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Original Article

Prognostic Significance of ERCC1 Expression in Resected Non Small Cell Lung Carcinoma

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Background and Aim: The effects of DNA repair pathway on survival were assessed by measuring the tumoral excision repair cross complementation 1 (ERCC1) expression in patients with resected non-small cell lung carcinoma (NSCLC). We aimed to determine the prognostic and predictive significance of ERCC1 in patients with completely resected NSCLC.

Methods: Immunohistochemistry (IHC) was used to assess the expression of ERCC1 in resected lung tumor samples obtained from 98 patients untreated without pre- or post-operative chemotherapy and/or radiotherapy. The median H score was used as a cut-off for ERCC1 IHC. Univariate and multivariate analyses were performed for factors influencing patient survival.

Results: The 5-year survival rates of patients for ERCC1 positive expression and ERCC1 negative expression were 76% and 49%, respectively; this difference was statistically significant ($p = 0.004$). Subsequent multivariate analysis suggested that ERCC1 expression (adjusted hazard ratio for death, 0.38; 95% CI, 0.18 to 0.78; $p = 0.008$) and pathological stage (2.2; 95% CI, 1.09 to 4.5; $p = 0.027$) were both independent prognostic factors.

Conclusion: The level of ERCC1 expression in tumors a strong predictor of survival in resected NSCLC patients untreated without pre- or post-operative chemotherapy and/or radiotherapy.

Key words: excision repair cross complementation 1, immunochemistry, non-small cell lung carcinoma.

Introduction

Most patients with non-small cell lung cancer (NSCLC) who survive for a long time have tumors at TNM stage I or II at the time of initial diagnosis. However, without giving up the basic principles of cancer surgery, the 5-year survival rates are not yet satisfactory even in these early stage patients for pathologic stages IA, IB, IIA, and IIB, 5-year survivals are approximately 70%, 60%, 55%, and 40%, respectively.[1)] Most patients with early stage NSCLC experience recurrence and die as a result of the disease, despite potentially curative treatment. Surgical resection is the standard treatment for these stages, and the efficacy of adjuvant therapy in this group of patients has not been confirmed.[2, 3)] However, early detection of a subgroup having early stage NSCLC with a higher risk of recurrence/metastasis and sensitive to chemotherapy may create a basis for aggressive adjuvant

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therapy, which may help to improve the survival rate in these patients.

Many factors considered affecting the prognosis of NSCLC in patients are still being investigated. Numerous reports suggest that a variety of tumor cell markers predict survival in patients who have early stage NSCLC.[4, 5] However, some reports conflict, and, in general, the associations do not appear sufficiently strong to be of value in formulating clinical treatment plans. Improvements in our understanding of the genetic and molecular basis of lung cancer in recent years have led to the discovery and assessment of new factors that may be of prognostic value. Recent works have demonstrated that genes involved in nucleotide metabolism and DNA repair are important determinants of the phenotypic behavior of early stage NSCLC; i.e., they are prognostic factors of patients' survival. Specifically, the excision repair cross-complementation group 1 (ERCC1), a component of the 5' nuclease involved in nucleotide excision repair (NER), are prognostic of patient outcome.[5–11]

The prognostic role of ERCC1 in solid tumours, including NSCLC, has been suggested for a long time.[12–16] These studies revealed that patients with low levels of ERCC1 expression had better outcomes and was found to be related to longer disease-free survival when treated with platinum-based chemotherapy. On the other hand, increased ERCC1 expression has been proved to be a double-edge sword in NSCLC,[17] being at the same time a significant and independent prognostic factor of survival in early stage NSCLC[6, 8, 9, 11] and also associated with increased resistance to platinum-based chemotherapy.[9, 15, 18] However various differences exists in these studies like, use of different methods for evaluating ERCC1 expression, use of different cut of values for ERCC1 positivity, inclusion of cases with heterogen stages, and inclusion of cases which have been treated before. Because of this disparity, we decided to study ERCC1 expression in NSCLC further. The present study was performed to assess the effects of the expression levels of the nucleotide excision repair complex markers ERCC1 in tumoral tissue, measured by immunohistochemical methods, on the survival of patients with early stage NSCLC.

