Tracking the Levels of Adiponectin and Interleukin-18 for Assessing the Efficiency of Chemotherapy in Suppressing Breast Cancer Progression

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Abstract

Breast cancer is a genetically and clinically heterogeneous disease. Cancerous cells multiplying abnormally in the breast, eventually spreading to the rest of the body if untreated. Breast cancer almost exclusively occurs in women. Cancer is a major public health problem worldwide with millions of new cancer patients diagnosed each year and many deaths resulting from this disease. Worldwide, female breast cancer has now surpassed lung cancer as the most commonly diagnosed cancer. SUBJECTS: Ninety two females were included in the current study, they were classified into three groups, depending on their health status (patients and healthy women) and the type of tumor suffered by the study patients: 32 female patients with cancerous breast tumors before receiving chemotherapy, 30 female patients with benign breast tumors (the pathological control group) and 30 females, who were included as a healthy control group. METHODS: Sandwich-ELISA method was applied to evaluate adiponectin and interleukin-18 concentrations in the sera of the study participants. RESULTS: Assessment of the adiponectin revealed a significant increase in the samples of malignant breast tumors group when compared with those of benign breast tumors (p=0.000) and healthy individuals (p=0.002). Interleukin-18 levels in the malignancy tumor group were significantly lower than their levels in the pathological (p=0.007) as well as healthy (p=0.000) controls groups. This study shows apparent slight decrease of adiponectin concentrations in cancerous patients group after getting approximately three consecutive doses of chemotherapy in comparison with its levels pretreatment, while non-significant difference was noted when comparing interleukin-18 levels after receiving the last dose of chemotherapy with its level before starting treatment. Although the levels of interleukin-18 increased after the end of the recommended course of chemotherapy, it did not rise to the levels of this protein in the healthy control group. The individual efficiency (sensitivity) of the evaluated criteria in the current study for distinguishing between cancerous and benign breast tumors was convergent. While the study recorded the highest specificity (93%) for adiponectin while the lowest specificity (40%) was recorded in interleukin-18. Adiponectin and interleukin-18 were able to distinguish 28 out of 32 breast cancer samples (88%).

Key words: Breast cancer, Adiponectin, Interleukin-18, chemotherapy

Introduction

Breasts are constantly going through change, from the time of their development. Benign breast conditions are very common and most breast changes are not cancerous. Through pregnancy and the menopause, this is because of the varying levels of the female hormones estrogen and progesterone. Most breast changes are likely to be normal or due to a benign breast condition, some of these conditions may cause problems and need treatment. The treatment of benign breast disease is usually simple and successful and does not involve simple surgical intervention in most complex cases [1]. Breast cancer is a genetically and clinically heterogeneous disease. Cancerous cells multiplying abnormally in the breast, eventually spreading to the rest of the body if untreated. Breast cancer almost exclusively occurs in women [2]. Cancer is a major public health problem worldwide with millions of new cancer patients diagnosed each year and many deaths resulting from this disease [3]. Worldwide, female breast cancer has now surpassed lung cancer as the most commonly diagnosed cancer. An estimated 2261419 new cases were diagnosed in women across the world in 2021 [4]. Cancer has been the second leading cause of death in the world and it has claimed 8.8 million lives, accounting for almost one in six deaths globally. Low-and middle-income countries accounted for nearly 70% of cancer deaths [5]. According to the latest statistic, breast cancer ranks the first number (2088849 new cases) of recorded cases worldwide. Breast cancer is a common disease that has a negative effect on women health, and is one of the leading causes of cancer related death, as it is the second most common cancer in women after lung, and it constitutes 23% of all cancer cases in women. Moreover; it is considered the first in global mortality (18.6% of cancer deaths, 626679 cases) [6]. For the Arab countries, the highest number of breast cancer patients is recorded in Syria. It is ranked 11th in the world, with 14.17 deaths, Lebanon with 12.19 deaths, Somalia with 12.18 deaths, Iraq with 11.70 deaths and 37th globally. This means that Iraq is in the first quarter, 11.30 and ranks 45th worldwide. Breast cancer is the highest cancer in women in Iraq, with a number of infections in 2018 (according to World Health Organization "WHO" statistics), ranged 5141 (20.3%) of all cancers, followed by lung cancer [7].

