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The Estimate of Cytokines and Fibroblast Growth Factors in Patients with Breast Cancer

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Abstract

Breast cancer is the most common cancer in women worldwide; it is a disease characterized by the growth of malignant cells within the mammary glands. It strikes males and females; there are diverse sorts of breast cancer. Cytokines and fibroblast growth factors that play a critical role in the development and progression of breast cancer.

The aim of the present study is to assess the role and the level of certain cytokines, and fibroblast growth factors in the serum of patients with breast cancer.

This is case-control study, blood samples were collected randomly from 60 newly diagnosed female patients, and 60 healthy female subject controls of all age groups. Venous blood samples were collected and analyzed for cytokines (Interleukin-19 and Interleukin-23) and fibroblast growth factors (FGF-18 and FGF-23) by using ELISA.

There was a highly significant difference ($p < 0.001$) detected in serum IL-19, IL-23, and FGF-18 levels of patients compared to healthy controls except for FGF-23, which was also recorded higher value in patients but not significantly compared to controls. There was a significant positive correlation ($p < 0.001$) between all these markers.

The present study reveals significantly higher concentrations of the markers detected in patients' blood serum compared to healthy individuals. The detection of the cytokines' levels and their relations with breast cancer will affect the diagnosis, knowledge about the disease immunology and thereafter effects on the treatments of patients with breast cancer depending on our findings.

1. Introduction

Breast cancer is a serious medical issue for women all around the globe. Approximately 1.7 million new cases and 522,000 mortalities happen every year because of breast cancer [1]. Globally, breast cancer-causing the second most leading death in peoples after lung cancer [2]. Breast cancer in the early phase commonly don't create any symptoms, yet as the tumor expands, delivered incorporate; lump in the breast, breast agony, knot under the armpit, swelling or thickness in the breast, the unconstrained release of the areola especially assuming bleeding and disintegration [3]. The cause of breast cancer is still unknown, but many factors like late pregnancy, late menopause, early menstruation, lack of breastfeeding, race, family history, fewer children, older age, the mutation in genes, and hormone therapies to determine the chance of breast cancer [4].

Within the immune system, the intercellular contact is the main objective mediated by interleukin (ILs). The interleukins are a superfamily of cytokines, and it is called as secretory immunomodulatory proteins which present complex immunological functions such as cell adhesion, maturation, proliferation and migration, and play a significant role in the inflammatory responses [5]. Interleukin -19 is a protein belongs to the IL-10 family. It is secreted by monocytes and induces fibronectin (FN), metastasis and cell proliferation in breast cancer cells to be assembled and expressed. Additionally, the IL-19 is the prognostic factor in a variety of cancers, particularly in breast cancer [6, 7].

Interleukin 23 (IL-23) belongs to the heterodimeric cytokine family IL-6 containing IL-12 p40, p19, and two disulfide-linked polypeptide chains. IL-23 is the potential therapeutic target for different kinds of chronic related immune-inflammatory disorders [8]. Interleukin (IL)-23/IL-23 receptor (R) gene expression levels are notably higher in breast cancer tissues. Furthermore, IL-23 and IL-23R expression levels are positively correlated with patients' tumor size, TNM stage and metastasis [9].

The family of 23 members with varied functions is the fibroblast growth factors (FGF). These members regulate various physiological and cellular processes including the development of skeleton and embryo, tumor growth, inflammation, morphogenesis, angiogenesis, tissue repair, growth, and cell differentiation [10, 11]. Fibroblast growth factor 18 is a protein that in humans is encoded by the FGF18 gene. FGF18 is mediate the proliferation of MDA-MB-231 cells through the ERK/c-Myc signaling pathway and induced epithelial-to-mesenchymal transition (EMT) factors to promote cancer migration and invasion. FGF18 plays an essential role in breast cancer's growth and metastasis via the ERK/c-Myc signaling pathway and EMT, indicating that FGF18 may be a potential molecular treatment target for breast cancer [12].

The important role of FGF18 in the development of limbs and skeletal growth has been investigated, probably through modulation of osteoblasts, chondrocytes, and osteoclasts [13]. In many types of cancers, the activation of the FGF pathway is the most common event. FGF18 expression was upregulated in the colon and ovarian tumors, and progression of a tumor as well as poor overall patient's survival is highly related to increased FGF18 mRNA and protein expression [14, 15].

