RESEARCH ARTICLE

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Investigation of Serum Folate-Receptor-1 in Patients with Non-Small Cell Lung Cancer

ABSTRACT

Objective: Histopathological overexpression of folate receptor-1(FOLR1) involved in folate transport in cell growth has been reported in various cancers. Increased serum FOLR1 (sFOLR1) has also been reported in epithelial ovarian cancer. The aim was to investigate sFOLR1 levels in non-small cell lung cancer(NSCLC) patients and the response prediction of the standard chemotherapy targeting folic acid metabolism.

Methods: In this prospective study, sFOLR1 levels were investigated in 30 healthy individuals and 60 patients with stage4 malign metastatic NSCLC before and after standard chemotherapy. The commercial immunoassay(ELISA) kit was used for the analysis of sFOLR1. Serum carcinoembryonic antigen(CEA), vitamin B12, and folate levels were also investigated.

Results: In NSCLC patients sFOLR1 levels were significantly higher($p<0.001$) than the healthy individuals. After 3 months of standard treatment, sFOLR1 was significantly lower than pre-treatment values in NSCLC patients($p<0.001$). Diagnostic accuracy was strong in the differentiation of NSCLC patients from healthy individuals(AUC= 0.966). with the cut-off point of 82.45 pg/ml, the sFOLR1 level was performed with 95% sensitivity and 99% specificity. Pretreatment sFOLR1 levels were significantly lower in patients with-response to standard chemotherapy($p<0.01$). The best predictive value was determined as 393.80 pg/ml. At the end of the 401 days, a significant difference was found in patients with high sFOLR1 predictive value. The median overall survival(OS) duration was 288 days for all patients (95% GA 198.13-377.87). Median progression-free survival(PFS) was 321 days(95% GA 211.90-430.10).

Conclusions: For monitoring standard chemotherapy with drugs targeting folic acid metabolism, sFOLR-1 levels may be an important biomarker.

Keywords: Folate receptor 1 (FOLR1), Carcinoembryonic Antigen (CEA), Non-Small Cell Lung Cancer (NSCLC), Chemotherapy, Biomarker.

Küçük Hücreli Dışı Akciğer Kanserli Hastalarda Serum Folat-Reseptör-1 Düzeylerinin Araştırılması

ÖZET

Amaç: Hücre büyümesinde folat taşınmasında rol oynayan folat reseptörü-1'in (FOLR1) histopatolojik aşırı ekspresyonu çeşitli kanserlerde bildirilmiştir. Artmış serum FOLR1(sFOLR1) epitelyal yumurtalık kanserinde de rapor edilmiştir. Amaç, küçük hücreli dışı akciğer kanseri (KHDAK) hastalarında sFOLR1 düzeylerini ve folik asit metabolizmasını hedefleyen standart kemoterapinin tahminini yanıtını araştırmaktı.

Gereç ve Yöntem: Bu prospektif çalışmada, standart kemoterapi öncesi ve sonrası evre4 malign metastatik KHDAK'li 60 hasta ve 30 sağlıklı bireyde sFOLR1 düzeyleri araştırıldı. Ticari immünolojik test (ELISA) kiti sFOLR1'in analizi için kullanıldı. Serum karsinoembriyonik antijen (CEA), vitamin B12 ve folat düzeyleri de araştırıldı.

Bulgular: KHDAK hastalarında sFOLR1 seviyeleri sağlıklı bireylere göre anlamlı derecede yüksekti($p<0,001$). 3 aylık standart tedaviden sonra hastalarda sFOLR1 anlamlı olarak daha düşüktü ($p<0,001$). KHDAK hastalarının sağlıklı bireylerden ayırt edilmesinde tanısal doğruluk güçlüydü (AUC= 0.966). Tanısal doğruluk sFOLR1 seviyesi 82.45 pg/ml kesme noktasında %95 duyarlılık ve %99 özgüllük gerçekleştirmiştir. Standart kemoterapiye yanıt veren hastalarda tedavi öncesi sFOLR1 düzeyleri anlamlı olarak daha düşüktü($p<0.01$). En iyi tahmin değeri 393.80 pg/ml olarak belirlendi. 401 günün sonunda sFOLR1 tahmin değeri yüksek olan hastalarda anlamlı fark bulundu. Medyan genel sağkalım (OS) süresi tüm hastalar için 288 gündü (%95 GA 198.13-377.87). Medyan progresyonsuz sağkalım (PFS) 321 gündü (%95 GA 211.90-430.10).

