Lung cancer is the most common cancer in the world-wide, and it is the most common reason for cancer-related mortality in women and men. Non-small cell lung cancer (NSCLC) constitutes 85% of all lung cancer cases.\(^1\) The causes of NSCLC have not been fully elucidated. According to current consensus, smoking is the main cause of lung cancer.
tumors, although not all patients with lung cancer have a history of smoking, suggesting that genetic variations are also involved in the development of this malignancy. Present research suggests that the occurrence of lung cancer is accounted for by the interaction between the environment and heredity. To improve disease outcome, it is crucial to implement biomarkers into the clinics which assist physicians in their decisions regarding diagnosis, prognosis, as well as prediction of treatment response.

The human fibroblast growth factor (FGF) family consists of 22 members. Fibroblast growth factor 23 (FGF 23), is a peptide hormone member of the FGF 15/19 subfamily, which is differentiated from the larger FGF family by virtue of its lacking the conventional FGF heparin-binding domain and by exhibiting endocrine function. Klotho is an essential cofactor for binding of FGF 23 to the FGF receptor (FGFR), it modulates bFGF signaling, inhibits the insulin and the insulin-like growth factor (IGF)-1 pathways, and regulates the activity of the transient receptor potential vanilloid type 5 calcium channel. It was shown that FGF 23 level was increased in hematologic malignancies, prostate cancer, breast cancer and ovarian cancer.

Fibroblast growth factor pathway is a new treatment target for urothelial cancer. In NSCLC patients without druggable alterations in genes as epidermal growth factor receptor (EGFR) or rearrangements of the anaplastic lymphoma kinase (ALK) or c-ROS oncogene 1 (ROS1), prognosis remains adverse with a median survival time of about 18 months. New treatment agents are needed in the specific population.

Methods

Study Population

This was a prospective, single-center study. Medical information was obtained from the archived files of newly diagnosed stage 3B and 4 NSCLC patients without druggable alterations in genes as EGFR, ALK or ROS1 in the medical oncology clinic of Okmeydani Training and Research Hospital, in 2019. A total of 97 NSCLC patients older than 18 years were scanned. Patients without pathology reports and laboratory test results and 18 fluorodeoxyglucose (18-FDG) positron emission tomography/computed tomography (PET/CT) were excluded. Also, we excluded patients with EGFR, ALK or ROS1 positive NSCLC, chronic kidney disease and vitamin D deficiency. Disease staging was performed according to the Tumor, Node, Metastasis eighth edition (TNM 8) staging system. We also enrolled healthy control in similar age, without any chronic disease and vitamin D deficiency in the study. The study was performed in accordance with the declaration of Helsinki. The ethical approval was received. The patients give a written informed consent before the study.

Fibroblast Growth Factor 23

Venous blood samples required for laboratory examination were taken in the morning following a 12-hour fasting. The samples were transported to the laboratory on an ice mold and the serum sample was separated by centrifuging at 4000 rpm, 5 minutes, and the samples were stored at -80°C. Human Fibroblast Growth Factor 23 ELISA (Enzyme-Linked-ImmuSorbentAssay) Kit (Catalog No: YLA-1509HU, YL Biotech Co. Ltd., Shanghai China) was used by ELISA method for intact FGF23 measurement. The readings were made spectrophotometrically with a 450 nm wavelength scanning device. Results are given in pg/ml.

Statistical Methods

SPSS 15.0 for Windows was used for the statistical analysis. Descriptive statistics were given as number and percentage for the categorical variables, average, standard deviation, minimum, and maximum for the numeric variables. The relations between the numerical variables were made using the Spearman Correlation Analysis since the parametric test condition could not be met. Two independent group comparisons of the numerical variables were made using the Mann–Whitney U test when normal distribution conditions were not achieved. The statistical significance level of alpha was accepted as p<0.05.

