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RESEARCH ARTICLE

Expression of Angiogenesis-related Genes in a Group of Iranian Cases of Breast Cancer

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Abstract: ***Aims*:** This study aims to design an angiogenesis gene expression profile; to study angiogenesis gene expression profile in breast cancer; and to map angiogenesis gene expression profile in individual participants.

***Background*:** In molecular etiology of each disease, there are some important molecules involved in the related pathways. From the viewpoint of precision medicine, molecular etiology of a disease is different person by person because of genetic variations of the genes involved in these pathways. This point of view intends researchers of drug development to design novel drugs for targeted therapy based on the exact etiology. In the case of angiogenesis, there is a drug profile parallel to the molecular profile. Bevacizumab, sunitinib and aflibercept are examples of anti-angiogenic drugs.

***Objectives*:** A hallmark of solid tumors is sustained angiogenesis. Vascular endothelial growth factors (VEGF), VEGF receptors (VEGFR) and placental growth factor (PlGF) are involved in angiogenesis. We aimed to study the gene expression profile of angiogenesis including *VEGF-A*, *VEGF-B*, *VEGF-C*, *VEGF-D*, *VEGFR-1*, *VEGFR-2*, *VEGFR-3* and *PlGF* in an Iranian group of patients undergoing breast surgery due to breast cancer and breast fibroadenoma.

***Methods*:** Tumor tissue samples of a group of patients with invasive ductal carcinoma (IDC) and a group of patients with fibroadenoma (Fib) were used. Gene expression was studied by real-time quantitative polymerase chain reaction (q-PCR) and fold changes (FC) with their 95% confidence intervals (CI) were reported based on calibration with normal breast tissue.

***Results*:** All the genes showed significant up-regulation in IDC group. The extensive up-regulation was for *VEGFR-2* (FC=52.68; 95% CI=17.96-154.47; P<0.001). In Fib group, *PlGF* showed a significant up-regulation (FC=10.41; 95% CI=5.35-20.26; P=0.002). Comparison of IDC group with Fib group showed significant up-regulation of *VEGFR-1*, *VEGFR-2* and *VEGFR-3* in IDC group (P<0.05).

***Conclusion*:** Malignancy of breast tumors is associated with overexpression of all the genes of this profile. However, only VEGFRs showed up-regulation in comparison to benign tumors. Individualized targeted therapy, according to this profile, should be studied in the future.

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1. INTRODUCTION

1.1. Background

According to pathology textbooks, a hallmark of solid tumors is sustained angiogenesis. In this process, cancer cells secrete angiogenic factors to have better access to blood circulation. Hence, the tumor can be survived *via* better nutrition and can be spread *via* better vascularization. A lot of factors are associated with increased induction of angiogenesis such as inflammation and hypoxia [1-3]. Oxidative stress increases angiogenesis, epithelial to mesenchymal transmission and silencing tumor suppressor genes [4, 5]. Chronic inflammation is another important factor that triggers angiogenesis [6].

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Breast cancer is the most common solid tumor and the most common malignancy in women. According to the report of 2009, the incidence of breast cancer in Iran was 24 per 100000 women [7]. A 10-year national registry (2015) in Iran reported an incidence of 22.1-23.1 per 100000. Breast cancer covered 24.6% of all cases of cancer in Iranian women [8]. A spatial study in Iran mentioned that different regions of the country had different relative risks and hence education and screening should have different policies in different provinces of Iran [9]. This cancer brings a lot of family problems, especially in the Iranian culture that a mother has a pivotal role in family [10]. Screening and early detection can decrease the mortality rate of breast cancer [11] whereas delay in diagnosis increases mortality rate and healthcare costs [12]. The early detection methods consist of breast self-examination, clinical examination and mammography [13].

Pathologically, breast cancer is usually ductal and lobular carcinomas. Sarcoma and lymphoma are rare types of breast malignancies. Invasive ductal carcinoma is the most common malignant tumor of breast [14] and fibroadenoma is the most common benign tumor of breast [15]. Invasive ductal carcinoma has a process of formation as ductal hyperplasia, atypical ductal hyperplasia, ductal carcinoma *in situ* (DCIS), DCIS with micro-invasion (DCIS-MI), and invasive ductal carcinoma [16]. Each specimen of breast tumor is subjected to immunohistochemistry (IHC) evaluation. The most commonly used markers for IHC are estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2). In some cases, evaluation of Her2 with *in situ* hybridization is indicated [17]. Investigation of biomarkers has opened a door for precision medicine in breast cancer [18].