Material and Methods

Between January 1999 and January 2007, 98 patients with NSCLC tumors, staged T1 or T2 and N0 or N1- M0 (stage I-IIA) from the pathological evaluation, underwent a successful and complete resection without operative mortality (no deaths in the hospital or 30 days after surgery) and without pre- or post-operative chemotherapy and/or radiotherapy. Since this was a retrospective study, institutional review board approval was not required. The scientific study committee of Yedikule Teaching Hospital for Chest Diseases and Thoracic Surgery reviewed and approved the database.

Study subjects were 9 women (10 %) and 89 men (90 %) with a mean age of 55.6 ± 9 years (range, 36–78). Mediastinoscopy was performed in 94 patients (96 %) as part of routine pre-thoracotomy mediastinal evaluation, and no mediastinal metastasis was detected. In the other 4 patients, computerized tomography of the thorax or positron emission tomography was used to eliminate the risk of mediastinal lymphatic metastasis. Neoadjuvant chemo and/or radiotherapy were not given. Systematic mediastinal lymphatic dissection was performed during thoracotomy along with appropriate lung resection. Lung resection consisted of lobectomy in 72 patients and pneumonectomy in 26 patients (**Table 1**). None of the patients received adjuvant therapy.

Resected specimens were sent to our pathology department for histopathological and immunohistochemical examination. Specimens were fixed in 10% formalin and embedded in paraffin. The longest diameter of the tumors was measured, and one or two 4-μm thick sections were obtained for each centimeter of the tumor from the areas containing viable tumor tissue at a maximum ratio with no (or minimum) hemorrhage or necrosis. Several sections were subjected to routine hematoxylin–eosin staining, while the others were kept for immunohistochemical staining. Tumors were staged after thoracotomy (pTNM) according to the International System for Staging Lung Cancer developed by the American Joint Committee for Cancer (AJCC) in 1997.[1] Histopathological tumor types were determined according to the classification of the World Health Organization.[19] Histopathological tumor types and differentiations, T and N stages, and tumor sizes are shown in **Table 1**.

Immunohistochemical staining (IHS)

Our study was done by Bond-Max (M 211-64) which is a fully automatic immunohistochemical and in-situ hybridization device. Among the prepared sections, one section which containing maximum tumor tissue and minimum –or no- necrosis and hemorrhage was chosen, for each patient. Sections were deparaffinized through a graduated xylene and alcohol series, and then rehydrated

Table 1 Prognostic factors revealed by univariate and multivariate analyses in patients with completely resected non-small cell carcinoma of the lung (n = 98)

	Number of patients (n = 98)	5-year survival rate (%)	Univariate *p* value	Multivariate *p* value HR (95% CI)
Sex			0.19	
Female	9 (10%)	44%		
Male	89 (90%)	68%		
Tumor size			0.48	
<20 mm	10 (10%)	81%		
21–30 mm	13 (13%)	66%		
31–50 mm	46 (47%)	55%		
50 mm	29 (30%)	53%		
Tumor localization			0.98	
Left	33 (34%)	65%		
Right	65 (66%)	67%		
T classification			0.49	
T1	23 (23%)	76%		
T2	76 (77%)	63%		
N classification			0.018	0.027
N0	57 (58%)	76%		2.2 (1.09–4.5)
N1	41 (42%)	49%		
Histology			0.013	0.28
Squamous cell	53 (54%)	80%		2.3 (1.01–5.23)
Adenocarcinoma	40 (40%)	49%		
Others	5 (6%)	60%		
Tumor differentiation			0.81	
Poor	45 (46%)	62%		
Moderate	39 (40%)	67%		
Well	14 (14%)	69%		
Surgical procedure			0.80	
Lobectomy	72 (73%)	69%		
Pneumonectomy	26 (27%)	66%		
Perineural invasion			0.4	
Positive	25 (26%)	63%		
Negative	73 (74%)	71%		
Blood vessel invasion			0.068	0.47
Positive	55 (56%)	57%		1.3 (0.5–3.03)
Negative	43 (44%)	75%		
Lymphatic vessel invasion			0.82	
Positive	79 (80%)	66%		
Negative	19 (20%)	62%		
ERCC1 expression			0.004	0.008
Positive	58 (59%)	76%		0.38 (0.18–0.78)
Negative	40 (41%)	49%		

HR, hazard ratio; ERCC1, excision repair cross complementation 1

in distilled water. Antigen retrieval was performed by adding citrate buffer (pH 6.0) and heating in a microwave oven (20 minutes, 100˚C). Incubation of sections in a 3% hydrogen peroxide solution removed endogen peroxidase activity, and then the sections were washed with a phosphate-buffered saline (PBS) solution. All of the procedures were performed in accordance with the antibody manufacturer's protocols. After incubation of sections with blocking solution for 20 min, the sections were incubated again with anti-ERCC1 primary antibodies at a 1:50 dilution (8F1) (GTX22356; Genetex). The reaction was visualized by use of a 3,3-diaminbenzidine (DAB) substrate system (08102; Novacastra). Then contrast staining was performed using Mayer's hematoxylin.

