoprotein, consists of inorganic iron compound and the primary intracellular iron storage protein in both prokaryotes and eukaryotes, keeps iron in a soluble and non-toxic form. In humans, it acts as a buffer against iron deficiency and iron overload. Studies have suggested that there was abundance of SF in tumor cells, and increasing SF expression levels can help diagnose malignant tumors.[2-5] However, the clinical significance of SF in advanced NSCLC has not been well investigated.

In this study, we examined SF expression levels in blood specimens from patients with advanced NSCLC and healthy subjects using the electrochemiluminescence method. Additionally, we investigated the correlations between SF expression levels and clinicopathological parameters as well as subsequently evaluated whether or not it can be used to predict the efficacy of platinum-based therapies in advanced NSCLC patients.

Methodology

It was a prospective, case control study conducted on 50 patients of non-small cell lung cancer and 50 healthy subjects over a period of 3 years at Institute of Respiratory diseases, SMS Medical College, Jaipur. Biopsy Proven NSCLC patients according to Tumor Node Metastasis (TNM) classification of the international union against cancer 2017, Edition 8th (TNM stage 3 and 4), patients age more than 18 years with Eastern cooperative oncology group (ECOG) performance status (PS) of 0-2 and with normal renal and hepatic function and patients with written informed consent were included in our study while Patients who had any other Malignancy, history of previous chemotherapy for Malignancy, history of any other disorders affecting Ferritin metabolism and history of recent treatment with iron therapy were excluded from our study.

Approval from ethics and research review board of SMS Medical College (No.175/MC/EC/2020) was obtained. Pre chemotherapy evaluation was done (hemoglobin >9 g/dl, neutrophil count >1.5 × 10^9 /l, platelet count ≥ 100 × 10^9 /l, bilirubin <1.5 times the normal upper limit, aspartate aminotransferase and alanine aminotransferase <2.5 times the normal upper

limit, creatinine clearance rate >50 ml/s and normal electrocardiogram). After prechemotherapy evaluation, all patients were treated with carboplatin based first-line chemotherapy regimens and assessed for tumor response after the end of 3rd and 6th cycle of chemotherapy. Response was defined as complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) according to The Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The ORR was defined as the sum of CR and PR rates.

In all patients, a 5 ml venous blood sample was collected in a plain vial at diagnosis prior to treatment. Similarly, blood samples were also obtained from 50 healthy subjects. Collected samples were kept at room temperature for a minimum of 30 min (and a maximum of 60 min), and serum samples were obtained after centrifugation at 4000 rpm at 4°C for 10 min from peripheral venous blood and immediately stored at −80° C until use. The expression levels of SF in blood specimens from patients with advanced NSCLC and healthy subjects were assayed using electro chemiluminescence method, and the reagent kit was from Roche Co., Ltd. (Germany). According to the manufacturer's instructions, the normal range of the SF was identified at 22-322 ng/ml for men and 10-291 ng/ml for women. Subsequently, patients were divided into two groups with normal (22-322 ng/ml in men and 10-291 ng/ml in women) and high (>322 ng/ml in men and >291 ng/ml in women) SF expression levels groups.

Student's t-test and Chi-square test were used to compare the difference for mean or rate between two or more than two groups.

Results

The expression levels of SF were significantly higher in patients with advanced NSCLC (402.49±285.80 ng/ml) than those in healthy subjects (162.55±60.66 ng/ml) ($t = 5.8217$, $p < 0.0001$) [Table 1]. All controls had SF levels within normal range while 27 patients had SF levels within normal range and 23 patients had high SF levels [Table 2].