1.2. Rationale

Angiogenesis is an important process in tumor formation and growth. A lot of molecules are involved in this process. Vascular endothelial growth factors (VEGF), VEGF receptors (VEGFR) and placental growth factor (PlGF) are some of the most important ones. Therefore, an example of a profile for angiogenesis includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGFR-1, VEGFR-2, VEGFR-3 and PlGF. This profile has been previously used by Masood *et al.* (2002) in Kaposi's sarcoma cell line of human [19]. In molecular etiology of each disease, there are some important molecules involved in the related pathways. From the viewpoint of precision medicine, molecular etiology of a disease is different person by person. This point of view intends researchers of drug development to design novel drugs for targeted therapy based on the exact etiology. In the case of angiogenesis, there is a drug profile parallel to the molecular profile. In this drug profile, bevacizumab targets VEGF-A, sunitinib targets VEGFRs, aflibercept targets VEGF-A, VEGF-B, VEGF-C and PlGF, and some other examples [20]. Despite a lot of progressions in this approach, there is an evidence gap for breast cancer in both angiogenesis profile and drug profile.

1.3. Objectives

According to the rationale that molecular profile study is useful in precision medicine, we aimed to study gene expression profile of angiogenesis including *VEGF-A*, *VEGF-B*, *VEGF-C*, *VEGF-D*, *VEGFR-1*, *VEGFR-2*, *VEGFR-3* and *PlGF* in an Iranian group of patients with breast cancer and breast fibroadenoma as a pilot study in order to have an idea for personalized medicine in future researches. We compared gene expression in cancer and fibroadenoma tissues with each other and with normal tissues of mammoplasty cases.

2. MATERIAL AND METHODS

2.1. Study Design

An analytical case series study was performed as pilot research for personalized medicine. According to the PICO model for evidence-based medicine, P (patient/population/problem) stood for cases of breast surgery with the details mentioned below, I (intervention/exposure) stood for exposure to the amount of expressed genes as the independent variable, C (comparison) was comparison of target genes with reference gene and also comparison of gene expression with normal tissue as a calibration, and O (outcome) stood for the malignancy of tumor tissue as the dependent variable. The domain of study was etiology/harm.

2.2. Patients

The samples were collected in an operation room of Firoozgar tertiary hospital (Tehran, Iran) from the patients who were candidates for breast surgery in mid-2019 *via* convenient sampling. The inclusion criteria were being Iranian, having invasive ductal carcinoma or fibroadenoma according to the pathology reports, and receiving neoadjuvant chemotherapy if malignancy. The exclusion criteria considered comorbid conditions, technical problems for tissue sampling or any ethical limitation. Therapeutic regimen was not important. According to this, firstly, 21 patients with invasive ductal carcinoma were selected by the researcher (SAY Ahmadi). Because of technical and ethical limitations, he could not take tumor tissue samples from 10 of them. These patients were those who had smaller tumor size as the whole tumor was needed for pathological diagnosis (permanent or frozen sections). This small tumor size was due to earlier stages or better response to neoadjuvant chemotherapy. This limitation was not predictable until the time of tumor tissue preparation, because the mammography was carried out

before neoadjuvant chemotherapy. In some of such cases, patients had wire and marker in their breasts and tumor tissue was not detectable by the naked eye. Finally, 11 patients with invasive ductal carcinoma were included in the study. In addition, 5 patients with fibroadenoma were included in the study. 4 cases of mammoplasty were selected for collection of normal tissue. Briefly, the groups of the study were invasive ductal carcinoma (IDC group) and fibroadenoma (Fib group).

2.3. Tumor Tissue Preparation

After resection of tumor or complete mastectomy in operation room, the researcher brought the resected parts to the pathology room. About 4 mm of tumor tissue was separated. In the cases of complete mastectomy, the tumor was simply resected by a scalpel. In the cases of partial mastectomy (tumor resection), firstly, the tumor was used for routine actions by a pathologist including frozen section of the margins and touch slide preparation of the margins. Then if the size of grossly detectable tumor was enough, the researcher separated tissue sample by a scalpel. The fibroadenoma and normal fibroglandular tissue samples were collected in the very operation room. The samples were washed in phosphate buffered saline (PBS) and immediately kept at -80°C till the gene expression study.