FGF23 is one kind of protein containing approximately 251 amino acids (32 kDa) with a canonical N-terminal FGF homology domain [16]. The presence of FGF23 is varied biological structures; it is primarily expressed in osteocytes [17]. In the advanced stages of cancer, the FGFR signaling plays a significant role, especially when cancer cells survive and become immune to hormonal agents such as prostate resistant to androgen and breast resistant endocrine therapy cancers [18, 19].

The primary purpose of this study was to find and investigate the role and the level of cytokines and fibroblast growth factors in the serum of patients with breast cancer. Besides, no studies have been conducted to identify and evaluate the correlation among these biomarkers themselves; therefore, this study pointed out the positive correlation among these cytokines. The data finding supports the hypothesis that these inflammatory biomarkers may have a role in breast cancer.

2. Material and Methods

Participants and characteristics

In this cross-sectional study, the serum samples obtained from 60 newly diagnosed breast cancer adult females (stage II, III, and IV breast cancer patients before taking chemotherapy or radiation therapy) and 60 healthy females without any clinical evidence of breast cancer.

-Patients with hepatic, renal, thyroid, irritable bowel syndrome, infection including viral infection and other inflammatory disorders as well as the pregnant and breastfeeding women all were excluded from this study. The adult female participants in this study were belonged to the same ethnic group with different ages. This study performed between August – November, 2019 at Hiwa hospital in Sulaimaniyah province of Iraqi Kurdistan Region.

Sample collection and preparation

Blood serum was collected and stored by using serum separator gel tubes by allowing blood to coagulate for 20 minutes at room temperature, centrifuged at 4000 rounds per minute (RPM) for 20 minutes. Sera collected and stored in Eppendorf in a freezer at - 70 ℃ till the day available for the analysis.

Determination of serum cytokines and FGF levels

Levels of IL-19, IL-23, FGF-18, and FGF- 23 in the patients' sera were determined by sandwich enzyme-linked immunosorbent assays (ELISAs) and absorbance was measured at 450 nm according to the manufacturer's instructions (Bioassay Technology Laboratory, Korean Biotech Co., Ltd)

Statistical analysis

All the statistics were calculated using non-parametric tests. Statistics mean and standard deviations of the markers calculated. In addition to the simple t-test for the differences between markers concentrations means of apparently healthy control and breast cancer patients, the confidence interval C.I 95% of each marker of apparently healthy control and breast cancer patients performed. Also, multiple correlations between the levels of IL- 19, IL- 23, FGF- 18, and FGF- 23 in the serum of breast cancer patients were conducted. All analysis was fulfilled using Prism 7.0 Software (Graphpad, La Jolla, California, USA). The values $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered statistically significant.

3. Results

Population characteristics

There were no statistically significant differences between apparently healthy controls and breast cancer patients in terms of family history, menopausal status, smoking, age group, children, breastfeeding, and contraceptive pills. Except for age (yr) (Mean± SD), body mass index (BMI). The demographic characteristics of the studied cases revealed that the mean age of patients was 49.57 ±9.47 years, while the mean age of control cases was 45.20±9.57, which was significantly lower than the patients with the $p = 0.013$. The associations between the other demographic characteristics and the incidence with the disease revealed that there were no statistically significant differences between them. Except the BMI which shows significant difference ($p = 0.05$) between the patients and control group, as most of the cases of control appeared to be underweight and normal, while the patients were mostly overweight (Table 1).

Serum level of IL-19, IL-23, FGF-18, and FGF- 23 in the studied cases

The detected concentrations of all studied biomarkers in the blood of breast cancer patients were significantly ($P \leq 0.01$) higher than those of the healthy individuals except the FGF-23 of the cancer patients which was non significantly higher than the FGF-23 value of the control individuals (Table 2). The study results also show the lower and upper limits of each biomarker through the confidence interval with a probability of 95% (Table 3).

Table 1**.** Demographic characteristics of breast cancer patients and healthy controls.