Results

In this study, 40 men newly diagnosed stage 3B and 4 NSCLC patients without druggable alterations in genes as EGFR, ALK or ROS1 and 24 healthy men were enrolled. All of patients had smoking history. The median age of patients and controls were 54.7 and 53.1 years. Histologic subtypes were squamous cell carcinoma in 22 (55.0%) patients, adenocarcinoma in 18 (45.5%) patients. The median tumor diameter was 5.3 cm. The number of patients with N1, N2 and N3 according to TNM 8 staging system were, 12 (30.0%), 20 (50.0%) and 8 (20.0%) respectively. The number of patients were 22 (55.0%) and 18 (45.0%) in stage 3B and stage 4 groups respectively. The most common metastasis sites were liver, bone and brain respectively (Table 1). The mean SUVmax was 14.1±6.1 in patients group. The mean phosphor level was 3.6±0.43 mg/dl and 3.7±0.38 in patients and control groups respectively. The mean FGF 23
level was calculated as 87.7±58.0 pg/ml in patients group and 63.1±11.4 pg/ml in control group (p=0.045) (Table 2). Fibroblast growth factor 23 levels were 85.5±42.5 pg/ml and 89.6±69.1 in metastatic and non-metastatic patients respectively (p=0.532). The median FGF 23 levels were 91.1±58.4 pg/ml and 92.5±60.8 pg/ml in squamous cell carcinoma and adenocarcinoma groups respectively (p=0.926) (Table 3).

In the correlation analysis, there was no any statistical significant correlation between FGF 23 and prognostic factors such tumor diameter, lymph node status, stage and SUVmax (rho=0.006 p=0.971, rho=-0.127 p=0.440, rho=0.029 p=0.858 and rho=0.299 p=0.090 respectively). There were the statistical significant correlation between FGF 23 and alanine aminotransferase and aspartate aminotransferase (rho=0.359 p=0.027 and rho=0.362 p=0.025 respectively) (Table 4).

Discussion

We planned this study to detect the clinical importance of serum FGF 23 in newly diagnosed NSCLC patients without druggable alterations in genes as EGFR, ALK or ROS1. We found that serum FGF 23 levels were higher in patients with NSCLC than healthy controls but there was no significant difference of FGF 23 levels in different prognostic groups. There are some trials focused on the relation between FGF 23 and cancer, in different tumor types. Firstly, in a study, FGF 23 level was evaluated in ovarian cancer and was found that serum FGF 23 concentrations are significantly higher in women with advanced-stage ovarian cancer compared with concentrations in women with early-stage ovarian cancer or benign disease or in healthy women.[15] In a trial published in 2015 was found that FGF 23 is expressed in prostate cancer at increased levels but FGF 23 is not correlated with clinical or pathological parameters. Also in this study, exogenous FGF 23 has been shown to increase proliferation and invasion of prostate cancer.[13] In another trial published in 2019, circulating FGF 23 level was evaluated in patients with urothelial carcinoma and was found that serum FGF 23 concentrations are significantly higher in women with advanced-stage ovarian cancer compared with concentrations in women with early-stage ovarian cancer or benign disease or in healthy women.[18] Also, FGFs genomic alterations were shown in many solid tumors. Fibroblast growth factor 23 interacts with FGFR 1,2,3,4 and FGFR 5.
α-Klotho co-factor. Fibroblast growth factor receptor genomic alterations were analysed in many solid tumors and FGFR1, FGFR3 and FGFR4 gene amplifications were found in NSCLC patients. In a study published in 2019, the interaction between FGFR signaling and EGFR was investigated, when the cells were stimulated with the FGFR4 specific factor FGF 19, the activation of both FGFR4 and EGFR was observed. Also this cooperation was found independent of EGFR activating mutations.

In our study, serum FGF 23 levels were higher in patients with NSCLC than healthy controls but there was no significant difference of FGF 23 levels in different prognostic groups. Considering high FGFR alteration rates and FGFR related EGFR activation in patients with lung cancer, our study suggests that high FGF-FGFR interaction may be causative for EGFR, ALK or ROS1 wild type non-small cell lung cancer.

This is the first prospective study focused on the clinical impact of serum FGF 23 levels in newly diagnosed NSCLC patients without druggable alterations in genes as EGFR, ALK or ROS1. There are some limitations in our study. Firstly, patients outcomes still are not mature to determine the prognostic impact of FGF 23 on lung cancer. Secondly, the patients number was not enough for subgroup analysis.

### Conclusion

Our study suggests that high FGF-FGFR interaction may be causative for stage 3B and 4 NSCLC without druggable alterations in genes as EGFR, ALK or ROS1 and is important in terms of suggesting the FGF pathway as a new treatment target in NSCLC patients. There are needed the studies focused on the FGF-FGFR interaction as a target for this specific cohort.

### Disclosures

**Informed Consent:** The patients give a written informed consent before the study.

**Ethics Committee Approval:** The Okmeydani Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 08.10.2019, number: 48670771-514.10).

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**Conflict of Interest:** The authors declare that there is no conflict of interest regarding the publication of this paper.


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