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# ANALYSIS OF ESTROGEN RECEPTOR ALPHA (ERα) EXPRESSION AND EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR) MUTATION AND THEIR RELATIONSHIP WITH HORMONAL AND MENSTRUAL FACTORS IN WOMEN WITH LUNG ADENOCARCINOMA

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**Abstract**

Lung cancer is the second most common cancer worldwide. The proportion of non-smoking women with lung cancer is higher than non-smoking men. Studies have found gender differences in estrogen receptor alpha expression and mutation in the human epidermal growth factor receptor in tumour tissue. The aim of the study is to analyze the expression of estrogen receptor alpha (ERα) and epidermal growth factor receptor (EGFR) in women with lung adenocarcinoma. The study is retrospective and includes 41 non-smoking women with lung adenocarcinoma. We studied the expression of ERα and EGFR mutations in tumour tissue and their relationship with the hormonal and menstrual factors. The positive expression of ERα in tumour tissue is 9.8%, and in these patients, the estradiol levels were many times higher. EGFR mutation expression is found in 26.8%. There is a correlation between the expression of the EGFR mutation and the time of menopause. The expression of ERα and EGFR in non-smoking women with lung adenocarcinoma is most likely related to the influence of sex hormones and menstrual factors.

**Key words:** lung cancer, women, estrogen-receptor alfa, epidermal-growth factor receptor

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**Introduction.** Lung cancer is the second most commonly diagnosed cancer worldwide. Despite advances in diagnosis and treatment, it has the highest mortality rate as compared to any cancer. Lung cancer is the cause of more deaths than breast cancer, prostate cancer, colorectal cancer, bladder cancer and melanoma combined [1, 2]. Since 1950, the death rate from lung cancer in women has increased by 600%. This fact is partly related to the increased use of cigarettes after 1940 [2]. Smoking is considered to be the main risk factor for both sexes for the development of the disease [3]. Nevertheless, the proportion of non-smoking women with lung cancer is significantly higher than non-smoking men. Mutations in the human epidermal growth factor receptor (EGFR) are significantly more common in females [4]. In the 21st century, the epidermal growth factor receptor (EGFR) mutation has been found to be the second most common mutation in non-small cell lung cancer. It is found in 10–20% of the Caucasian and 50% of the Asian population [5]. In women, higher expression of estrogen receptor alpha (ER$\alpha$) was also found in the tumour tissue [4]. Nuclear expression of ER$\alpha$ is found in 17% of the patients with lung adenocarcinoma and can be used as an independent marker for subsequent relapse and the possibility of anti-estrogen therapy [6]. This study aims to analyze the expression of the estrogen receptor alpha (ER$\alpha$) and the mutation of the epidermal growth factor receptor (EGFR) in non-smoking women with lung adenocarcinoma.

**Materials and methods.** A retrospective study was conducted at the Clinic of Thoracic surgery, St. George University Hospital, Plovdiv and the Department of Special Surgery at Medical University – Plovdiv in the period January 2016-September 2019. Immunohistochemical expression of ER$\alpha$ and EGFR mutation in tumour tissue was studied in 41 non-smoking women with adenocarcinoma. The results were compared with the age of the patients and some hormonal and menstrual factors.

To determine the expression of EGFR mutation, a molecular analysis was performed at the National Genetic Laboratory – Sofia. Materials from paraffin resection and endoscopic biopsies were examined. Cases with more than 50% tumour cell material in the slice were selected for DNA analysis. The EGFR mutation study is based on real-time PCR technology to detect 29 somatic mutations in the EGFR gene, exons 18–21, of which T790M; deletion in exon 19; L858R; L861Q; G719X; S768I; insertion into exon 20. Samples were considered positive if one of the 29 activating mutations of EGFR was detected. The study did not find variants in the EGFR gene associated with resistance to tyrosine kinase inhibitor therapy.

A ready-to-use rabbit monoclonal antibody assay was provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L sodium acetate to determine estrogen receptor alpha expression. The clone used in the study was: DAKO Code IR084, Monoclonal Rabbit Anti Human Estrogen Receptor $\alpha$ Clone EP1. A positive result is defined as nuclear staining in $> 1\%$ of tumour cells.