2.4. Gene Expression Study

From each tissue sample, an amount of about 200 µg was separated. Then the samples were powdered in a burette under exposure with liquid azote. Then the powdered sample was solved in buffer Skp for columnar method of RNA extraction according to the instruction of the kit (Norgen, Canada). After the extraction of RNA, the concentration of RNA was calculated in the range of about 200-300 ng/µl using nanodrop spectrophotometry. An amount of 4 µl of RNA was used for cDNA synthesis using random hexamer primers according to the instruction of the kit (Tekta Tajhiz Azma, Iran). The primers were chosen according to the paper by Masood *et al.* [19]. These primers were checked in NCBI database and oligo7 program. Since *VEGF* genes had a lot of alternative splicing and isoforms, the primers cover almost most of them. *Beta-actin* was used as internals control. SYBR green master mix (Norgen, Canada) was used for amplification of the genes *via* real-time polymerase chain reaction (real-time PCR).

2.5. Analysis of Data

Mean of expression of the genes in the normal tissues was considered as the calibrator for calculation of fold change (FC). Statistical analysis was performed on -ΔΔCTs and then the FCs were calculated with geometrical 95% confidence interval (CI). Each group of IDC and Fib was separately compared with the calibrator using one sample t test (-ΔΔCT=0 or the very FC=1 was the null hypothesis). In addition, IDC and Fib groups were compared with independent t test. Two tailed P value less than 0.05 was considered as a significant level. In order to show the individual results and personalized interpretation of the data, heat plot was used. In this plot, z-axis was the t score of -ΔΔCT based on the normal calibrator group. Since there were 4 samples in the normal group, t scores ±3.182, ±5.481 and ±12.924 were considered as the cutoff points of two tailed p values 0.05, 0.01 and 0.001, respectively. Software Excel 2013 (Microsoft, US), SPSS 24 (IBM, US) and Stata 14 (StataCorp LLC, US) were used for data analysis.

2.6. Ethical Considerations

Informed consent was obtained from the participants. Secrecy was regarded for medical and biological information of the patients. As we mentioned above, we took care that our research did not interfere with the routine diagnostic and therapeutic procedures of the participants. The protocol was registered in the ethics committee of Lorestan University of Medical Sciences with number IR.LUMS.REC.1398.194.

3. RESULTS

A total of 11 patients with invasive ductal carcinoma were studied. The mean age was 46.18 ranged 31-64. ER was positive in 8 patients. PR was positive in 6 patients. Her2 was positive in 5 patients. There was 1 triple negative patient. Familial history was positive in 3 patients. One patient had metastasis. Six patients had lymph node involvement. The ethnicities of the participants are shown (Table **1**).

All the *VEGF* and *VEGFR* genes as well as *PlGF* showed significant up-regulation in IDC group in comparison to calibrator (FC>1, P<0.05). The most up-regulation was for *VEGFR-2* (FC=52.68; 95% CI=17.96-154.47; P<0.001) followed by *VEGFR-1* (FC=45.86; 95% CI=14.66-143.41; P<0.001). In Fib group, only *PlGF* showed a significant up-regulation (FC=10.41; 95% CI=5.35-20.26; P=0.002). Comparison of IDC group with Fib group showed significant up-regulation of *VEGFR-1* (P=0.004), *VEGFR-2* (P=0.022) and *VEGFR-3* (P=0.015) in IDC group. No further significant result was detected. The only down-regulation was for *VEGFR-3* in Fib group; but it was not statistically significant (P=0.374) (Table **2**, Fig. **1**).

The heat plot (heat map) shows the individual gene expression profiles of angiogenesis. The order of patients is sorted by the similarity of the ethnicities (Table **1**). According to this, patients with ethnicity Turk/Azeri (IDC4-IDC7) showed the most similar gene expression barcode. All the patients of Fib group (Fib1-Fib5) showed heterogenic barcodes. Regarding

Table 1. Individual participant data on invasive ductal carcinoma patients.