Parameter	**Response**	**Patien t**	**Control**	**X^2_{stat}**	**P-value**
Age (yr) (Mean±SD)		49.57± 9.47	4 5.20±9.57	$t_{stat=.51}^{*}$	0.013
Family history	Yes	11	9	$0.24^{n.s}$	0.62
	NO	49	51		
Menopausal status	I am in/have been through the menopause	29	27	$0.14^{n.s}$	0.71
	I have not been through the menopause	31	33		
Smoking	Yes	5	4	$0.12^{n.s}$	0.73
	No	55	56		
Age group	30-40 years	12	15	$1.24^{n.s}$	0.26
	40-50 years	25	26		
	50-60 years	12	12		
	Older than 60 years	11	7		
BMI (kg/m^2)	Underweight (≤ 18.5)	1	4	7.76^{*}	0.05
	Normal (18.5 - 24.9)	22	31		
	Overweight (25 -29.9)	15	14		
	Obese (≥ 30)	22	11		
Children	Yes	52	48	$0.96^{n.s}$	0.32
	NO	8	12		
Breastfeeding	Yes	40	42	$0.15^{n.s}$	0.70
	NO	20	18		
Contraceptive pills or HRT	Yes	10	7	$0.62^{n.s}$	0.43
	NO	50	53		

Table 2. Means, Standard deviations, and confidence interval (C.I 95%) of studied markers.

Marker	Control	Patient	t-stat	P-Value
IL-19 ng/L	17.022±11.756	34.600±30.446	4.172	=0.001
95% C.I	14.047- 19.996	26.896- 42.304	**	
IL-23 ng/L	23.615±22.111	108.923±93.641	6.868	=0.001
95% C.I	18.020- 29.209	85.228 -132.617	**	
FGF-18 ng/L	150.068±48.745	215.690±20.374	9.621	=0.001
95% C.I	137.73 - 162.402	210.535 - 220.845	**	
FGF-23 ng/L	64.485±40.955	160.512±461.550	1.605	
95% C.I	54.122- 74.849	43.724- 277.300	n.s	=n.s

n.s not significant P>0.05, ** Highly significant P≤0.01.

Table 3. The correlation coefficient among the studied markers of breast cancer patients.

Patient	IL-19 ng/L	IL-23 ng/L	FGF-18 ng/L	FGF-23 pg/ml
IL-19 ng/L	-	0.461**	0.584**	0.748**
IL-23 ng/L	-	-	0.529**	0.405**
FGF-18 ng/L	-	-	-	0.529**

Authentication of four differentially expressed markers in patients with breast cancer and healthy controls are shown in (Figure 1). It shows the mean concentrations and the standard deviations of the markers in serum blood of breast cancer patients and healthy controls, all the markers exhibited higher values in breast cancer patients as compared to the healthy individuals without exceptions.