The statistical processing of the results was performed with the statistical package SPSS v. 23; SPSS Inc., Chicago, IL, USA.

**Descriptive and evaluation methods:**

- Variation analysis: standard deviation, minimum, maximum and range;
- Frequency analysis of qualitative variables (nominal and rank): absolute frequencies, relative frequencies (in percentage), cumulative relative frequencies (in percentage);
- Graphic images;

**Methods for testing hypotheses:**

1. Parametric:
   - 1.1 One simple T-test – check for equality of two means.
2. Non-parametric methods:
   - 2.1 Mann–Witney method – comparison of averages in two groups of one quantitative variable when the distribution is not normal;
   - 2.2 Kruskal—Walis H method – comparison of average values of three or more groups of one quantitative variable when the distribution is not normal;
   - 2.3 One-way analysis of variance (One-Way ANOVA) – comparison of arithmetic mean values of the same observed feature in two or more samples;
   - 2.4 Chi-square test or Fisher's exact test – search for a relationship between two qualitative variables;
   - 2.5 Median test – comparison of the medians in two groups of one quantitative variable.

The critical level of significance we use is $\alpha = 0.05$. The corresponding null hypothesis is rejected when the $P$-value is less than $\alpha$.

Opinion of the Ethics Committee: This study fully meets the standards and criteria for science and ethics and is approved by the Commission on Scientific Ethics at the Council for Research at MU-Plovdiv.

**Results.** The distribution by age groups is presented in Table 1.

The highest percentage of women with lung adenocarcinoma is observed in the age group 71–80 years – 12 (29.30%), followed by the group of 61–70 years – 11 (26.8%). All of these women have had stage IIIB and IV of lung cancer. Positive ER$\alpha$ expression was observed in 4 (9.8%) of the studied patients.

Table 1

Frequency distribution by age groups

| Age groups | Number $N$ | Valid percentage, % | Cumulative percentage, % |
|---|---|---|---|
| 40 years | 2 | 4.90 | 4.9 |
| 41–50 years | 4 | 9.80 | 14.6 |
| 51–60 years | 8 | 19.50 | 34.1 |
| 61–70 years | 11 | 26.80 | 61.0 |
| 71–80 years | 12 | 29.30 | 90.2 |
| > 80 years | 4 | 9.80 | 100.0 |
| Total | 41 | 100 | |

Table 2

Values of estradiol in the study groups, according to ERα expression

| | Mean ($X \pm$ SE) | Min | Max | $P$ |
|---|---|---|---|---|
| ERα – negative | $145.46 \pm 38.04$ | 36 | 1011 | 0.002 |
| ERα – positive | $2126 \pm 2029.24$ | 52 | 8213 | |

One-way analysis of variance One-Way ANOVA found a statistically significant difference in the mean values of estradiol in these patients compared with negative ones for ERα ($F = 10.756$; $P = 0.002$) (Table 2).

Of interest is the fact that positive ERα is observed in women who have a late onset of menarche (first menstruation). The mean age of menarche in women with positive ERα expression was $15.50 \pm 1.73$ and in women negative for ERα – $14.73 \pm 1.97$. There was a statistically significant difference in the mean age of onset of menarche in the study groups ($P = 0.02$).

Only in one of the studied women with positive expression of ERα, positive expression for EGFR mutation was observed.

Regarding the expression of EGFR mutation, it is found in 11 (26.8%) patients with lung adenocarcinoma. The one-way analysis of variance One-Way ANOVA found a statistically significant difference in the mean age of onset of menopause in women with positive receptor expression and those with negative ($F = 4.567$; $P = 0.03$). In 66.7% of patients positive for the EGFR mutation, menopause occurred before the age of 50 years. It is noteworthy that 80% of women with positive EGFR expression gave birth to their first child before the age of 25, and 81.8% of them had a late onset of menarche.

One simple T-test showed a statistically significant difference in estradiol values in the two study groups ($P = 0.0005$) (Table 3).

