Research Article

The Clinical Importance of Fibroblast Growth Factor 23 on Breast Cancer Patients

Ruhper Cekin, Serdar Arici, Muhammed Mustafa Atci, Saban Secmeler, Sener Cihan

Department of Medical Oncology, University of Health Sciences, Okmeydani Training and Research Hospital, Istanbul, Turkey

Abstract

Objectives: To investigate the relation between serum fibroblast growth factor (FGF) 23 levels and clinicopathologic features of breast cancer patients, by comparing healthy control group.

Methods: This was a prospective, single-center study. Newly diagnosed stage 1–4 breast cancer patients and healthy control in similar age, without any chronic disease and vitamin D deficiency were enrolled in the study. Fibroblast growth factor 23 levels were compared between groups.

Results: Thirty eight women newly diagnosed stage 1 and 4 patients and 40 healty women were enrolled. The median age of patients and controls were 54 and 53.1 years. The number of patients were 7 (18.4%), 9 (23.7%), 13 (34.2%), 5 (13.2%), 1 (2.6%) and 3 (7.9%) in stage 1A, 2A, 2B, 3A, 3B and stage 4 groups respectively. The mean FGF 23 level was calculated as 167.4±177.2 pg/ml in patients group and 63.1±11.4 pg/ml in control group (p=0.0004).

Conclusion: Our study suggests that high FGF-FGFR interaction may be causative for breast cancer and is important in terms of suggesting the FGF pathway as a new treatment target in breast cancer patients.

Keywords: Breast cancer, fibroblast growth factor 23, solid tumor

Globally, breast cancer is the second most frequently diagnosed malignancy just behind lung cancer.[1] The incidence rates are highest in high-income countries.[2] These international differences are likely related to societal changes (eg, changes in fat intake, body weight, age at menarche, and/or lactation, and reproductive patterns such as fewer pregnancies and later age at first birth). Studies of migration patterns are consistent with the importance of cultural and/or environmental changes.[3] Ten percent of breast cancer cases are associated with family history. In addition, risk may be modified by demographic, lifestyle, and environmental factors, although their association with breast cancer risk has not been clearly demonstrated. It is very important that clinicians benefit from biomarkers in predicting the response to diagnosis, prognosis and treatment. This is effective in improving the outcome of the disease.

The human fibroblast growth factor (FGF) family consists of 22 members. 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.[4] 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.[5–8] It

was shown that FGF 23 level was increased in hematologic malignancies, prostate cancer, breast cancer and ovarian cancer.[9–12] Fibroblast growth factor pathway is a new treatment target for pancreatic cancer and urotelhial cancer.[13] New treatment agents are needed in the specific population. There is no study investigated the relation between breast cancer and FGF 23.

We aimed to investigate the relation between serum FGF 23 levels and clinicopathologic features of breast cancer patients, by comparing healthy control group.

Methods

Study Population

This was a prospective, single-center study. Medical information was obtained from the archived files of newly diagnosed stage 1–4 and breast cancer patients in the medical oncology clinic of Okmeydani Training and Research Hospital, in 2019. A total of 80 breast cancer patients older than 18 years were scanned. Patients without pathology reports and laboratory test results were excluded. Also, we excluded patients 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-ImmunoSorbentAssay) Kit (Catalog No: YLA1509HU, 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 Analysis

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, 38 women newly diagnosed stage 1-4 breast cancer patients and 24 healthy women were enrolled. The median age of patients and controls were 54 and 53.1 years. Histologic subtypes were invaziv ductal carcinoma (IDC) in 31 (81.6%) patients, invaziv lobuler carcinoma (ILC) in 5 (13.2%) patients, invaziv papiller carcinoma (IPC) in 1(2.6%) patients and invaziv mixt carcinoma (IDC+ILC) in 1(2.6%) patients. The median tumor diameter was 3.1 cm. The number of patients were 1 (2.6%), 18 (47.4%) and 19 (50.0%) in grade 1, 2 and grade 3 respectively. Estrogen receptor (ER) status were negative in 13 (34.2%) patients, positive in 25(65.8%) patients. Progesterone receptor (PR) status were negative in 32(84.2%) patients, positive in 18(47.2%) patients. Cerb-b2 status were negative in 32 (84.2%) patients, positive in 6 (15.8%) patients. LVI was absent in 13 (59.1%) patients and in 9 (43.9%) patients. PNI was absent in 19 (86.4%) patients and in 3 (13.6%) patients. The number of patients with N0, N1 and N2 according to TNM 8 staging system were, 16 (43.2%), 18 (48.6%) and 3 (8.1%) respectively. The number of patients were 7 (18.4%), 9 (23.7%), 13 (34.2%), 5 (13.2%), 1 (2.6%) and 3 (7.9%) in stage 1A, 2A, 2B, 3A, 3B and stage 4 groups respectively (Table 1).