Sonuç: Folik asit metabolizmasını hedefleyen ilaçlarla standart kemoterapiyi izlemek için sFOLR-1 seviyeleri önemli bir biyobelirteç olabilir.

Anahtar Kelimeler: Folat Reseptörü 1 (FOLR1), Karsinoembriyonik Antijen (CEA), Küçük Hücreli Olmayan Akciğer Kanseri (KHDAK), Kemoterapi, Biyobelirteç.

INTRODUCTION

The most common cause of cancer-related death is lung cancer (LC) (1) and approximately 80% of LCs are NSCLC (2). Although molecular targeted treatment research is intensive, chemotherapy is still a treatment option for patients with advanced NSCLC (3). Platinum-based doublet, usually cisplatin or carboplatin, is the standard treatment for advanced NSCLC (4).

Folate receptor-1 is a glycosylphosphatidylinositol-associated glycoprotein binding to folic acid and its derivatives with strong affinity. FOLR1 mediates the transport of folate through receptor-mediated endocytosis. Histopathological overexpressed FOLR1 in various solid tumors such as breast, ovarian, pancreatic, kidney, and lung cancer, especially NSCLC and high-grade osteosarcoma, was caused by the increased metabolic needs of folates to feed nucleic acid synthesis and cellular growth (5–11). FOLR1 could be transferred from the localized cell surface to the bloodstream as a soluble form of sFOLR1(12,13). In patients with malignant epithelial ovarian cancers to distinguish them from benign patients and healthy subjects, sFOLR1 has been reported as a potential biomarker (6).

Biomarkers for differential diagnosis, prognosis, or follow-up of lung cancer are quite limited. The expression of CEA in pulmonary adenocarcinoma and lymph node metastasis was higher than in other types of NSCLC (14). Therefore, only CEA levels were used to detect the efficacy of chemotherapy and early relapses in NSCLC. Nevertheless, CEA was not effective in identifying an early-stage disease or differential diagnosis (14).

There is a need for good predictive markers for the clinic evaluation of NSCLC. It would be useful to define laboratory tests for diagnosis and prognosis. The purpose of our study was to define sFOLR1 levels and evaluate its use in follow-up of NSCLC patients.

MATERIAL AND METHODS

Sixty (60) patients with metastatic stage4 NSCLC and 30 healthy individuals as a control group were included in this prospective study at the medical oncology clinic of the tertiary research hospital. The local institutional review board approved the project and this study conformed to the provisions of the 1995 Helsinki Declaration. All participants provided written informed consent before sample collection. This study adheres to the REMARK guidelines (15,16).

Demographic characteristics of participants (age, gender, height, weight, smoking, alcohol, diabetes mellitus, hypertension) were investigated (Table 1). Standard chemotherapy could be a choice in metastases such as bone or liver of NSCLC. Combinations of doublet chemotherapy drugs such as cisplatin, carboplatin, paclitaxel, etoposide, and pemetrexed or with a single chemotherapy drug to treat especially for people with poor overall health or who cannot tolerate combination chemotherapy well, such as the elderly could often constitute (17).

Table 1. Characteristics of study participants.

	Healthy (n:30) Mean ±SD/ Median (min-max)	**NSCLC patients Pre-treatment (n:60)** Mean ±SD/ median (min-max)	**NSCLC patients Post-treatment(n:60)** Mean ±SD/ median (min-max)
Demographic Data	**n (%)**	**n (%)**	
Smoking (current)	0	50 (83.3%)	
Drinking (current)	0	20 (33.5%)	
Diabetes mellitus	0	12 (20%)	
Hypertension	0	25 (41.6%)	
Age(year)	57.3±12.06	60.38±6.28 (p=0.295)	
BMI	26.94 ±0.64	25.46 ±0.43 (p=0.210)	
Laboratory data			
Vitamin B12 (pg/mL)	502.8(348-687)	**383.36 (103.2-893)[a]**	
Folate (ng/mL)	13.65(5.9-19.6)	**3.24(1.3-6.98)[a]**	
CEA (ng/mL)	1.71(1.2-2.81)	**15.59 (1.07-105.9)[a]**	**12.32(1.04-77.91)[b]**
sFOLR1 (pg/mL)	230.50(203.5-346.0)	**518.95 (206.18-1342)[a]**	**325.04(195.2-838.13)[b]**