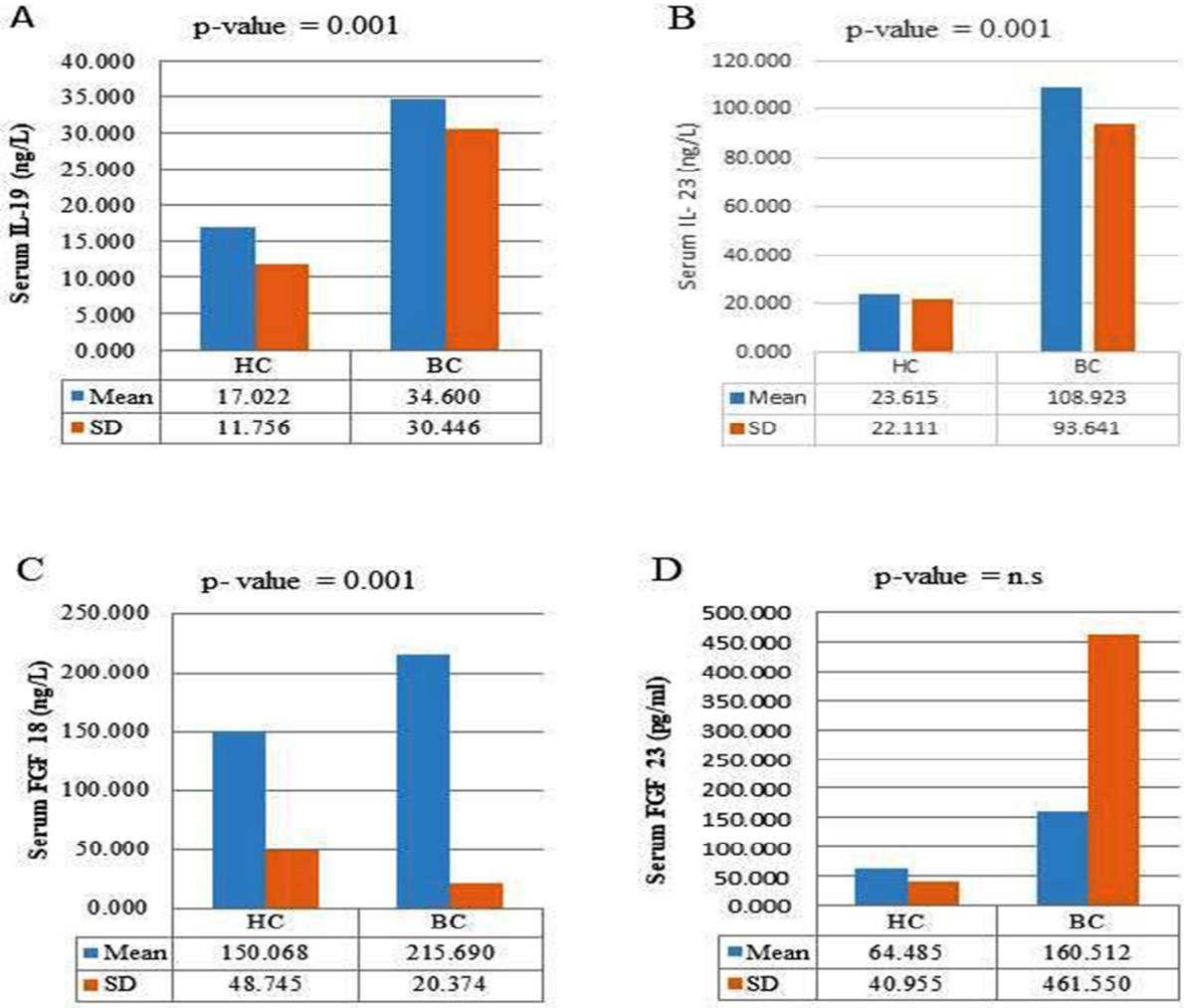

Figure 1. Histograms show the mean and standard deviations of (A) IL-19, (B)IL-23, (C) FGF18 and (D) FGF-23 concentration in the sera of breast cancer patients and healthy controls.

Correlation between the level of IL- 19, IL- 23, FGF- 18 and FGF- 23 in breast cancer patients

The results of correlations between the studied biomarkers in the blood sera of breast cancer patients emphasized that all studied biomarkers were related positively and significantly to each other (Figure 2).