The mean FGF 23 level was calculated as 167.4±177.2 pg/ml in patients group and 63.1±11.4 pg/ml in control group (p=0.0004) (Table 2). FGF 23 levels were 162.4±183.8 pg/ml and 218,2±179,5 in IDC and ILC patients respectively (p=0.325). The median FGF 23 levels were 228.0±231.7 pg/ml and 201.6±192.0 pg/ml in LVI positive and LVI negative groups respectively (p=0.920). Fibroblast growth factor 23 levels were 92.6±69.9 pg/ml and 231.3±212.9 pg/ml in PNI (perineural invasion) positive and PNI negative groups respectively (p=0.920). Fibroblast growth factor 23 levels were 170.2±168.3 pg/ml and 162.1±200.2 pg/ml in ER (estrogen receptor) positive and ER negative groups respectively (p=0.590). Fibroblast growth factor 23 levels were 140.2±115.1 pg/ml and 192.0±219.0 pg/ml in PR (progesterone receptor) positive and PR negative groups respectively (p=0.792). Fibroblast growth factor 23 levels were 96.0±50.7 pg/ml and 180.8±189.5 pg/ml in Cerb-b2 positive and Cerb-b2 negative groups respectively (p=0.317) (Table 3).

In the correlation analysis, there was no any statistical significant correlation between FGF 23 and prognostic factors grade, tumor diameter, Ki-67 levels and stage (rho=-0.231

Table 1. Patients Characteristics

	n	%
Histology		
IDC	31	81.6
ILC	5	13.2
IPC	1	2,.6
IDC+ILC	1	2.6
Grade		
1	1	2.6
2	18	47.4
3	19	50.0
Diameter Mean±SD (Min-Max)	3.10±1.70 (1–9)	
CEA Mean±SD (Min-Max)	2.09±1.72 (0.37–8)	
CA 15.3 Mean±SD (Min-Max)	30.2±92.3 (4–550.7)	
Ki-67 Mean±SD (Min-Max)	23.4±19.3 (2–90)	
LI		
Negative	13	59.1
Pozitive	9	40.9
VI		
Negative	13	59.1
Pozitive	9	40.9
PNI		
Negative	19	86.4
Pozitive	3	13.6
ER		
Negative	13	34.2
Pozitive	25	65.8
PR		
Negative	20	52.6
Pozitive	18	47.4
Cerb-b2		
Negative	32	84.2
Pozitive	6	15.8
T		
1	11	29.7
2	20	54.1
3	5	13.5
4	1	2.7
N		
0	16	43.2
1	18	48.6
2	3	8.1
M		
0	35	92.1
1	3	7.9
Evre		
1a	7	18.4
2a	9	23.7
2b	13	34.2
3a	5	13.2
3b	1	2.6
4	3	7.9

IDC : Invasive ductal carcinom; ILC: Invasive lobular carcinom; IPC: Invasive papillary carcinom; FGF: Fibroblast growth factor; LI: Lymphatic invasion; VI: Vascular invasion; PNI: Perinoral invasion; ER: Estrogen receptor; PR: Progestereon receptor; T: Tumor; N: Lymph node; M: Metastasis; CA: Cancer antigen; CEA: Carcinoembryogenic antigen.

Table 2. FGF-23 levels in patients and controls

	Patient	Control	p
FGF 23	167.4±177.2	63.1±11.4	0.0004

FGF: Fibroblast growth factor.

Table 3. FGF-23 levels in subgroups

	Mean±SD	Median	p
Histology			
IDC	162.4±183.8	88.2	0.325
ILC	218.2±179.5	190.4	
IPC	100.5		
IDC+ILC	136.3		
LI			
Negative	201.6±192.0	129.6	0.920
Pozitive	228.0±231.7	172.8	
VI			
Negative	201.6±192.0	129.6	0.920
Pozitive	228.0±231.7	172.8	
PNI			
Negative	231.3±212.9	136.3	0,114
Pozitive	92.6±69.9	60.17	
ER			
Negative	162.1±200.2	87.31	0.590
Pozitive	170.2±168.3	100.5	
PR			
Negative	192.0±219.0	100.2	0.792
Pozitive	140.2±115.1	98.1	
Cerb-b2			
Negative	180.8±189.5	98.1	0.317
Pozitive	96.0±50.7	99.8	

IDC : Invasive ductal carcinom; ILC: Invasive lobular carcinom; IPC: Invasive papillary carcinom; FGF: Fibroblast growth factor; LI: Lymphatic invasion; VI: Vascular invasion; PNI: Perinoral invasion; ER: Estrogen receptor; PR: Progestereon receptor.

p=0.162, rho=-0.116 p=0.486, rho=-0.071 p=0.681and rho=-0.197 p=0.236 respectively). There were the statistical significant correlation between FGF 23 and calcium and basofil (rho=0,423 p=0,018 and rho=0.447 p=0.005 respectively) (Table 4).