Statistically significant p values are marked in **bold**. a: between healthy and NSCLC; **b:** between pre-treatment and post-treatment.
BMI: Body mass index; CEA: Carcinoembryonic Antigen; sFOLR1: serum Folate Receptor-1; SD: standard deviation; min: minimum; max: maximum

The response was evaluated with modified Response Evaluation Criteria in Solid Tumors (mRECIST) (18) in all patients with NSCLC three months after standard chemotherapy. The largest diameter measured in primary tumors and the shortest diameter measured in metastatic lymph nodes were evaluated. After treatment, the change in the size of the primary tumor was evaluated for the response. Lesions were grouped into complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) (18,19). According to the treatment response of lesions, patients have been grouped as " with-response" and "without-response"(18). "With-response" patients had lesions with CR and PR, and "without-response" patients had stable (SD) and progressive (PD) lesions.

Laboratory Assessments: Peripheric venous blood samples were collected from patients before treatment and three months after standard

chemotherapy (usually 80 mg/m2 cisplatin). After centrifugation at 3000 rpm for 10 minutes, the sera were stored at -80 ° C until the analysis. CEA, vitamin B12, and folate levels were examined by immunofluorescent method with Cobas e601 analyzer (Roche diagnostics; Geneva, Switzerland). sFOLR1 levels were analyzed with Sun Red Biotechnology Company's Human FOLR1 Elisa kit (Catalog No: SRB-T-87946).

Statistical Assessments: The Kolmogorov-Smirnov test was applied to all groups and the parametric/non-parametric distribution of parameters was figured out. The difference between groups in parameters; student t-test for parametric distribution and Mann-Whitney U for non-parametric distribution were performed. Correlation analysis and the relationships between parameters were evaluated. All statistical analyses were performed with SPSS22.0 (SPSS Inc., Chicago, IL) program and p values less than 0.05 were considered statistically significant. Optimal cut-off and area under the curve (AUC) levels for serum FOLR1 and CEA were figured out using the receiving operator characteristics curve (ROC), for the difference between healthy and NSCLC patient groups and between with-response and without-response groups. At the end of the follow-up period, OS and PFS of higher or lower than cut-off values groups were evaluated with Kaplan Meier analysis.

RESULTS

Demographic data of NSCLC patients and healthy individuals were presented in Table 1. All subjects were male and the age and body mass index (BMI) of patients with metastatic NSCLC were like healthy subjects (p=0.295, p=0.210, respectively). Serum vitamin B12 and folate levels were significantly lower in the patient group (both, p<0.001). Serum CEA and FOLR1 levels were significantly higher in patients (both, p<0.001). After 3 months of treatment in the patient group, serum CEA and FOLR1 levels were significantly lower than pre-treatment levels (both, p<0.001) (Table 1).

The efficacy of CEA and sFOLR1 levels in the separation of NSCLC patients from healthy examined with ROC analysis. The diagnostic competence of both CEA (AUC= 0.949) and sFOLR1 (AUC= 0.966) was strong (AUC>70.0) with 90% sensitivity and 90% specificity for CEA, and 95% sensitivity and 99% specificity for FOLR1. Optimal cut-off values (CEA= 2.11 ng/ml and sFOLR1=282.45 pg/ml) were determined for the difference between patients with NSCLC and healthy groups (Table 2).

Table 2. Area under the curve of parameters at diagnosis and after treatment.

