ORIGINAL ARTICLE

HIF-1α Levels in patients receiving chemoradiotherapy for locally advanced non-small cell lung carcinoma

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SUMMARY

AIM: *To examine the relationship between treatment response and hypoxia-inducible factor-1 alpha (HIF-1α) levels in patients with locally advanced non-small cell lung cancer (NSCLC) who received chemoradiotherapy (CRT).*

METHODS: *Eighty patients with NSCLC were included in the study and treated at Acibadem Mehmet Ali Aydınlar University Medical Faculty. HIF-1 α levels were measured before and after CRT by the enzyme-linked immunosorbent assay (ELISA) method.*

RESULTS: *Patients' stages were as follows; stage IIIA (65%) and stage IIIB (35%). Squamous histology was 45%, adenocarcinoma was 44%, and others were 11%. Chemotherapy and radiotherapy were given concurrently to 80 patients. Forty-five (56%) patients received cisplatin-based chemotherapy, and 35 (44%) received carboplatin-based chemotherapy. Serum HIF-1α levels (42.90 ± 10.55 pg/mL) after CRT were significantly lower than the pretreatment levels (63.10 ± 10.22 pg/mL, p<0.001) in patients with locally advanced NSCLC.*

CONCLUSION: *The results of this study revealed that serum HIF-1α levels decreased after CRT. Decrease of HIF-1α levels after the initiation of CRT may be useful for predicting the efficacy of CRT.*

KEYWORDS: *Carcinoma, Non-Small-Cell Lung. Chemoradiotherapy. Hypoxia-Inducible Factor 1, alpha Subunit.*

INTRODUCTION

Lung cancer is the primary cause of cancer deaths worldwide in both men and women[1]. Non-small cell lung cancer (NSCLC) elucidates most lung cancers (approximately 85%)[2]. Treatment depends on the cell type (small cell versus non-small cell), the patient's overall medical condition, and the tumor stage in lung cancer. Stage III NSCLC comprises a diverse group of patients with dissimilarities in the extent and localization of disease[3]. For most patients with clinically evident N2 disease, the approach is concurrent chemoradiotherapy, using platinum-based chemotherapy plus full-dose radiotherapy (RT)[4].

Lung cancer is characterized by hypoxia and inflammation. Hypoxia-inducible factor-1 alpha (HIF-1α) takes part in the initiation, appraisal, and prognosis of lung cancer, and starts metastasis and angiogenesis by the transcription of multiple genes[5]. HIF-1α is related to the resistance to chemothera-

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peutic agents[6]. HIF-1α plays a role in the formation of lung cancer according to cigarette smoking, and mechanisms that may be effective on this pathway at the cellular level in the prevention of oxidative damage are studied[7,8]. It is thought that an antimetastatic effect can be achieved by acting on this mechanism[9].

In recent years, some studies have suggested that HIF-1α may be prognostic in lung cancer and be associated with tumor aggression[10-12]. It is thought to be related to chemo-resistance in patients with lung cancer[13]. In the literature, it has been shown that hypoxia-persuaded glutamine metabolism is convoluted in drug resistance in lung cancer, and the hypoxia-induced expression of glutamate dehydrogenase (GDH) depends on the upregulation of HIF-1α[14]. This could be reversed by the death domain-associated protein (Daxx), which negatively regulates hypoxia-persuaded cell invasion by inhibiting the HIF-1α/Histone deacetylase 1 (HDAC1) /Slug pathway[15].

Studies have found that hypoxia is the most common feature in the progression of all solid tumors, and thus it has become a central issue in tumor physiology and cancer treatment. Therefore, we wanted to examine the relationship between treatment response and HIF-1α levels in patients with locally advanced lung cancer who received chemoradiotherapy (CRT).