ID	Age	ER	PR	Her2	Ethnicity	Familial History	Metastasis	Lymph Node Involvement
IDC1	41	+	+	-	Lur/Lak	+	-	+
IDC2	35	-	-	+	Lur/Lak	-	+	+
IDC3	63	+	-	+	Lur/Lak	-	-	+
IDC4	64	+	+	+	Turk/Azeri	-	-	-
IDC5	45	+	+	+	Turk/Azeri	+	-	-
IDC6	33	-	-	-	Turk/Azeri	-	-	-
IDC7	39	-	-	+	Turk/Azeri	-	-	+
IDC8	31	+	+	-	Mazani	-	-	+
IDC9	57	+	-	-	Fars	-	-	+
IDC10	57	+	+	-	Fars	-	-	-
IDC11	43	+	+	-	Fars	+	-	-

Table 2. Gene expression study of the participants (statistical analysis on -ΔΔCTs).

Group		Number of Cases	Fold Change (95% CI)	One Sample P Value	Effect Direction	Two Sample P value	Effect Direction
VEGF-A	IDC	11	7.80 (3.14-19.34)	0.001**	UP	0.871	NS
	Fib	5	6.54 (0.58-73.71)	0.203	NS		
VEGF-B	IDC	11	23.65 (7.99-69.99)	0.000***	UP	0.082	NS
	Fib	5	2.15 (0.11-41.15)	0.639	NS		
VEGF-C	IDC	11	8.25 (2.66-25.62)	0.004**	UP	0.125	NS
	Fib	5	1.20 (0.11-13.48)	0.888	NS		
VEGF-D	IDC	11	27.01 (6.86-106.33)	0.001**	UP	0.384 #	NS
	Fib	5	2.03 (0.01-333.18)	0.800	NS		
VEGFR-1	IDC	11	45.86 (14.66-143.41)	0.000***	UP	0.004**	UP
	Fib	5	1.33 (0.28-6.29)	0.741	NS		
VEGFR-2	IDC	11	52.68 (17.96-154.47)	0.000***	UP	0.022*	UP
	Fib	5	2.11 (0.12-36.86)	0.636	NS		
VEGFR-3	IDC	11	7.89 (2.86-21.78)	0.003**	UP	0.015*	UP
	Fib	5	0.17 (0.01-5.56)	0.374	NS		
PlGF	IDC	11	14.91 (6.30-35.29)	0.000***	UP	0.616	NS
	Fib	5	10.41 (5.35-20.26)	0.002**	UP		

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; NS: non-significant; UP: significant up regulation. # Unequal variance was assumed.

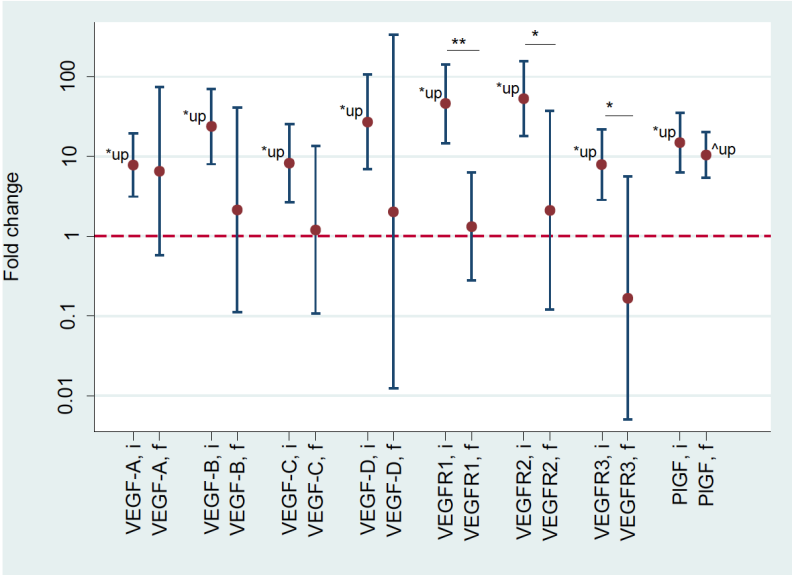

Fig. (1). Gene expression plot. Each pair of a gene shows its expression in IDC group (shown by I at the left) and in Fib group (shown by F at the right). Fold change =1 is the null hypothesis (the red hyphenated line). * Significant at P<0.05 for comparison of IDC and Fib groups. ** Significant at P<0.01 for comparison of IDC and Fib groups. *up: significant up-regulation in IDC group in comparison to the reference line. ^up: significant up-regulation in Fib group in comparison to the reference line. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

T score=12.924 as the cutoff point of overexpression, *VEGFR-1* had the most overexpression. The barcodes were not related to age, ER and Her2; however, gene overexpression seems to be more frequent in PR positive patients. Expression of *VEGF-C*, *VEGF-D* and *VEGFR-3* seems not to be related to lymph node involvement in contrast to previous literature (Fig. **2**). Graphical abstract of the results is shown (Fig. **3**).