Table 1: Baseline Comparison of Serum Ferritin levels between cases and controls

Serum Ferritin level (ng/ml)	Case Group (N=50)	Control Group (N=50)	t-test value	p- value
Mean ± SD	402.49 ±285.80	162.55 ±60.66	5.8217	<0.0001

Table 2: Baseline Characteristics of cases and controls

Study Population	Serum Ferritin Level (ng/ml)	
	Normal	High
Controls	50	0
Cases	27	23

After receiving 3 cycles of platinum-based chemotherapy, 24 patients (48%) had SF expression levels within the normal range, whereas 26 patients (52%) had higher SF expression values. 47 patients received 6 cycles of platinum-based chemotherapy whereas 3 patients died, 28 patients (59.57%) had SF expression levels within the normal range, whereas 19 patients (40.42%) had higher SF expression values. As seen in Table 3, correlation of SF expression levels and patients alive status up to 1 year was found to be statistically significant ($\chi 2 = 6.48$, $p = 0.0022$)

Among cases, we compared the overall response rate (ORR) between normal and high SF expression levels groups after patients received 3 and 6 cycles of platinum-based chemotherapy. Our study showed that, after receiving 3 cycles of CT, the ORR was 79.16% (19/24) in patients with normal SF expression levels

group, which was significantly higher than that was 38.46% (10/26) in high SF expression levels group ($\chi 2$ = 8.489, p = 0.0036) and after receiving 6 cycles of CT, the ORR was 64.28% (18/28) in patients with normal SF expression levels group, which was significantly higher than that was 26.31% (5/19) in high SF expression levels group. ($\chi 2$ = 6.531, p = 0.0106). [Table 4 and 5]

Using 322 ng/ml as cut-off value as per previous studies, we determined the sensitivity and specificity of serum ferritin for lung cancer using ROC curve. The value of sensitivity is 73.68% and specificity is around 82%. Therefore, it can be interpretated that there is 73.68% chance that patients will be tested positive for malignancy if SF value is more than 322 ng/ml. Similarly, 82% specificity indicated that there is 18% chance of patients to be tested negative for malignancy if SF value is more than 322 ng/ml. Hence, serum Ferritin is found to have good accuracy with respect to outcome. [Figure 1]

Table 3: Correlation of SF expression levels and patients alive status up to 1 year

Alive status	Case group (N=50)	Serum Ferritin Level (ng/ml)		Chi square Test value	p-value
		Normal	High		
Yes	28	21	7	9.3233	0.0022
No	22	7	15		

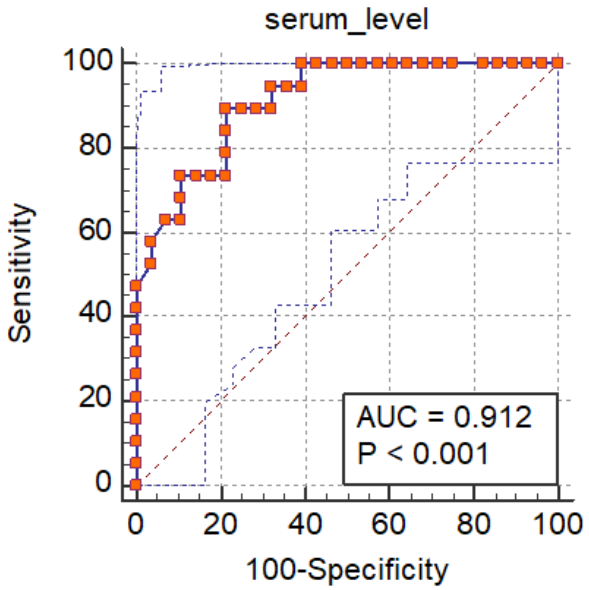
Table 4: Efficacy of platinum-based chemotherapy of advanced NSCLC patients in normal and high Serum Ferritin (SF) expression levels group after 3rd cycle

Group	CR+PR (%)	SD+PD (%)	Total	Chi-square test	p-value
Normal	19 (79.16%)	5 (20.83%)	24	8.489	0.0036
High	10 (38.46%)	16 (61.53%)	26		

Table 5: Efficacy of platinum-based chemotherapy of advanced NSCLC patients in normal and high SF expression levels group after 6th cycle

Group	CR+PR (%)	SD+PD (%)	Total	Chi-square test	p-value
Normal	18 (64.28%)	10 (35.72%)	28	6.531	0.0106
High	5 (26.31%)	14 (73.69%)	19		

Fig 1: AUROC curve for serum ferritin level



Discussion

Ferritin is a 24-subunit protein that is composed of two types of subunits, termed H and L, which is a protein of crucial importance for human iron metabolism, which can be synthesised in most cells of the human body.[6,7] Stites et al.[8] mentioned that the source of ferritin in airways in conditions such as inflammation is postulated as stemming from the transudation of serum iron into airways. Ferritin was proved to be measurable and present in high concentrations in exhaled breath condensate (EBC) of lung diseases such as asthma and cystic fibrosis, however seldom has been studied in EBC of patients with lung cancer.[9]

Our study showed that the mean age was 61.3 years among cases and 60.91 years in control group. Our

study showed that Adenocarcinoma was the most common (58%) pathological type of lung cancer in both male patients and female patients followed by Squamous cell carcinoma (40%) & Large cell carcinoma (2%).