Test Result Variable(s)	Cut-off	Area Under Curve (AUC)	Asymptotic Significance	Asymptotic 95% Confidence Interval		Sensitivity (%)	Spesificity (%)
				Lower Bound	Upper Bound		
CEA1	2,11	0,949	**<0,001**	0,895	1,000	90	90
sFOLR1 (pre-treatment)	282,4	0,966	**<0,001**	0,919	1,000	95	99
sFOLR1 (post-treatment)	393.8	0.870	**<0,001**	0.762	0.977	79	67

According to the standard chemotherapy response, patients were grouped as "with-response" and "without-response". The six patients in the with-response group were CR and the other ten were in the PR group. In the without-response group, twelve patients were SD and the other twelve were PD (Figure 1) with mRECIST criteria. There was no significant difference in pre-treatment CEA levels between the with-response and the without-response groups (p>0.05). On the other hand, pre-treatment sFOLR1 levels were significantly lower in patients with-response than without-response group patients (p<0.01) (Figure 1). The best predictive value was decided as 393.80 pg/ml with 79% sensitivity and 67% specificity in ROC analysis (Figure 2).

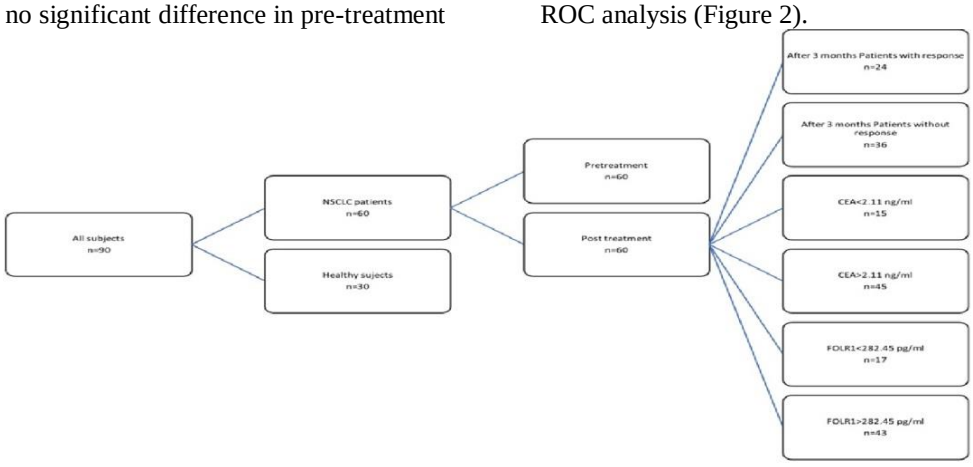

Figure 1. Participants in study groups

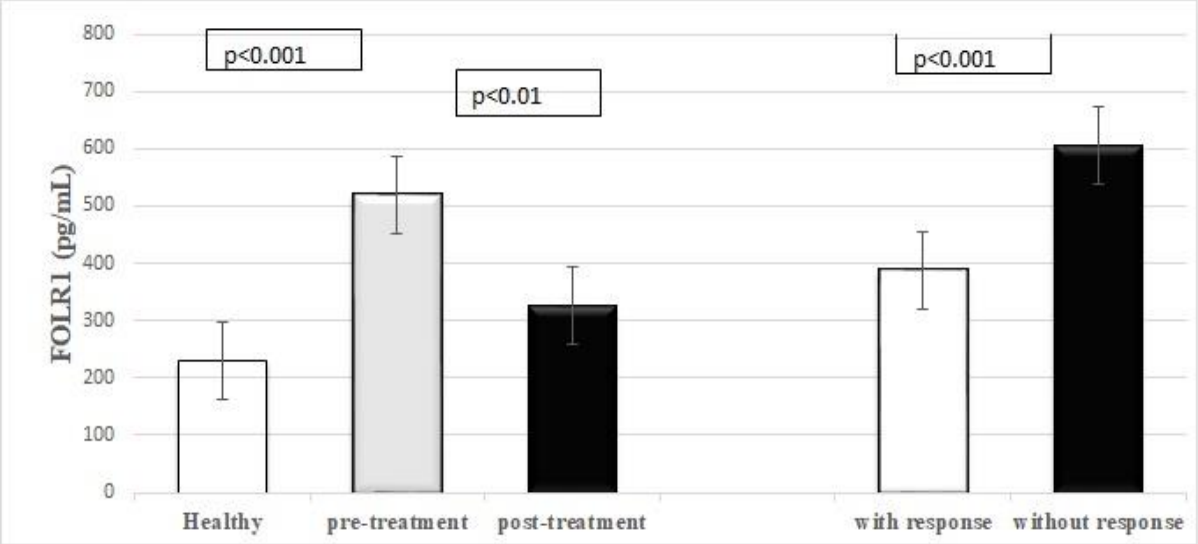

Figure 2. Serum FOLR1 levels of groups.