4. DISCUSSION

In the present study, we aimed to reach a gene expression profile for our patient. We found overexpression of all the genes if comparing IDC group with normal calibrator. However, if comparing IDC group with Fib group, *VEGFR* genes had overexpression. The IDC group patients had passed their last course of neoadjuvant chemotherapy at least for two weeks before. It indicates that our tumor cells had repaired their malignant tendencies considering our gene expression study as a method of investigating these malignant tendencies.

Linardou *et al.* (2015) in Greece performed a gene expression study on *VEGF-C* and *VEGFR-1*. They found that overexpression of these two genes was associated with increased relapse, mortality and tumor marker expression [21]. In our study, heat plot showed more frequency of PR in patients with overexpression of *VEGFR-1*; however, further study is needed for better judgement. Lohri *et al.* (2014) accounted VEGFR-1 as a negative regulator of angiogenesis [22] while some other researchers believed that VEGFR-1 was angiogenesis promoting in adults tissue especially in tumor angiogenesis and in ischemia [23]. According to the literature, VEGFR-1 was associated with increased vascular permeability, VEGFR-2 was associated with increased endothelial cell survival and migration, and VEGFR-3 was associated with lymphangiogenesis and vasculogenesis [24]. In our study, all *VEGFR* genes showed significant up-regulation in IDC group in comparison to Fib group. It showed that the progression of tissue (from normal or benign tumor tissue) toward malignancy was associated with all of the above mechanisms. Sunitinib is a VEGFR inhibitor monoclonal antibody used in some cancers. Interestingly, literature did not support the beneficial effects of sunitinib as an inhibitor of VEGFRs. Elgebaly *et al.* (2016), in a meta-analysis, reported that sunitinib did not have a beneficial effect either alone or in combination with adjuvant chemotherapy [25]. Personalized medicine can respond this controversy. Our heat plot gives pilot information for future studies. Briefly, 5 out of 11 individuals of IDC group had *VEGFR-1* up-regulation at the probability of T score > ±threshold (P≥|T|) 0.001 in comparison to normal breast tissue. Among them, 4 individuals had simultaneous up-regulation of *VEGFR-2* and *VEGFR-3* at P≥|T| 0.01 and 0.05, respectively. It seems that VEGFR-3 is more dangerous than VEGFR-2 and VEGFR-2 is more dangerous than VEGFR-1 malignancy wise. Among PR+ patients, most of them had up-regulation of *VEGFR-1* at P≥|T| 0.01 and 0.001. Literature has shown that progesterone has a close association with angiogenesis [26-28]. It gives an idea to use percentage of PR positivity as a replacement biomarker for angiogenesis.

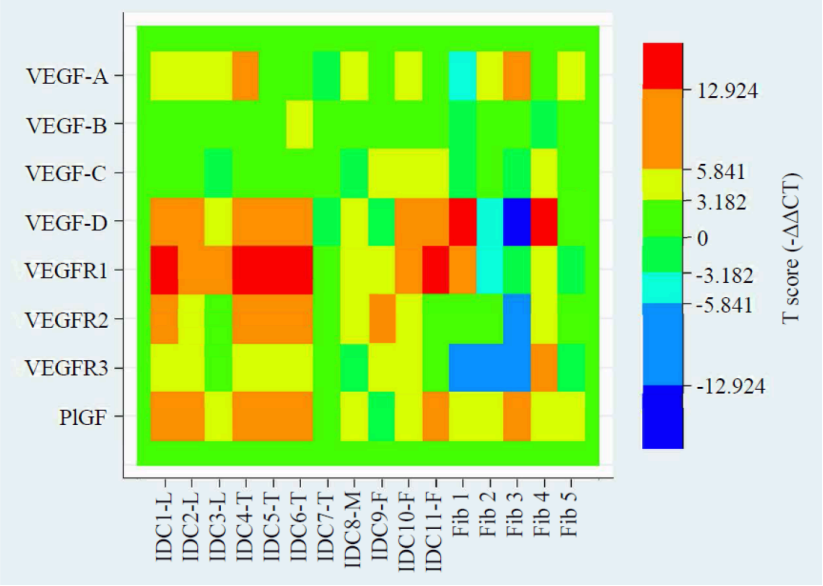

Fig. (2). Heat plot of gene expression for individual patients. (L): Lur/Lak ethnicity; (T): Turk/Azeri ethnicity; (M): Mazani ethnicity; (F): Fars ethnicity. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