METHODS

The serum samples of 80 NSCLC patients who were referred to the Acibadem Mehmet Ali Aydınlar University Medical Faculty, Department of Medical Oncology, and Pulmonary Diseases from May to November 2018 were obtained. All patients had histologically confirmed NSCLC diagnosis and had not received any treatment within the last six months. The staging was determined according to the American Joint Committee on Cancer (AJCC) and International Union against Cancer (UICC) staging systems. Stage IIIA and IIIB patients were included in the study; all had taken curative CRT. Patients with another malignancy, early and terminal stage disease, any hypoxic disease, such as diabetes and ischemic heart disease, any infection, and who had had surgery within the last six months were excluded.

Detailed clinical history, physical examination, and a series of biochemistry tests were done before the treatment phase. Those with the Eastern Cooperative Oncology Group (ECOG) performance status of 1 or less and appropriate blood chemistry tests received chemotherapy concurrently with radiotherapy. Chemotherapy and radiotherapy were given concurrently to 84 patients, of whom 45 (56%) received cisplatin-based chemotherapy, and 35 (44%) received carboplatin-based chemotherapy. Concurrent CT regimens were cisplatin 50 mg/m^2 on days 1, 8, 29, and 36 with etoposide 50 mg/m^2 on days 1–5 and 29–33; paclitaxel 50 mg/m^2 weekly with carboplatin dose of area under the curve (AUC) 2 and, for non-squamous tumors, only cisplatin 75 mg/m^2 on day 1 with pemetrexed 500 mg/m^2 on day 1, every 21 days for three cycles. All patients (n = 80) received a total radiation dose of at least 60 Gy (range 50–66 Gy) in 2.0 Gy daily fractions.

Blood samples were obtained from patients in the morning after 12 hours of fasting before the initiation of CRT and after one week of treatment completion. Medical histories of the patients were also recorded on the initiation of therapy. Serum samples were stored at −80°C until final analyses were carried out.

Measurement of serum hypoxia-inducible factor-1 (HIF) levels

and Serum HIF-1a levels were measured using the sandwich-enzyme-linked immunosorbent assay method with the Human ELISA kit (Elabscience, Catalog Number: E-EL-H1277, Wuhan, Hubei Province, China). A preliminary experiment was conducted to verify the validity of plasma samples. The coefficients of intra- and inter-assay variation were 4.8 % (n=15) and 6.1 % (n=15), respectively.

Statistical analysis

Statistical analysis was carried out using SPPS 21.0 software (SPSS Inc., Chicago, IL., USA). Continuous variables were categorized using median values as the cut-off point. For group comparison of categorical variables, One-way ANOVA or Chi-square tests were used, and Mann–Whitney U test or Kruskall–Wallis tests were used for the comparison of continuous variables. All statistical tests were carried out two-sided, and a p-value ≤0.05 was considered statistically significant.

RESULTS

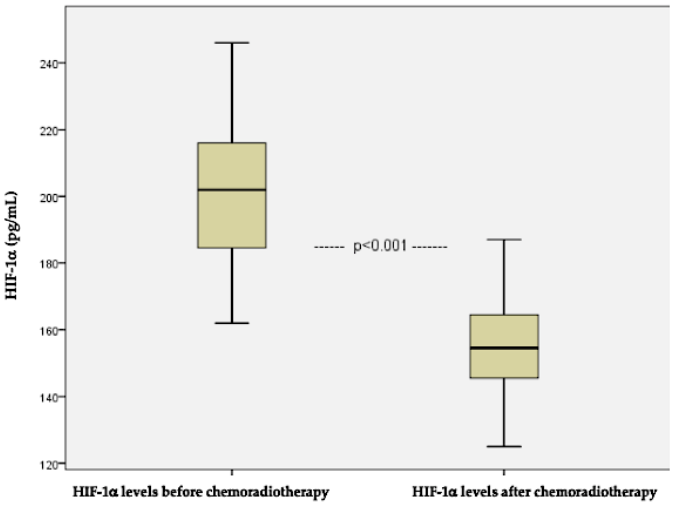
The general data of the 80 NSCLC patients are shown in Table 1. The median age of the patients was 63 (45–79) years. Most of the patients were male

TABLE 1. GENERAL CHARACTERISTICS AND TREATMENT MODALITIES OF NON-SMALL CELL LUNG CANCER (NSCLC) PATIENTS.