In the group of women positive for EGFR mutation, estradiol was 3.6 ($115.90 \pm 60.29$) times lower than in the groups where no EGFR mutation was observed – $420.37 \pm 271.97$.

**Discussion.** The epidermal growth factor receptor (EGFR) is a transmembrane protein that plays an important role in the signal transduction of the human

Table 3

Values of estradiol in the study groups, according to EGFR expression

| | Mean ($X \pm$ SE) | Min | Max | $P$ |
|---|---|---|---|---|
| EGFR – negative expression | $420.37 \pm 271.97$ | 36 | 8213 | 0.0005 |
| EGFR – positive expression | $115.90 \pm 60.29$ | 37 | 716 | |

cell. The fact that EGFR is expressed in a large proportion of women with non-small cell lung cancer shows the importance of this study in deciding how to treat these patients.

In our study, EGFR mutation expression was found in 11 (26.8%) patients with lung cancer.

A multicentre study in France found a higher incidence of the EGFR mutation – 44%. Similar to our study, a positive correlation was found between the EGFR mutation and the later onset of menarche, but no statistically significant association was found between the age of menopausal onset and the expression of this alteration. A higher incidence of the mutation was found in patients who gave birth to their first child after the age of 30, while in our study it was found in patients who had their first child at an earlier age [7]. Another study in Japan confirmed that females were at higher risk for developing EGFR mutations, and also found that menstrual and reproductive factors (age of menarche, menopausal status, number of pregnancies) affected the risk of EGFR mutation [8].

In our study 66.7% of the EGFR-positive patients experienced menopause before the age of 50, 80% gave birth to their first child before the age of 25, and 81.8% had their first regular period after the age of 15.

Mazieres et al. [9] confirmed the higher frequency of the EGFR mutation in non-smoking women and found a positive correlation between this alteration and estrogen receptor alpha expression. Such a connection was not established in our study.

The estrogen receptor $\alpha$ (ER$\alpha$) is a characteristic immunohistochemical marker for breast cancer and is commonly used for distinguishing breast cancer from adenocarcinoma with other primary sites, including lungs. However, there is growing evidence of the role of estrogen in the development and progression of lung cancer [10, 11].

A study analyzing the expression of ER$\alpha$ in a normal and in tumour lung tissue found that it was significantly higher in the cancerous tissue of women compared to men. It was also found a significantly higher ER$\alpha$ expression in the tumour tissue compared to the normal one [12].

Mazieres et al. [9] analyzed the expression of ER$\alpha$ and found significantly higher expression in non-smokers (11.1–23.8%), depending on the method used. For comparison – in smokers these values were 3.9–10.4%, respectively.

In our study, the positive expression was lower – 9.8%, which may be due to the smaller number of patients enrolled in the study.

More and more data accumulate on the importance of ER-α as a prognostic factor. A study in Norway examined the expression of ER-α and found a significantly better prognosis in women with ER-α-positive tumours on pemetrexed/carboplatin treatment compared to gemcitabine/carboplatin (20/4.6 months). According to the authors, ER-α is an independent prognostic factor in patients with advanced non-small cell lung cancer [13]. On the other hand, a study conducted at the University of Lübeck in 2000–2005 found that ERα expression was associated with a worse prognosis in patients with non-small cell lung cancer undergoing radiation therapy [14].

Comparing data from different studies, a higher frequency of ER-α expression was found in non-smoking women with lung cancer. Regarding the role of this estrogen receptor as a prognostic factor, the data are contradictory. Most likely, this heterogeneity is due to the insufficient number of studies in this area. Further research of the role of ER-α in lung adenocarcinoma could provide important answers regarding treatment options and prognosis in women with this cancer. On the other hand, the EGFR mutation is also a therapeutic option in patients with lung cancer. Further studies in this direction could establish a link between estrogen receptor alpha (ERα) expression, epidermal growth factor receptor (EGFR) mutation, and the influence of hormonal and menstrual factors in the pathogenesis of non-small cell lung cancer.

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