Evaluation of ERCC1 expression level

Two pathologists (NB and NF) who were unaware of clinical data independently evaluated ERCC1 staining.

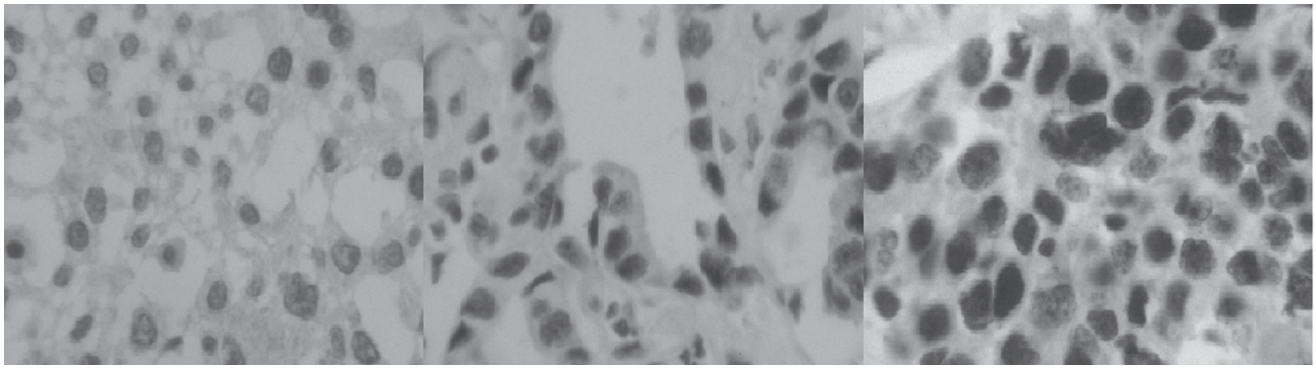

Fig. 1 Examples of ERCC1-expression scores by immunohistochemistry; scores 1 (**a**), 2 (**b**), and 3 (**c**) correspond to weak, moderate, and strong
ERCC1, excision repair cross complementation 1

Tumor nuclear staining intensity was graded on a scale of 0–3 (**Fig. 1).** The percentage of positive tumor nuclei was evaluated, and a proportion score was attributed (0 if 0%; 0.1 if 1%–9%; 0.5 if 10%–49%; 1.0 if ≥50%), as previously described.[20] The intensity and proportion scores were then multiplied to give the semiquantitative H-score. The median value of all the H scores was a priori chosen as the cutoff point for separating ERCC1-positive tumors from ERCC1-negative tumors.

Patients were followed up with regard to survival or recurrence at 6-month intervals routinely. Additionally, patients were called by phone, and some new information was received, during the preparation of the manuscript. The mean follow-up period was 46.5 ± 21.5 months (2–94 months). The survival period was calculated using the day of lung resection as the first day and the day of death or the last follow-up as the last day.

Statistical analysis

Cases were evaluated for demographic, surgical, and pathological variables, and the distributions of these variables were compared using the χ^2 test or Fisher's exact test (Student's t test was used to compare means). Correlations were determined using the Spearman rank correlation test. Patient survival was analyzed by the Kaplan–Meier method, using time zero as the date of thoracotomy and death as the endpoint. Differences in survival were determined by log-rank test in the univariate analysis, and prognostic factors with P-values less than 0.15 were included in a multivariate analysis using the Cox proportional hazards regression model. Results were considered significant at $p < 0.05$.

Results

The overall 5-year survival rate of the patients was 65%. The 5-year survival rate was 76% for patients with stage I disease (N0) and 49% for patients with stage II (N1) disease. This difference was statistically significant ($p = 0.018$). The effects of histopathological type on survival were assessed, and the 5-year survival rate was found to be 80% for patients with squamous cell carcinoma, 49% for patients with adenocarcinoma, and 60% for patients with other types of NSCLC. Differences among groups were statistically significant ($p = 0.013$). T, Gender, tumor size, tumor differentiation, and type of lung resection did not affect survival. In addition, no significant differences were observed in the survival rates of patients with and without lymph vessel or perineural invasion (**Table 1**). The 5-year survival rate was 57% for patients with invasion of the tumor to the tissue vasculature and 75% for patients with no vascular invasion ($p = 0.069$).