Characteristic	No. of cases (%)	Plasma HIF-1α level (pg/mL) (Mean ± SD)	*p*
Gender			
Female	18 (23)	62.56 ± 11.30	>0.05
Male	62 (77)	63.42 ± 9.76	
PS			
0	45 (56)	66.27 ± 9.69	>0.05
1	31 (39)	63.09 ± 10.38	
2	4 (5)	61.75 ± 0.83	
cStage			
IIIA	52 (65)	63.00 ± 9.72	>0.05
IIIB	28 (35)	63.38 ± 10.40	
Histopathology			
Adenocarcinoma	35 (44)	61.25±9,12	>0.05
SqCC	36 (45)	67.83±4.81	
Other	9 (11)	63.00±11.21	

NSCLC = Non-small cell lung cancer; PS = performance status; ECOG-PS = Eastern Cooperative Oncology Group PS; SqCC = squamous cell carcinoma; cStage = clinical stage; HIF-1α = hypoxia-inducible factor-1 alpha.

FIGURE 1. CHANGES OF SERUM HYPOXIA-INDUCIBLE FACTOR-1 ALPHA (HIF-1A) LEVELS BEFORE AND AFTER CHEMORADIOTHERAPY IN PATIENTS WITH LOCALLY ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC).



(77%). Patients' stages were as follows: stage IIIA (35%) and stage IIIB (65%). Squamous histology was 45%, adenocarcinoma was 44%, and others were 11%.

The changes in serum HIF-1α levels before and after CRT in patients with locally advanced NSCLC are shown in Fig. 1. Serum HIF-1α levels (42.90 ± 10.55 pg/mL) after CRT were significantly lower than the pretreatment levels (63.10 ± 10.22 pg/mL, $p < 0.001$) in patients with locally advanced NSCLC.

DISCUSSION

Lung cancer is a highly lethal cancer worldwide. Although different treatments are available, the survival of patients is still poor. Oxygen supply is necessary for the growth of cells and is often diminished, especially inside the tumor mass in solid tumors. Tumor hypoxia contributes to radiotherapy- and chemotherapy-resistance of cancer cells, thus predicting an aggressive behavior by promoting neoangiogenesis[16]. The current study showed that serum HIF-1α levels in patients with locally advanced NSCLC decreased significantly after CRT.

HIF-1 and HIF-2 coordinate the cellular response to hypoxia and are essential nuclear transcription factors for solid tumor growth and survival. When there is hypoxia, HIF-1α heterodimerizes with the HIF-1β subunit within the tumor nucleus and binds to the hypoxia-response element (HRE). This way, HIF-1 stimulates several genes, such as erythropoietin or vascular endothelial growth factors (VEGFs), that are involved in angiogenesis, migration, and survival[17,18]. These genes are found to be explicated in tumor cells and are also involved in tumor progression[19]. Tumor and endothelial cell-specific HIF1α are found to have conflicting roles in thrombosis of cancer patients[20]. Furthermore, during lung injury due to sodium nitrite, antioxidants reverse this injury by downregulating HIF-1α[21].

In cervical and oropharyngeal carcinoma patients who are treated with radiotherapy, HIF-1a overexpression has been associated with poor outcome[22,23]. Hypoxia-induced resistance is multiplex. HIF-1 plays an important role in the conversion of cells into the hypoxic conditions, which precisely brings about the chemo-resistance of tumors[24-33]. Patients with shorter survival in early staged cancers are associated with overexpression of HIF-1α[34]. Cisplatin and doxorubicin are the drugs for which hypoxia-induced drug resistance has been reported for lung cancer[26]. Furthermore, it has been shown that multidrug resistance in colon cancer can be reversed by HIF-1 inhibition[35], and when compared with wild-types, HIF-1α knockout cells are more sensitive to cytostatic and irradiation[36].