The average follow-up time was 401 days (range 91-452 days). At the end of the follow-up period, OS and PFS were evaluated with Kaplan-Meier analysis. A significant difference was detected in terms of OS and PFS in patients with pre-treatment FOLR1 levels above the predictive cut-off value. High sFOLR1(≥393.80 pg/ml) levels predicted significantly poor response than low sFOLR1 (<393.80 pg/ml) levels (Figure 3). Those with low sFOLR1 levels (<393.80 pg/ml) predicted good response than those with high (≥393.80 pg/ml) levels. Median OS time was 288 days (95% GA 198.13-377.87) and PFS was 321 days (95% GA 211.90-430.10).

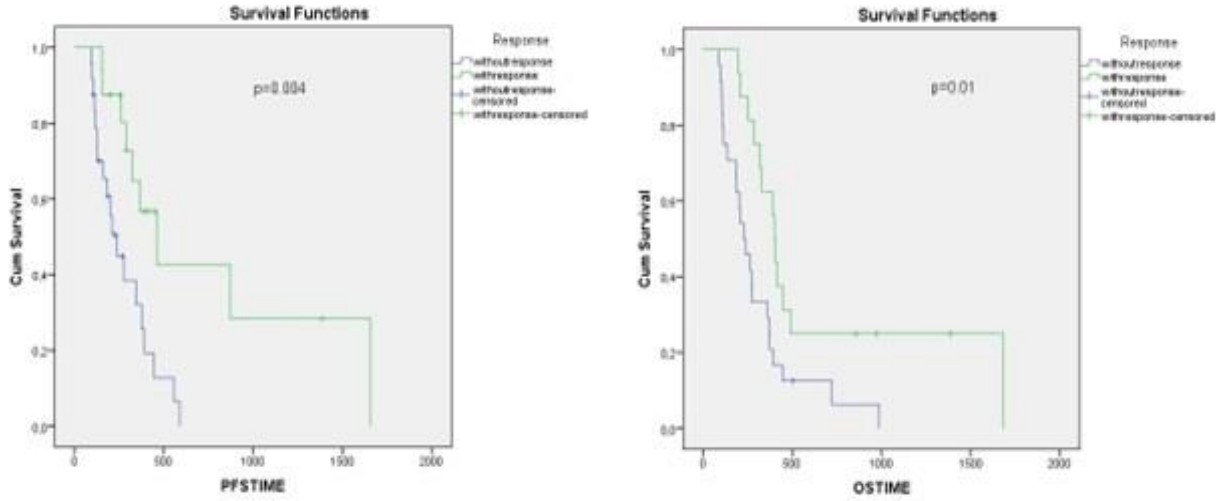

Figure 3. Progression free survival (PFS) and overall survival (OS) of with response and without response groups.

DISCUSSION

Histopathological FOLR1 (FRα gene) mRNA expression has been reported to be significantly higher in cancers such as mesothelioma, lung, pancreas, ovarian, and colorectal cancers (8–11). Over-expression histopathological FRα has been reported in adenocarcinoma compared to squamous cell cancers (7,20). Similarly, membrane carrier FOLR1 and reduced folate carrier-1 proteins were often reported overexpressed in NSCLC patients (3,21). However, there has been no study evaluating the role of sFOLR1 levels as a potential biomarker for lung cancers. In our study, sFOLR1 levels were significantly higher in NSCLC patients than in healthy individuals (Table 1). This result is consistent with the expression results reported in other studies and this study is the first study reporting serum levels. Although there are no studies on sFOLR1 levels in NSCLC; It has been reported as a biomarker for ovarian cancer and has been reported to be significantly higher than healthy controls (12,22). This supports our results in terms

of serum FOLR1 levels that could be used as tumor markers. Therefore, an easy-to-obtain and fast-achievable marker such as serum can provide a great advantage in the follow-up of patients.