Adiponectin is mainly secreted by adipose tissue and in limited quantities by fat brown, salivary gland, cardiac tissue, cerebrospinal fluid, bone marrow. Adiponectin is synthesized as a single subunit with a primary sequence of 244 amino acids divided into four domains: at N-terminus a signal peptide, followed by a short hyper-variable region, a collagenous region containing 22 *Glycin-X-Y, where X and Y are occupied by Pro or Hyp more frequently than other residues* (Gly-X-Y) repeats, and at C-terminus a globular domain that interacts with the receptors [8]. Adiponectin exerts different biological functions, such as the regulation of glucose uptake and insulin sensitivity, and the stimulation of fatty acid oxidation. Moreover, adiponectin decreases the pro-inflammatory cytokines production (TNF-a and interleukin-6),

prevents monocytes migration, affects vascular endothelium, and mediates anti-atherogenic actions through the inhibition of sub-endothelial cholesterol accumulation [9]. The response to adiponectin is related to the structural heterogeneity of serum adipokine isoforms and to the target tissues. Indeed, adiponectin activates two of main seven transmembrane receptors, adiponectin receptor 1 (Adipo-R1) and 2 Adipo-R2; which they detected in almost every tissue, but generally with a different expression ratio and affinity for the adipokine oligomers [10]. Physiologically, adiponectin contributes to the 0.05% of total proteins present in the systemic circulation, with a concentration ranging from 3 to 30 μg/mL, depending on hormonal, inflammatory, pharmacological and dietary factors [11]. Increase adiponectin levels have been linked to an increased risk of developing type 2 diabetes, metabolic syndrome, insulin resistance, hypertension, cardiovascular disease, and different malignancies, including breast cancer. Adiponectin and breast cancer pre-clinical studies with different experimental approached highlight the role of adiponectin on angiogenesis along breast tumorgenesis [12]. Many epidemiological studies confirmed to the importance of adiponectin in the regulation of breast cancer cell growth and proliferation, many investigations have attempted to elucidate the role of adiponectin accordingly to estrogen receptor-α (ER-α) expression [129]. Several experimental models have documented the anti-proliferative and pro-apoptotic effects exerted by adiponectin in ER-α negative breast cancer cells [13].

Interleukin-18 is a multidirectional cytokine that regulates the immune response in various ways; in 1989, interleukin-18 was first described as an “interferon-inducing factor” [14]. Interleukin-18 plays an essential role in stimulating natural killer cells, and cellular anticancer activities also enhance the expansion of type of T helper cells (Th1) and cell activation [15]. Furthermore, interleukin-18 increases the expression of adhesion related molecules, nitric oxide synthase enzyme synthesis, and chemokine production. Interleukin-18, in combination with interleukin-2, causes a Th2 cell response and the production of interleukin-4 and interleukin-13 [16]. Simultaneously, interleukin-18 can reduce antitumor immunity in a programmeddeath-1 (PD-1) dependent manner. PD-1 is a co-inhibitory receptor that constitutes one of the top checkpoints [17]. Many polymorphisms in the interleukin-18 promoter region affect the transcript factor binding locations, which could be interleukin-18 expression quantitative trait loci [18].

The Study Individuals

Within ten months (from the beginning of October 2021 to the end of July 2022) 92 females were included in the current study, they were classified into three groups, depending on their health status (patients and healthy women) and the type of tumor suffered by the study patients. The first group included 32 female patients with cancerous breast tumors before receiving chemotherapy, their ages ranged between 32 and 67 years, while the second group included 30 female patients with benign

breast tumors, whose ages ranged between 31-68 years (the pathological control group). The third group included 30 females, who were included as a healthy control group, their ages ranged from 32-62 years. According to the questionnaire that was prepared based on the opinion of specialized doctors, which included complete information on: age, place of residence, occupation, period of onset of symptoms, medical history, stage of malignancy, location of the breast (as illustrated in **Figure 1**) and treatments used by patients, tumor cases were collected. Samples of patients with malignant tumors were collected from the National Hospital for Oncology and Hematology before receiving chemotherapy and were followed up during the chemotherapy period. Patients with cancerous tumors underwent surgical treatment 3-5 weeks before receiving first chemotherapeutic dose. Thirty of the cancer patients were married and had at least one child.

(A) **(B)**

Figure 1: Stage (A) and Location (B) of Malignant Tumor

While, cases of benign breast tumors were collected from a group of hospitals and centers in Najaf Governorate, namely: Breast Cancer Early Detection Unit in Al-Sadder Medical City, Private Al-Ameer Hospital, Private Al-Ghadeer-2 Hospital, Private Al-Najaf Hospital, and Private Al-Batoul Hospital.The patients with benign breast tumors were married and had 2-7 children. Healthy samples were collected from the study population environment, such as housewives, postgraduate students, as well as workers in the centers and hospitals where infected samples were collected. Finally, some cases underwent Cesarean delivery only as a surgical intervention prior to injury.