No ERCC1-positive cells were observed in 20 of the 98 tumors examined by immunohistochemical staining. A semiquantitative approach was used to calculate a score for each tissue section. Of 98 patients, 40 had an H score <1.0, considered IHC negative, and 58 had an H score ≥1.0, considered IHC positive. The 5-year survival rates of patients was 76% for ERCC1 positive expression and 49% for ERCC1 negative expression; this difference was statistically significant ($p = 0.004$, **Fig. 2**). An analysis of ERCC1 was frequent in squamous cell carcinoma (73%) for squamous cell carcinomas versus 45% for adenocarcinomas, $p = 0.01$). No statistical significance was found between ERCC1 expression and age, sex, tumor

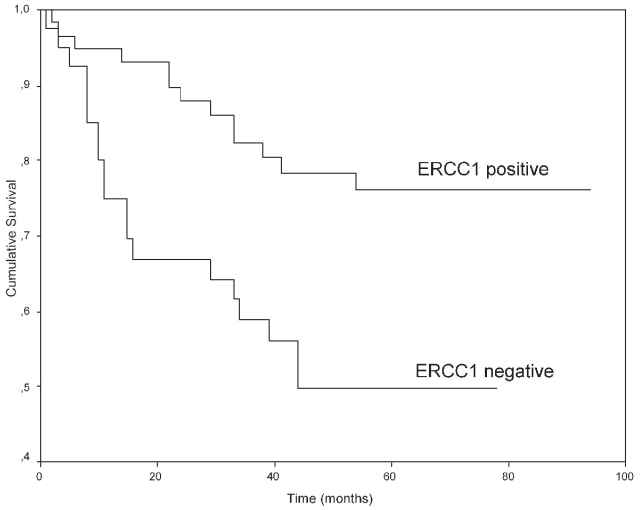

Fig. 2 Survival curves of patients having NSCLC with positive or negative expression of ERCC1.
ERCC1, excision repair cross complementation 1; NSCLC, non-small cell lung carcinoma

stage, T stage, N stage, vascular invasion, tumor differentiation, or tumor size.

Factors that were found to affect the survival rate on the univariate analysis (p <0.15) (i.e., histopathological tumor type, blood invasion, N stage, and ERCC1 expression) were used in a Cox regression analysis for multivariate analysis of the factors that may affect the survival rate. The analysis showed that expression of ERCC1 (p = 0.008), and pathological stage (p = 0.027) were both independent prognostic factors (**Table 1**).

Discussion

Many patients with NSCLC die from a relapse of disease though surgical treatment is potentially curative. Staging helps us to predict the overall survival of a group of patients; however, our prediction of the prognosis of NSCLC in a specific patient is not that reliable. Thus, we require new prognostic factors to determine the subgroup of patients who have a relatively poor prognosis for NSCLC. The discovery of effective factors in a study may be impeded by the inclusion of patients in all stages of cancer, treated with different modalities. The effects of these new factors may be relatively weak compared to the effects of T, N, and M variables, which are known to affect survival strongly. We included patients with early stage tumors without mediastinal lymph node or distant organ metastasis and without preoperative or postoperative chemo and/or radiotherapy to minimize the effects of these primary survival markers. This was expected to reveal the effects of other factors.

Many factors influence the prognosis of NSCLC in patients who have undergone resection, but none is being used in treatment decision making. Currently, the most frequently reported candidate marker is ERCC1, implicated in DNA repair. The expression level of ERCC1 has recently been reported to be a prognostic factor in the survival of patients with surgically resected NSCLC.[6, 8, 9, 11] Patients with tumors expressing high levels have a better outcome that those with tumors expressing low levels. In the present study, we evaluated the effect of intratumoral ERCC1 expression on the survival of patients with surgically resected NSCLC. We have shown that patients with ERCC1 positive tumors survived significantly longer after surgery than those with ERCC1 negative tumors.