Discussion

We planned this study to detect the clinical importance of serum FGF 23 in newly diagnosed breast cancer patients. We found that serum FGF 23 levels were higher in patients with breast cancer 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. First-

Table 4. The analysis of correlation between FGF-23 and clinicopathologic features of patients

	FGF-23 (pg/ml)	
	rho	p
Disease Features		
Grade	-0.231	0.162
Size	-0.116	0.486
Ki-67	-0.071	0.681
T	-0.082	0.631
N	-0.249	0.137
M	0.031	0.853
Stage	-0.197	0.236
CEA	0.162	0.401
CA 15.3	0.317	0.068
CBC		
WBC	0.238	0.155
NEU	0.289	0.083
LYM	0.158	0.342
MON	0.296	0.071
EOS	0.119	0.476
BAS	0.447	**0.005**
HGB	-0.024	0.888
MPV	0.019	0.912
PDW	-0.235	0.155
PLT	0.220	0.190
Biochemistry		
Urea	0.078	0.645
Creatinine	0.127	0.447
GFR	-0.152	0.362
AST	0.203	0.221
ALT	0.075	0.661
LDH	-0.047	0.829
Sedimentation	0.164	0.489
CRP	0.112	0.602
Sodium	-0.065	0.697
Potassium	0.185	0.272
Calcium	0.423	**0.018**
Phosphorus	-0.091	0.779

MON: Monocyte; EOS: Eosinophil; BAS: Basophil; CRP: C reactive protein; CEA: Carcinoembryogenic antigen; CA: Cancer antigen; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood urea nitrogen; CBC: Complete blood count; GFR: Glomerular filtration rate; FGF: Fibroblast growth factor; HGB: Hemoglobin; LDH: Lactate dehydrogenase; LYM: Lymphocyte; M: Metastasis; MPV: Mean platelet volume; N: Lymph node; NEU: Neuthrophil; PDW: Platelet distribution width; PLT: Platelet; T: Tumor; WBC: White blood cell.

ly, 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.[12] 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.[10] In another trial published in 2019, circulating FGF 23 level was evaluated in patients with urothelial carcinoma and was found that FGF 23 is significantly higher in patients group than control group but FGF 23 levels are similar in, different grades, tumor sites and stages.[14] Also, FGFs genomic alterations were shown in many solid tumors. Fibroblast growth factor genomic alterations were found 46% in squamous cell lung carcinoma and 39% in lung adenocarcinoma.[15–17] Fibroblast growth factor 23 interacts with FGFR 1, 2, 3, 4 and 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.[18] 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.[19] Breast cancer is divided into molecular subgroups defined by genomic changes causing tumor progression, and patient groups that can be effectively treated with targeted agents can be identified.[20] İn a study published in 2015 where FGF family aberrations were investigated in a different group of cancer types, the diagnosis of breast cancer correlated with the aberrations in FGF/FGFR. There was also a significant relationship between FGF/FGFR aberrations and liver metastases.[11] Another study published in 2012 showed that FGF-10 and its receptors, FGFR1 and FGFR2, play a role in breast cancer sensitivity and progression, so that the FGF signal can be selected by breast cancer cells. In the same study, it was found that the blocking of granzyme B responsible for cleavage blocks the transfer of FGFR to the nucleus and the promigrating effect of FGF stimulation. As a result of these findings, it has been shown that FGF signaling can regulate cancer cell behavior and may be a new treatment target in the treatment of invasive breast cancer.[21] Multiple genetic changes have been demonstrated in FGF receptors in breast cancer. For example, amplification of FGFR1 (8p11–12) is found in 2–15% of all breast cancer and 16–27% of luminal type B breast cancer.[22] These amplifications cause FGFR1 overexpression and are associated with resistance to endocrine therapy and poor prognosis.[23,24] In 2013, a study supporting this situation was published by F. Andre et al.[25] FGFR1, FGFR2 and FGFR3 blocker, dovitinib, was used in hormone receptor positive, Her-2 negative and metastatic breast cancer that amplified FGFR. While FGF 23 level increased, estradiol level decreased. While breaking

the endocrine resistance, antitumor activity was detected. İn a smilar study conducted in our clinic and published in 2020, suggested that high FGF-FGFR interaction may be causative for stage 3B and 4 NSCLC without druggable alterations in genes as EGFR, ALK or ROS 1.[26]

Conclusion

Our study suggests that high FGF-FGFR interaction may be causative for breast cancer and is important in terms of suggesting the FGF pathway as a new treatment target in breast cancer patients. There are needed the studies focused on the FGF-FGFR interaction as a target for this specific cohort.

Disclosures

Ethics Committee Approval: The study was performed in accordance with the declaration of Helsinki and was reviewed and approved by the Ethics Committee of the University of Health Sciences, Okmeydani Training and Research Hospital. Informed consent was not required from the participants for the study, because the analysis used anonymous clinical data obtained after each patient agreed to treatment by written consent (08/10/2019-48670771-514.10).

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