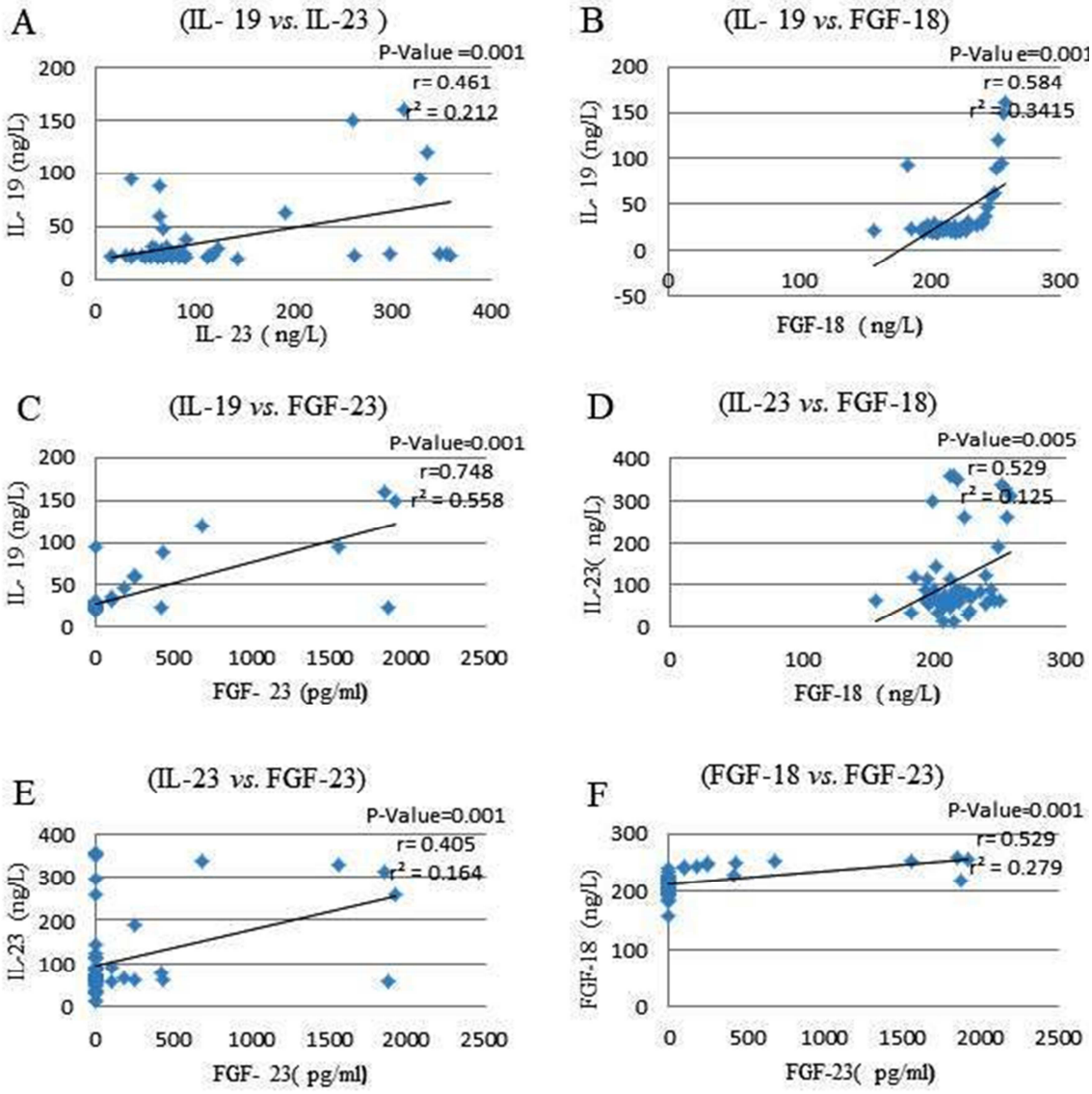

Figure 2. Positive correlations between different markers (A) IL-19 vs IL-IL-23, (B) IL- 19 *vs.* FGF-18, (C) IL-19 *vs.* FGF-23, (D) IL-23 *vs.* FGF-18, (E) IL-23 *vs.* FGF-23 and (F) FGF-18 *vs.* FGF-23 detected in the blood serum of breast cancer patients.

4. Discussion

Cytokines have different biological functions, and the detection of the cytokines' levels and their relations with breast cancer will affect the diagnosis, knowledge about the disease immunology and thereafter effects on the treatments of patients with breast cancer depending on our findings. In the present study, we found that the concentration of IL- 19 in the serum of breast cancer patients is significantly higher than that from healthy individuals, indicating that IL- 19 plays a crucial role in the progression and the development of breast cancer [20].

Moreover, animal studies were consistent with our study results, which revealed the high expression of IL-19 associated with advanced tumor stage and poor survival. In vivo, studied mice injected with IL-19-overexpressing breast cancer cell lines appeared more giant tumor cells and increased the metastatic

micronodules in the lung. High IL-19 articulation in breast cancer malignancy tissue is related to poor survival. IL-19 is vital in the pathogenesis of breast cancer cell growth [21].

In the present study, the level of IL-23 in patients with breast cancer was significantly higher than the healthy control individuals. Our findings supported by other research results with the same results and supposed patients with shorter live survival presented higher IL-23 values and suggested poor prognoses [22].

IL-23 is an important pro-inflammatory cytokine, and other research revealed that induction of IL- 23 inhibits breast cancer cell apoptosis through pre-treatment of patients with polyclonal antibody (PAb) IL-23p19 which is a neutralizing antibody specific for IL-23, may influence the effects of IL-23 on cancer cell behavior results suggested that PAb IL‑23p19 decreased IL-23-triggered cell proliferation whereas induced IL‑23 inhibited cellular apoptosis. Western blot analysis applied for the detection of molecules that may be liable for the changes. Outcomes indicated that PAb IL‑23p19 treatment decreased IL‑23‑caused up-regulation of B‑cell lymphoma‑2 protein expression and activation of the Janus kinase 2/signal transducer and activator of transcription three signaling pathway. The level of IL-23 positively correlated with the tumor size, TNM tumor stage, and advanced metastasis. IL- 23 may probably be the critical marker for prognosis and the management of breast cancer patients [9, 23].