The predictive and/or prognostic role of ERCC1 in solid tumors, including ovarian, gastric, colorectal, and, NSCLC has been recognized for the last 10 years.[12–18] These studies revealed that increased ERCC1 expression, however, has also been shown to be a predictor for cisplatin resistance. Therefore, it is associated with decreased survival of patients with gastric, ovarian, and colorectal cancers, and NSCLC. Results in studies of patients with resected NSCLC suggest a different, but not contradictory, prognostic significance for ERCC1 in treated and untreated patients.[6–11] Indeed, the prognosis of early-stage disease in untreated patients with a high level of ERCC1 in the tumor is better than that of patients with a low level of ERCC1. Furthermore, the response of patients to platinum-based therapy is unlikely in the setting of a high level of ERCC1. Several potential mechanisms may contribute to this. Impaired DNA repair could lead to genomic instability that in turn could lead to a malignant phenotypic behavior of tumors.[21] This could explain the poorer prognosis of disease in untreated early-stage patients with low levels of ERCC1 in tumors. Another, and possibly complementary mechanism, may be the increased ability of overexpressing cells to repair DNA damage. Thus, increased DNA repair function may be a disadvantage for platinum-based therapy.

Most studies evaluating the ERCC1 level in tumor tissue of resected NSCLC found that ERCC1 was an independent factor of survival.[6, 8, 9, 11] On the other hand, some of the studies could not document the effect of the ERCC1 level on survival (**Table 2**).[10, 16] In the study done by Olaussen et al.[9] with the greatest number of patients, the lack of detectable ERCC1 protein expression, as

Table 2 Results of studies investigating ERCC1 as a prognostic factor for overall survival in surgically resected non-small cell lung cancer patients

Studies (years)	n	Stage	Treatment	Technique	Cut-off level for ERCC1 positivity	Highest ERCC1 positivity according to histopathology	HR for death [95%CI] in ERCC1 positive cases
Rosell et al.[16] (2004)	67	IIB-IIIB	NACT + surgery	RT-PCR	2.73 to 12.31	NR	1.51(0.55–4.1), $p = 0.42$
Simon et al.[8] (2005)	51	I-III	Surgery	RT-PCR	50	AC ($p = 0.04$)	0.24 (0.07–0.77), $p = 0.016$
Olaussen et al.[9] (2006)	761	I-III	Surgery (n: 372) Surgery + ACT (n: 389)	IHC	*H*-score of ≥1	EDC ($p < 0.001$)	0.66 (0.49–0.90), $p = 0.009$ 1.16 (0.86–1.56), $p = 0.34$
Zheng et al.[11] (2007)	184	I	Surgery	IHC	65.9	NS	(Improved OS, $p = 0.01$)
Lee et al.[6] (2007)	133	I-III	Surgery	IHC	*H*-score of ≥10	EDC ($p < 0.001$)	0.59 (0.35–1.001), $p = 0.051$
Okuda et al.[10] (2008)	149	I-IV	Surgery +ACT (n = 90) Surgery (n:59)	IHC	*H*-score of ≥1	NS	2.18 (1.16–4.07), $p = 0.014$ $p = 0.24^{\pm}$
Current series (2009)	98	I-IIA	Surgery	IHC	*H*-score of ≥1	EDC (p = 0.01)	0.38 (0.18–0.78) $p = 0.009$

ERCC1, excision repair cross complementation 1; RT-PCR, reverse transcriptase polymerase chain reaction; IHC, immunohistochemistry; NACT, neoadjuvant chemotherapy; ACT, adjuvant chemotherapy; NR, not recorded; AC, Adenocarcinoma; EDC, epidermoidcarcinoma; HR, hazard ratio; OS, overall survival, NS, non-significant; n, number of cases; ± univariate analysis was applied.

determined by immunohistochemistry (IHC), was found to be related to overall survival after cisplatin-based adjuvant chemotherapy in the International Adjuvant Lung Cancer Trial Biology (IALT BIO) study. Interestingly, in the group that was not administered adjuvant chemotherapy, patients with ERCC1-positive tumors showed better survival. In other studies done by Zheng et al.,[11] Lee et al.[6] and also by Simon et al.[8] who used a different method for the detection of ERCC1 measurement (RNA expression) positivity of ERCC1 expression showed improved survival of patients with early-stage NSCLC. In agreement with these studies, we demonstrated that ERCC1 expression was an independent predictive factor of overall survival in patients with early-stage NSCLC. Also, multivariate analysis suggested that ERCC1 expression is a marker for longer survival using as covariates clinicopathologic variables, such as N stage. On the other hand, in the studies done by Okuda et al.[10] and Rosell et al.,[16] no effect of ERCC1 expression on the survival was detected. Intervariance between the results of the studies can be due to the use of different methods in the evaluation of ERCC1 expression, acceptance of different threshold levels for ERCC1 positivity, and heterogenous distribution of the patients in the study. In addition, most studies, investigating the prognostic importance of ERCC1 in resected NSCLC, have a retrospective design with inherent bias and enroll small numbers of patients, which impacts the power of the study. This leads to difficulties in making a direct comparison between the various studies. Besides all these disadvantages, our study showed that ERCC1 can be used as a prognostic factor in early-stage NSCLC patients. Prognosis is poorer in patients with early-stage NSCLCthan in patients with positive ERCC1 expression., On the other hand, a positive effect of chemotherapy on survival was proved in advanced stage NSCLC patients with negative ERCC1 expression.[15–18] These findings can be interpreted as follows: chemotherapy can also be given in patients with early NSCLC and negative ERCC1 expression to increase their survival. However, further studies are needed to clarify this issue.