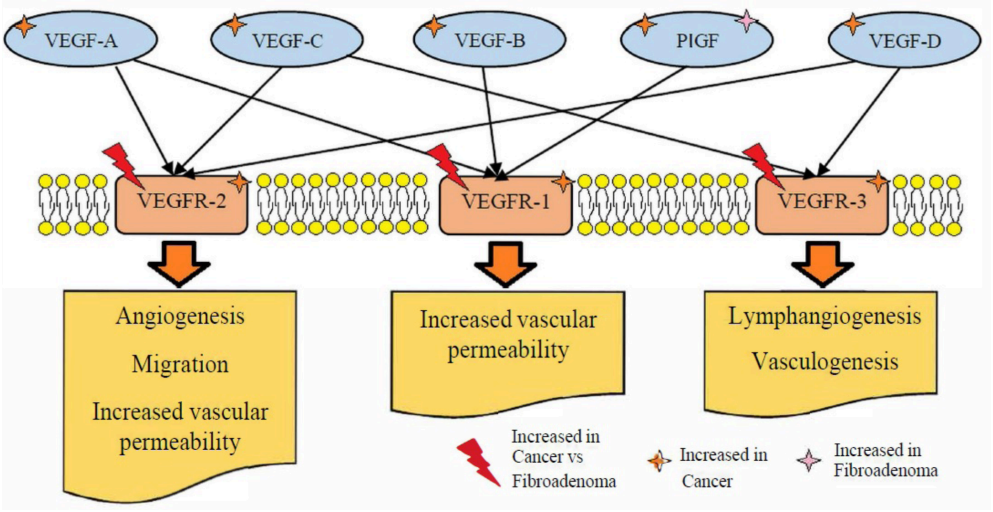

Fig. (3). Graphical abstract of the study. Breast cancer is associated with all of the above pathways. Fibroadenoma is associated with PlGF and VEGFR-1 pathway. The base of this figure was adapted from conceptualization of Meza-Junco and Sawyer [24]. We originally redesigned this figure. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The results of Fib group showed up-regulation of *PlGF* as the only significant change. Since there was no significant change for other genes, it was necessary to talk about the power of analysis in Fib group especially because of lower sample size in comparison to IDC group. *VEGF-C* and *VEGFR-1* showed 1<FC<2. *VEGF-B* and *VEGF-D* showed 2<FC<3. These small effect sizes and high P values indicated a possibility for lack of a real association. *VEGF-A* showed an enough effect size and low P value. It seemed that this gene might have an up-regulation in real and occurrence beta error. *VEGFR-3* showed down-regulation with enough effect size, indicating a possibility of beta error occurrence. In 3 out of 5 patients of Fib group, *VEGFR-3* showed down-regulation at P≥|T| 0.01 according to the heat plot. It indicated that this receptor did not have high activity in benign tumors. According to the graphical abstract, PlGF affects mainly *via* the VEGFR-1 pathway, which is the safest receptor of angiogenesis in comparison to the two other receptors. VEGF-A also acts in VEGFR-1 and VEGFR-2 pathways. PlGF is a member of VEGF family expressed by the placenta and a little by some other tissues. PlGF also helps other molecules of VEGF family for binding to VEGFR-1 and VEGFR-2 that VEGFR-2 has more tyrosine kinase activity for angiogenesis of embryo [29]. In the patho-

genesis of tumors, PlGF plays a role in chemotaxis and inflammation [30]. In addition, it has an important role in the pathogenesis of breast cancer [31]. However, there was not enough literature on its role in breast fibroadenoma. Our study showed PlGF as a common angiogenic factor in breast cancer and breast fibroadenoma.