Limitations of the Study

The current study required exclusion the following cases: All participators (patients with breast tumors or healthy controls) who had suffered chronic diseases, *i.e.*; liver, renal, cardiovascular diseases, diabetes, hypertension and morbid obesity from participating in the current study. Breast patients hadn’t tumor before diagnosis breast tumor. Smoker women. Patients whose disease symptoms coincided with taking oral or intravenous contraceptives or who took oral contraceptives for 3 consecutive years before the onset of symptoms. Cases who underwent surgery within 5 years.

Samples Collection

After a fasting period of not less than 8 hours, 5 milliliters of venous blood samples were collected from the study subjects (patients and healthy ones) using gel tubes. After separating the serum from the study samples using a centrifuge at 5000 xg for 5 minutes. Serum samples were preserved using Eppendorf tubes at -20°C and stored until use.

Assessment of Adiponectin and Interleukin-18 in the Sera of Patients and Control Groups

Sandwich Enzyme Linked Immune Sorbent Assay (Sandwich-ELISA) method was applied to evaluate adiponectin and interleukin-18concentrations in the sera of the study participants.

Results and Discussion

Assessment of the adiponectin in the three study groups revealed a statistical significant increase of this marker concentration in the samples of malignant breast tumors group when compared with those of benign breast tumors (p=0.000) and healthy individuals (p=0.002). On the other side, the comparisons between breast tumors and healthy controls (p=0.121) failed to show same results. Interleukin-18 levels were evaluated in the samples of patient and healthy individuals of the current work, then the outcomes were analyzed statistically using ANOVA test.Interleukin-18 levels in the malignancy tumor group were significantly lower than their levels in the pathological (p=0.007) as well as healthy (p=0.000) controls groups. Furthermore, when the groups of benign breast tumors and healthy controls compared together; interleukin-18 levels didn't show significant differences between the two groups, as illustrated in **Table 1**.

Table 1: Levels of Adiponectin and Interleukin-18 in the Samples of Study Individuals

Parameters	Subjects (N) Mean ± S.D. Minimum-Maximum			*p-value*
	Malignant Tumors 32	Benign Tumors 30	Controls 30	
Adiponectin (ng/mL)	8.747±1.719 4.19-10.50	3.981±2.112 1.14-9.13	2.186±1.765 0.17-6.23	0.000 For M *vs* B 0.002 For M *vs* C 0.121 For B *vs* C
Interleukin-18 (ng/mL)	62.823±46.457 11.590-173.370	114.536±56.466 26.910-232.890	159.491±48.064 115.830-250.970	0.007 For M *vs* B 0.000 For M *vs* C 0.19 For B *vs* C

The mean difference is significant at the 0.05 level. M: Malignant Tumors, B: Benign Tumors, and C: Healthy Controls

Adiponectin and interleulin-18 levels were followed during three consequence chemotherapeutic doses. **Figure 2** shows apparent slight decrease of adiponectin concentrations in cancerous patients group after getting approximately three consecutive doses of chemotherapy in comparison with its levels pretreatment. **Figure 3** shows that there is a non-significant difference when comparing interleukin-18 levels after receiving the last dose of chemotherapy with its level before starting treatment. Although the levels of interleukin-18 increased after the end of the recommended course of chemotherapy, it did not rise to the levels of this protein in the healthy control group.