In the studies examining ERCC1 as a prognostic factor in patients with resected NSCLC, the relation of the ERCC1 expression and the tumor histological type was also evaluated, but the results were controversial. Only the study of Simson et al., showed a higher ERCC1

expression levels in the adenocarcinomas.[8] However, no differences in histological types were detected in the remaining three studies.[10, 11, 16] In our study, significantly more ERCC1 expression was detected in the epidermoid type (%56) than in adenocarcinoma (%36) (p <0.01), supporting the results of the two other studies.[6, 9] Differences in results can be explained by the use of different methods in the measurement of ERCC1 and differences in threshold values for ERCC1 positivity.

Problems exist in the measuring of ERCC1 levels in NSCLC. Marked differences in methodology exist regarding monoclonal antibodies and ERCC1 detection techniques. Currently, ERCC1 analysis is done by immunohistochemistry and ERCC1 mRNA expression. The relationship between these two techniques of ERCC1 assessment remains uncertain. Indeed, Zheng et al. could not demonstrate a correlation between the ERCC1 mRNA (quantitative, real time RT-PCR) and protein expression (AQUA).[11] In the studies done in the resected NSCLC, the role of ERCC1 has been assessed by both methods: mRNA was measured by RT-PCR in two studies[8, 16] and protein expression, by immunohistochemical techniques in four studies.[6, 9–11] In our study, expression of ERCC1 in tumor tissue was evaluated by immunohistochemical methods.

Because the immunohistochemical method of detecting ERCC1 protein is simple and can be easily performed in almost every pathology laboratory, the present findings could be widely applicable in clinical medicine. However, standard IHC with visual scoring in an attempt to quantify protein expression has significant technical limitations. These include the nonquantitative chemistry of routine immunoperoxidase stains and the subjective light intensity perception of the human eye. Therefore, it is difficult to identify an optimal cut-off value for ERCC1 using immunohistochemistry in prospective trials. Besides, the evaluation was subjective, and the laboratory conditions also effect the staining. To minimize these limitations, as in other studies[6, 9, 10] a scoring system (H scores) was used in our study. In this context, we thought that the studies might become more reliable if ERCC1 gene expression were measured in a more objective way. In addition, the ERCC1 level was determined by the ratio of stained cells in several studies, and staining intensity of the cells was added as an additional factor in some other studies. In the present study, we determined both the ratio and the intensity; adding the intensity score.

Like as in our study, in studies evaluating ERCC1 expression in early stage resected NSCLC cases by immunohistochemical metods, H scoring was done by interpretting staining intensity of cells with ERCC1 and ratio of stained cells, and the calculated median value of H score was accepted as the cut-off value for the positivity. On the other hand, different from our study and two other studies,[9, 10] in the study done by Lee et al.,[6] different scoring was used for the measurement of ratio of stained cells. Because of this, the cut-off value found in this study (H score = 10) was found to be different from our study and other studies (H score = 1) as expected.

In conclusion, the survival of a patient with NSCLC depends on numerous factors, and accurately determining the prognosis of disease in a patient is not possible. ERCC1 expression in early-stage NSCLC is a reliable indicator of disease prognosis and local or distant recurrence of these tumors. Because of the poor prognosis, adjuvant chemotherapy may be appropriate for patients with NSCLC and low ERCC1 expression. The present study provides a basis for designing future studies to investigate the efficacy of adjuvant chemotherapy in these patients.

Conflicts of Interest

The authors declare no conflicts of interest.

Financial Disclosure

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