There were some pilot studies similar to our project. Georgiou *et al.* (2013) designed a pilot study in Ioannina a city in Greece to investigate the impact of breast surgery on circulating biomarkers of angiogenesis. They took blood samples of 10 cancer and 6 fibroadenoma patients before surgery and at days 3 and 7 after surgery. They assayed plasma level of VEGF-A and interleukin (IL)-8 by Elisa as well as a set of angiogenesis related transcripts by real-time PCR. VEGF-A increased on day 3 and declined on day 7. IL-8 declined on day 7. They used a heat plot and found that different patients had a different profile of angiogenesis [32]. Valtola *et al.* (1999) designed a pilot study at the University of Helsinki on tissues of 6 patients with ductal carcinoma, 6 patients with lobular carcinoma, 8 patients with intraductal carcinoma and 12 normal tissues. They studied VEGF-C, VEGF-D and VEGFR-3 by IHC. They found that VEGF-C positive and VEGFR-3 positive vessels are more in cancer tissues. They believed that VEGFR-3 was also seen in lymphatic vessels; however, they did not find any association with lymphangiogenesis [33]. In our study, heat plot did not show any possible association for the genes of the above molecules and lymph node involvement. A meta-analysis showed that IHC positivity of VEGF-A and VEGF-C was associated with lymph node metastasis [34].

In general, there are a lot of studies about the role of angiogenesis in breast cancer. Triple negative breast cancer (TNBC) had always been a challenge. The most important challenge was the treatment; because there was no positive receptor to be targeted. Role of angiogenesis and anti-angiogenic agents has been discussed in this study. Thus, suppression of angiogenesis in TNBC cases is a therapeutic approach. Unfortunately, current evidence does not support the beneficial effect of anti-angiogenic agents. Therefore, literature believes that this therapeutic approach should be individualized [35, 36]. There are a lot of meta-analyses on the role of angiogenesis in breast cancer. A meta-analysis on clinical trials reported that sunitinib (a monoclonal antibody against VEGFRs) did not have a beneficial effect either alone or in combination with adjuvant chemotherapy [25]. Another meta-analysis on clinical trials reported that bevacizumab (a monoclonal antibody against VEGF-A) could decrease progression-free and event-free survival rates in patients with higher levels of plasma VEGF-A. However, data for overall survival was not available. This meta-analysis indirectly showed the role of personalized medicine in the treatment of breast cancer [37].

The strength of our study was to use a profile, whereas some previous studies had used some limited genes of *VEGF* and *VEGFR* families. We studied gene expression (real-time PCR) instead of protein expression (IHC) to show the recent tendencies of the tumor cells before surgery. However, there are some limitations to the clinical use of real-time PCR. The first one is that the primers are not uniform around the world. In this case, these gene families have a lot of polymorphisms and a lot of isoforms. These variations result in a heterogenic PCR product with different melting curves and peaks and various bands on gene electrophoresis [38]. In such conditions, if we use more specific primers we may miss some important isoforms. The second one is that there is not a uniform method for the calibration of gene expression. The third one is that it is not possible to calculate confidence interval for fold change of gene expression for individual patients in clinics. Using heat map for each individual may be helpful. If we use Z score we should gather a lot of normal tissues from general population. Instead, we can use T score for each patient from his/her 4 random samples of normal breast tissue. Hence, the reference tissue will be uniform and the problem of primers will be solved. We suggest personalized clinical trials according to the individual results of this T score based method of heat map.

CONCLUSION

Malignancy of breast tumors is associated with over expression of all the genes of this angiogenesis profile. However, only VEGFRs showed up-regulation in comparison to benign tumors. This profile can be used in other populations and other cancers. Individualized targeted therapy according to this research should be studied in future.

LIST OF ABBREVIATIONS

CI	=	Confidence interval
DCIS	=	Ductal carcinoma *in situ*
ER	=	Estrogen receptor
FC	=	Fold change
Fib	=	Fibroadenima
Her2	=	Human epidermal growth factor receptor 2
IDC	=	Invasive ductal carcinoma
IHC	=	Immunohistochemistry
PBS	=	Phosphate buffered saline
PCR	=	Polymerase chain reaction
PlGF	=	Placental growth factor
PR	=	Progesterone receptor
VEGF	=	Vascular endothelial growth factor
VEGFR	=	VEGF receptor

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The protocol was registered in the ethics committee of Lorestan University of Medical Sciences with number IR.LUMS.REC.1398.194. Other than the ethics committee of LUMS, the ethics committee of IUMS approved our protocol (IR.IUMS.REC.1397.983).

HUMAN AND ANIMAL RIGHTS

No Animals were used for studies that are base of this research. The reported experiments on human are in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013 (http://ethics.iit.edu/ecodes/node/3931).

CONSENT FOR PUBLICATION

Informed consent was obtained from the participants.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the study is available within the article.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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