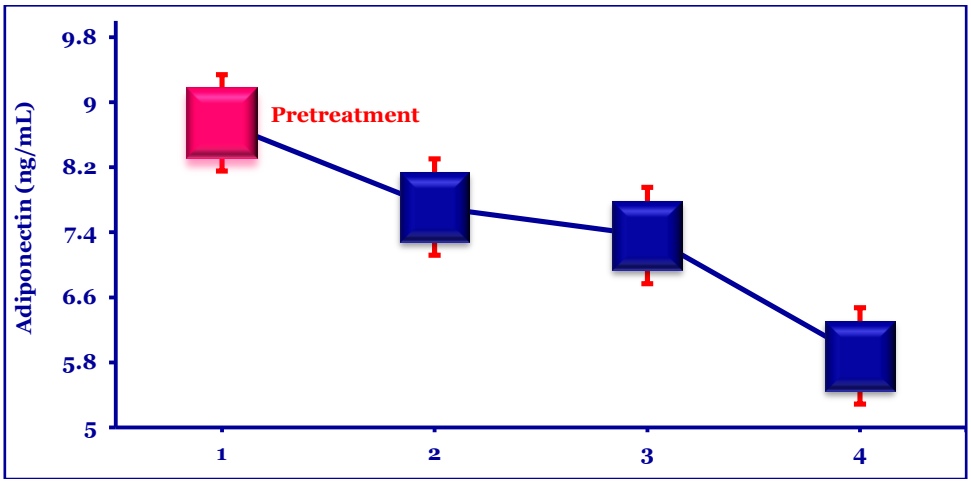

Figure 2: Follow-up Adiponectin Levels during Successive Chemotherapy

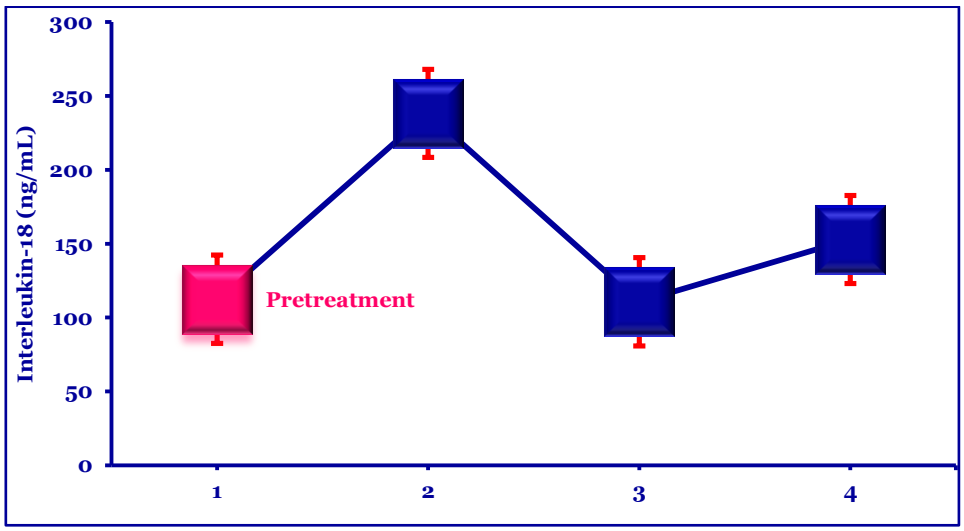

Figure 3: Follow-up Interleukin-18 Levels during Successive Chemotherapy

Adipose tissue is a bioactive endocrine organ that secretes a wide array of soluble factors, called adipocytokines, and contributes significantly to the development of the normal mammary gland and to breast carcinogenesis [19]. Adipose tissue plays a crucial role in signaling pathways that influence breast cancer development and progression. This depends not only from the adipose tissue mass but

also on breast adipocytes that surround breast tumor epithelial cells, which is the most abundant cell type in stroma of mammary gland [20].Among adipocytokines, adiponectin has a critical role in detecting breast cancer when it is elevated, especially in obesity-related cancers, including breast cancer. Many studies have demonstrated the relationship between hyperadiponectinemia and increased breast cancer risk, highlighting how patients with breast cancer who have higher adiponectin levels show a more aggressive phenotype [21].Direct evidence has been reported supporting the role of adiponectin as an inhibitory factor for breast cancer development and how it attenuates the growth of MDA-MB-231 cells by inhibiting cell proliferation and inducing apoptosis [22]. On the other hand, adiponectin does not increase DNA fragmentation and apoptosis in T47D cells, suggesting that its pro-apoptotic effect results from a cell type–specific response [23].

Interleukin-18 is a marginally pro-inflammatory cytokine belonging to interleukin -1 family. biologically it inactive form of 24 kDa and transformed to the active form of 18kDa by proteolytic cleavage mediated by interleukin-1B converting enzyme (ICE) [24] Interleukin-18 was identified as an interferon-γ-inducing factor, enhancing the Th1 immune-response by stimulation of natural killer (NK) and cytotoxic T cells. It has been additionally described as an important player in inflammation, autoimmunity and anticancer immune response. More recently, it was described as an important element in the inflammation system that is a complex of intracellular cysteine proteases that activatecaspase-1 and leads to the activation of the inflammation process [25].The major biological function of interleukin-18 is to increase IFN-γ production by T cells and to promote the differentiation of IFN-γ-producing (Th1) CD4+ T cells. These effects of interleukin-18 are synergistic with interleukin-12 and the up-regulation of interleukin-18Rα gene and surface expression by interleukin-12 contribute to this effect [180].In the present study, it is believed that the changes in the levels of interleukin-18 before receiving chemotherapy and during the treatment phase is due to the inflammatory response resulting from receiving chemotherapy after the first dose, given that the treatment is a chemical that stimulates the immune system, followed by the stage of inflammatory suppression and a decline in interleukin-18 levels to approximately its levels before start treatment.