Cancer stem cells (CSC) are thought as drivers of tumor growth and are responsible for unresponsiveness to therapy, recurrence, and metastasis. In hypoxia, CSCs are shown to be regulated by HIF-1α and HIF-2α for survival and protection of tumor growth[37]. The expression of a CSC marker which is called CD133, in both small cell lung cancer (SCLC)

and NSCLC cells, was correlated with the hypoxia-induced up-regulation of HIF-1α[38]. Moreover, Hu et al.[39] have tested F-fluoroerythronitroimidazole PET/CT to evaluate the prognosis in NSCLC patients as an assessment for tumor hypoxia. A clinical study showed a decline in HIF-1 protein and mRNA levels in some of its target genes in tumor cells[40]. Additionally, Kummar et al.[41] proved that a chemotherapy drug called topotecan decreases the expression of HIF-1α and some HIF-1 genes in different solid tumors.

Furthermore, Zonta et al.[42] indicated that the regulatory signaling of melatonin is mediated via its receptor MT1, suggesting melatonin as an adjuvant strategy against angiogenesis in ovarian cancer (OC). Regarding reproductive cancers, overexpression of HIF-1a has also been linked to poor prognosis in OC, and treatment with melatonin reduced the levels of HIF-1α, VEGF, and VEGF receptor (VEGFR2).

He et al.[43] have shown that the plasma HIF-1α levels in NSCLC patients are higher than in healthy volunteers. The current research and this study all found that the plasma levels of HIF-1α in NSCLC patients were higher than those of healthy people[43]. The reason for this might be that tumor tissues with high HIF-1α protein expression experienced tissue necrosis, which resulted in a huge amount of HIF-1α entering the bloodstream, or that there was a special regulation mechanism in the hematological system of NSCLC patients, such as CSCs[43-49].

Since hypoxia is closely associated with chemo- and radio-resistance, we investigated HIF-1a levels during CRT in patients with stage III NSCLC. We found that the levels of HIF-1a decrease during CRT. If these levels start to increase after CRT, following hypoxia and tumor progression, this means that HIF-1a levels can be used to detect tumor progression and metastasis. Decreased HIF-1α levels after the start of CRT may also be useful for predicting the efficacy of CRT. New hypotheses can be produced, and future studies are needed to prove this theory.

Conflict of interest: All authors declare no conflict of interest.

RESUMO

OBJETIVO: *Examinar a relação entre a resposta ao tratamento e os níveis de fator 1 induzida por hipóxia (HIF-1α) em pacientes com câncer de pulmão de células não pequenas localmente avançado (NSCLC) que receberam quimiorradioterapia (CRT).*

MÉTODO: *Oitenta pacientes com NSCLC foram incluídos no estudo e foram tratados na Faculdade de Medicina da Acibadem Mehmet Ali Aydınlar University. O nível de HIF-1α foi medido antes e depois da TRC pelo método de ensaio imunoenzimático (ELISA).*

RESULTADOS: *Os estágios dos pacientes foram os seguintes; estágio IIIA (65%) e estágio IIIB (35%). A histologia escamosa foi de 45%, o adenocarcinoma de 44% e o outro de 11%. Quimioterapia e radioterapia foram dadas simultaneamente a 80 pacientes. Quarenta e cinco (56%) pacientes receberam quimioterapia à base de cisplatina e 35 (44%) receberam quimioterapia à base de carboplatina. Os níveis séricos de HIF-1α (42,90 ± 10,55 pg / mL) após a TRC foram significativamente menores do que os níveis pré-tratamento (63,10 ± 10,22 pg / mL, p <0,001) em pacientes com NSCLC localmente avançado.*

CONCLUSÃO: *Os resultados deste estudo revelaram que os níveis séricos de HIF-1α diminuíram após a TRC. A diminuição dos níveis de HIF-1α após o início da TRC pode ser útil para prever a eficácia da TRC.*

PALAVRAS-CHAVE: *Carcinoma Pulmonar de Células não Pequenas. Quimiorradioterapia. Subunidade alfa do Fator 1 Induzível por Hipóxia.*

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