The role and the level of FGF-18 in breast cancer disease have not been broadly studied in humans. No previous studies have shown the significant differences in serum cytokine levels of breast cancer patients compared to healthy controls, so the pathway of the carcinogenic mechanism of FGF- 18 remains indistinct. Fortunately, in this present study, we have revealed significant expression of FGF-18 in breast cancer patients compared to healthy controls. This mediates that FGF-18 may play a critical role in the development of breast cancer.

Some researchers found that Quantitative PCR is used to detect the transcription level of FGF- 18 under hypoxic conditions. A wound-healing assay was performed to evaluate cell relocation. Akt/GSK3β/β-catenin pathway protein was explored by using the western blotting pathway. FGF18 stimulated the epithelial-mesenchymal transition (EMT) and relocation of breast cancer cells through the initiation of the Akt/GSK3β/β-catenin pathway [24]. FGF18 exhibition may play an essential role in the progression of breast cancer. Moreover, it could be a predictive biomarker and potential molecular treatments for breast cancer [24, 25].

Surprisingly, the data from the present study revealed that the serum FGF-23 level did not significantly differ between breast cancer patients and healthy controls. However, the serum FGF-23 marker exhibited higher value in patients as compared to the healthy control individuals. The level of FGF-23 in breast cancer has not been widely studied in humans. Some of the research findings agree with the result of our study, and a study supports our finding in which the level of FGF-23 in those subjects who recorded as a carrier for breast cancer susceptibility gene (BRCA) mutation is higher than control subjects but not significantly different $p > 0.05$ [26].

The dysregulation of FGF signaling in cancer well understood, and it is becoming increasingly more probable that certain tumors grow based on activation of this pathway for their growth and survival. FGF/FGFR dependence provides the hope of creating new therapeutic methods that selectively target the FGF/FGFR axis in patients whose tumors recognized to affect FGF/FGFR dysregulation. This research achieves the determination of many: to treat the proper patient with the proper drug. However, the FGF/FGFR signaling axis is so intimately involved in many biologic processes that it will additionally be disturbed by therapeutic intervention. Furthermore, this requires extensive focused effort in the coming years to develop this new therapeutic possibility in cancer treatment [27].

The relationship among these cytokines were thoroughly analyzed, and a strong positive correlation have been detected among all these biomarkers (IL-19, IL-23, GFG-18 and, FGF 23). Thus, these cytokines IL-19, IL-23, FGF-18, and FGF-23 might act synergistically in the initiations and the developments of breast cancer tumors. This finding suggesting to use the ELISA technique in the screening of these inflammatory markers

and serve as a novel finding in breast cancer patients because these relationships among these four biomarkers have not reported previously.

Further researches are needed to determine the long term effects of the cytokine on a large number of cases before and after receiving the chemotherapy and/or hormonal therapy. Additionally, the serum level of cytokines and fibroblast growth factors are not reflecting tissue expression.

5. Conclusion

The investigation of the biomarkers (IL-19, IL- 23, FGF -18, and FGF- 23) in this study group of apparently healthy individuals and breast cancer patients using ELISA, revealed a significant expression level of (IL-19, IL- 23 and FGF- 18) in breast cancer patients in comparison to healthy controls except for FGF- 23, which was non significantly expressed; (IL-19, IL- 23 and FGF- 18), therefore, it could probably be used for the future diagnostic aspects. Such significant differences in the levels of these biomarkers (IL-19, IL- 23 and FGF- 18) among breast cancer patients and healthy individuals, furthermore the significant positive correlation between these cytokines (of IL-19, IL- 23, FGF -18, and FGF- 23) themselves; increase our understanding of the disease regarding the immunology to put specific future treatments based on our findings.

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Ethical approval

All procedures performed in studies involving human participants were accordance of the College of Pharmacy at University of Sulaimani research committee.

Information consent

All the participants included in this study provided written consent.

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