SF is non-specifically elevated in some conditions, including chronic disease, inflammation, and malignancy.[2,10] It has been reported that SF expression levels are higher in many malignancies, such as renal cancer,[11] colorectal cancer,[12] lung cancer,[3] prostatic cancer.[13] The presence of high SF expression levels in cancer patients may be related to a multi factorial mechanism, such as enhanced growth and proliferation with increased necrosis and lysis of tumor cells and release of ferritin, malignant tumors causing ferric ions to accumulate in cells of the reticuloendothelial system, resulting in ferritin synthesis.[12] Our study showed that the expression levels of SF were significantly higher in patients with advanced NSCLC (402.49±285.80 ng/ml) than those in healthy subjects (162.55±60.66 ng/ml) ($t=5.8217$, $p<0.0001$). Therefore, the elevated SF expression levels may be a common phenomenon in cancer patients.

Singh[4] measured the expression levels of SF in 32 patients with untreated renal cell carcinoma and 32 normal controls, and found that the mean concentration of SF in cancer patients was significantly higher than that in healthy controls. In addition, the higher expression levels of SF were associated with advanced cancer stages and grades. Lorenzi[12] analyzed the relationship between preoperative SF levels and clinicopathological parameters of patients with colorectal cancer and concluded that patients with metastasis had higher SF expression levels (but not significantly) compared to the group without secondary lesions. Kakari et al.[14] reported significantly higher SF expression levels in patients with extensive than with limited disease in patients with SCLC.

Several studies[3,12] showed that SF expression levels were associated with the survival of patients with advanced colorectal cancer and lung cancer. However, no previous study has tested the relationship of SF expression levels with the efficacy of platinum-based chemotherapy for advanced NSCLC. Our study showed that the ORR was 79.16% in normal SF expression levels group, which was significantly higher than that of 38.46% in high SF expression levels group ($\chi2 = 8.489$, $p = 0.0036$) after 3rd CT cycle. Shi H et al.[1] in 2014 concluded that the ORR between normal and high SF expression levels groups according to the result of computed tomography scanning after patients received two cycles of platinum-based chemotherapy was 57.1% in normal SF expression levels group, which was significantly higher than that of 28% in high SF expression levels group ($\chi2 = 3.998$, $p = 0.046$). Our study also showed that the ORR was 64.28% in normal SF expression levels group, which was significantly higher than that of 26.31% in high SF expression levels group ($\chi2 = 6.531$, $p = 0.0106$) after 6th CT cycle. Nevertheless, the findings need to be investigated further in a larger number of patients at 3rd & 6th CT cycles, in order to assess the value of this relationship.

Our study reveals that there exists a clinical relationship between the expression levels of SF and the tumor progression as well as the efficacy of platinum-based therapies for advanced NSCLC patients. However, it is necessary to perform progressive studies with larger samples for the internal mechanisms.

Conclusion

Early detection of lung carcinoma could change the disease outcome. Hence, the survival rate can increase dramatically. In the effort to improve early detection,

Ferritin is an interesting inflammatory marker studied recently in lung cancer. Our results suggest that the SF expression levels of healthy subjects were significantly lower than those in patients with advanced NSCLC. Using the cut off value of 322 for serum Ferritin, the sensitivity was 73.68% and specificity was 82%. Hence, SF test is found to have good accuracy with respect to outcome. We conclude that estimating serum ferritin level in advanced NSCLC patients is a non-invasive and inexpensive method. SF may be a valuable blood marker for predicting the tumor progression and the efficacy of platinum based therapies for advanced NSCLC patients.

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