Diagnostic efficacy and AUC values were remarkably high in ROC analysis, which evaluated the diagnostic effectiveness of sFOLR1 levels in the patient group (AUC= 0.966). At the highest level of sensitivity and specificity, the optimal cut-off value was 282.45 ng/ml.

On the other hand, CEA has been routinely used as a serum biomarker of lung cancer follow-up. High false-positive rates have been reported in lung cancer due to their low specificity for CEA levels, screening, or early diagnosis followed by traditional markers (12). However, it has proven to be a poor diagnostic indicator of sensitivity and specificity for LC. Thus, additional biomarkers are needed. In this study, the AUC value for CEA(AUC=0.949) was lower than sFOLR1. Therefore, sFOLR1 was a potential candidate to compensate for a lack of biomarkers needed in LC. To support our findings, sFOLR1 levels showed higher specificity and higher sensitivity than CA125 in the detection of epithelial ovarian cancer based on ROC analysis (6). A combined analysis of CEA and sFOLR1 may be useful for the early diagnosis and the treatment response of NSCLC. Additionally, such a combination could improve specificity and prediction of treatment efficacy.

In this study, sFOLR1 levels were significantly decreased 3 months after standard chemotherapy (Table 1). Over the past decades, FOLR1 has attracted much attention in antitumor therapy (23). Like our findings, in ovarian cancer cells, high expression of FOLR1 levels has been reported as a useful therapeutic application to increase sensitivity to cisplatin treatment (23), and reported also that FOLR1 was highly expressed in ovarian cancer but was reduced following multidrug resistance (23). At the same time, FOLR1 has been reported as a potential target for evaluating the response to treatment of human carcinomas with pemetrexed, a thymidylate synthase (TS) inhibitor (24). There have been reports that FOLR1 was highly expressed in NSCLC (3) and FOLR1 expression was associated with the prognosis of patients with NSCLC (21,25). However, to our knowledge, few studies were performed to explore the association between FOLR1 expression and drug resistance in NSCLC. The data we provide here that recommend, sFOLR1 levels were a key marker in monitoring standard chemotherapy treatment of NSCLC. When the patient groups' response to chemotherapy treatment was examined: there was no significant difference in pre-treatment CEA levels between the with-response and without-response groups, while pre-treatment sFOLR1 levels were significantly lower in patients with-response (Figure 1). According to the ROC analysis, in determining patients' good response to treatment: those with sFOLR1 levels below 393.80 pg/ml were the better response.

All patients were monitored for 401 days to assess survival and a significant difference was detected in terms of OS and PFS in patients with above sFOLR1 predictive value. Survival time decreased in those with sFOLR1 levels above 393.80 pg/ml. To support our findings, Kurosaki et al. high sFOLR1 levels in epithelial ovarian tumors predicted shorter PFS (6,24). Similarly, O'Shannessy, et al. (2012) reported shortened survival of those with high histopathological FOLR1 overexpression in patients with pulmonary adenocarcinoma (25). Combined detection of CEA and sFOLR1 may be useful for the early diagnosis and the treatment response of NSCLC. Additionally, such a combination could improve specificity and treatment prediction.

Our study should be interpreted with its limitations. The small sample size was the major limitation. The uncertainty of the factors affecting sFOLR1 levels, and to the best of our knowledge the lack of studies on serum levels in NSCLC patient groups were other limitations. However, the data we provide here suggest that FOLR1 may be a useful predictive biomarker for NSCLC. The results obtained in the NSCLC patients would be valuable for the potential role of sFOLR1 as a candidate biomarker. Our findings on FOLR1 are an important addition to the literature in this field. Further research is warranted to develop better prediction tools in NSCLC.

CONCLUSION

Serum FOLR1 levels were significantly higher in NSCLC patients than in the healthy subjects. Serum FOLR1 levels were significantly lower in patients with-response to standard treatment, and OS and PFS durations were significantly longer in those pretreatment sFOLR1 levels under 393.80 pg/ml. As a result, sFOLR1 levels appear to be a potential biomarker candidate in NCSLC patients' predicting the response to treatment. It will be appropriate to support our findings with data from larger samples.

Clinical Trial Number: 2018.110.08.01 of the Relevant Ethics Committee

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