Calculation of sensitivity and specificity used for assessing the efficiency of the tested parameters to be suggest them as tumor markers. Receiver operating characteristic (ROC) was applied to evaluate the diagnostic efficiency of each criteria in the current work (**Figures 4** and 5 for adiponectin and interleukin-18; respectively). **Table 2** shows the area under the curve and cutoff value for the evaluated criteria in the current study**.** The individual efficiency (sensitivity) of the evaluated criteria in the current study for distinguishing between cancerous and benign breast tumors was convergent. While the study recorded the highest specificity (93%) for adiponectin while the lowest specificity (40%) was recorded in interleukin-18.

Table 2:ROC Analysis of the Adiponectin and Interleukin-18 as Diagnostic Tumor Markers for Breast Cancer

Parameters	AUC	SE	*p-value*	Cutoff value	Sensitivity%	Specificity%
Adiponectin	0.950	0.011	0.000	7.11	88	93
Interleukin-18	0.631	0.107	0.045	13.17	88	40

AUC: Area Under Curve, SE: Standard Error

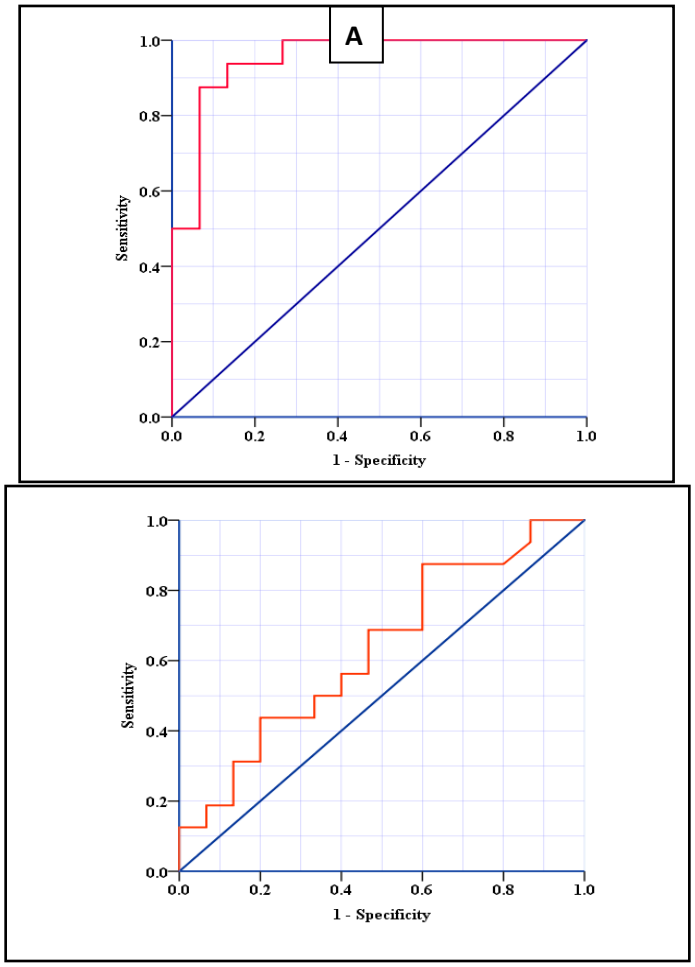

Figure4: ROC Curve of A: Adiponectin, B: Interleukin-18

Combined sensitivity of the two parameters were examined in the group of breast tumors, as summarized in the **Table 3**. Adiponectin and interleukin-18 were able to distinguish 28 out of 32 breast cancer samples (88%).

Table 3: Combined Sensitivity of the Evaluated Parameters

Markers	Adiponectin	Interleukin-18
Adiponectin	88	91
Interleukin-18	91	88

Increasing the sensitivity of the criteria presented in the current work by linking them to each other offers an opportunity to raise use them as tools to distinguish

cancer than other breast diseases. On the other hand, this association may provide a mechanism to distinguish the different stages of cancer which contributes for strengthening the ability of the physician to determine the method of treatment to be followed more accurately. These findings reinforced the objective of the present study to investigate the possibility of using parameters as diagnostic tools for breast cancers. **Table 4** shows the combination of the evaluated parameters was 93%.

Table 4: Combined Specificity of the Studied Criteria

Markers	Adiponectin	Interleukin-18
Adiponectin	**93**	**93**
Interleukin-18	**93**	**40**

Conclusion

Adiponectin and interlukin-18 are efficient diagnostic tools for distinguishing malignant from benign breast tumors. Moreover adiponectin is effective follow-up marker to assess the efficiency of chemotherapy in suppressing cancer progression, finally; the efficiency of tumor markers in diagnosing tumors increases when the criteria are evaluated together for the examined sample.

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