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ORIGINAL ARTICLE

The regulatory effects of interleukin-12 on interleukin-18 and interferon-γ production in Egyptian breast cancer patients

Soheir R. Demian [a], **Mona Hamdy** [a], **Inas M. Ali** [b,*]

[a] *Immunology Department, Medical Research Institute, Alexandria University, Egypt*
[b] *Cancer Management and Research, Medical Research Institute, Alexandria University, Egypt*

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KEYWORDS

Interleukin;
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Abstract *Background:* Breast cancer is the commonest form of cancer in women throughout the world. The outcome of malignant neoplasia in human is often accompanied by defective cellular immunity.
Objective: To investigate the regulatory effects of IL-12 on IL-18 and IFN-γ production in patients with breast cancer. In addition, the correlations between IL-18 and IFN-γ levels with different prognostic factors.
Methods: This study included 40 female patients with histologically proved breast cancer. They were divided into two groups metastatic and non-metastatic. Peripheral blood mononuclear cells were isolated from freshly drawn heparinized blood of each subject and cultured with and without recombinant IL-12 supplementation. IL-18 and IFN-γ levels assessed using ELISA before and after the addition of IL-12.
Results: The level of IL-18 in culture supernatants ranged from 0.69 to 0.95 ng/ml with mean value of 0.8 ng/ml in non-metastatic patients, whereas IL-18 in metastatic patients levels varied from 0.5 to 0.7 ng/ml with a mean value of 0.6 ng/ml after supplementation with IL-12. In non-metastatic patients the levels of IFN-γ ranged from 1.2 to 1.9 ng/ml with mean value 1.4 ng/ml while in

* Corresponding author. Mobile: +20 10 6566583.
E-mail address: Inas.el.badry@hotmail.com (I.M. Ali).

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metastatic patients the levels ranged from 0.7 to 1.2 ng/ml. Statistical analysis of data revealed that the supplementation of culture with IL-12 significantly increased the mean values of IL-18 IFN-γ in different clinicopathological parameters.

Univariate analysis by age, tumor size, grade, number of lymph nodes, ER-PR, IL-18 level, and HER2 neu. Indicated that patients with IL-18 level higher than the median at the diagnosis had higher survival results than those with lower IL-18 concentration. In multivariate analysis IL-18 did not have an independent influence on the survival.

Conclusion: Findings provide insights that interleukin-12 may be a potent stimulant of the immune response by enhancing the production of IL-18 and IFN-γ. We can suggest that, IL-12 could be used as a therapeutic agent for improving prognosis associated with breast cancer.

1. Introduction

Breast cancer is an important public health problem. It is the commonest form of cancer in women throughout the world.[1] In Egypt, it ranked first among malignancies in females, with incidence of 37.3%.[2]

Multiple factors are associated with increased risk of its development. Breast cancer has unpredictable course and characterized by long duration and heterogeneity among patients. However, there has been decline in breast cancer mortality overall which is attributed to both success of early detection programs and advances in treatment.[3]

Cell mediated immunity plays an important role in the host defense mechanisms to malignant neoplasia. It is enhanced by T helper cells and has different effects on the destruction of malignant cells of the numerous tumor models.[4,5]

Interleukin-12 (IL-12) and IL-4 induce differentiation of naïve T cells towards Th1 and Th2 cells, respectively.[6]

Previous reports demonstrated the shift from Th1 to Th2 in peripheral blood mononuclear cells (PBMCs) from cancer bearing patients.[7]

Malignant neoplasia in humans is often accompanied by defective cellular immunity.[8]

There is substantial evidence for immune defects in patients with breast cancer. They have lower absolute numbers of peripheral blood lymphocytes,[9] but increased numbers of functionally suppressive CD4+ CD25+ Treg.

Studies of these cells and NK cells have revealed molecular and cellular mechanisms of immunosuppression.[10]

The prevalence of T (reg) is increased in the peripheral blood as well as in the tumor microenvironment in patients with invasive breast cancer. These T (reg) may mitigate the immune response against cancer, and may partly explain the poor immune response against tumor antigens.[11]

Interleukin-12 (IL-12) is an important cytokines that induce the activation of natural killer (NK) cells, T cells and expression of anti angiogenic genes.[12]

Decreased IL-12 production may be an important factor for progressive tumor growth.[13] IL-12 has the ability to produce a distinct sequence of molecular and phenotypic changes in the tumor leading to anti tumor response.[14] It up regulate Fas expression in human breast cancer cells which plays a role in the elimination of metastatic tumor activity.[15]

It was found that IL-12 treatment of a murine model breast cancer lead to tumor regression and systemic anti tumor immunity.[16]

Furthermore IL-12 induces naïve CD4+ T helper cells to develop into Th-1 cells *in vivo* and *in vitro*. Resting T cells do not express interleukin-18 receptor (IL-18R) and fail to produce IFN-γ. Pre-treatment of T cells and B cells with (IL-12) rendered them responsive to IL-18 by activation of STAT4 (a protein inside the cell plays an important role in IFN-γ production) and induction of interleukin-18 receptor (IL-18R).[17]

These IL-12 stimulated cells had both high and low affinity IL-18R and an increased IL-18R mRNA expression. IL-18 receptor transduces a signal that activates a kinase and consequently explains their important direct effect on IFN-γ production.[18]

IL-18 is a multifunctional cytokines. It plays an important role in the manifestations of T cell mediated immunity in cancer patients through activating natural killer (NK) cells and IFN-γ production by peripheral blood mononuclear cells (PBMC's) which exhibits a number of immune regulatory effects.[19]

Many studies revealed that IFN-γ helps to restore the cellular immunity and shift the balance from Th2 to Th1. Also its production by T cells and natural killer cells is central to the scenario of IL-18 induced anti-tumor response.[20]

Mononuclear cells of patients with breast cancer have a defective IL-12 and IFN-γ production capability while generating higher amounts of IL-10 and IL-4.[21,22] This may explain why the anti-tumor response to breast cancer is impaired. Although information is steadily accumulating describing the functional activities of these cytokines, very few data have been reported on the regulation of their production.[23]

Therefore, the characterization of different cytokines production is important for investigating the impaired cellular immunity and the application of immunotherapeutic principles to treatment of breast cancer.

The aim of this work is to investigate the regulatory effects of IL-12 on IL-18 and IFN-γ production in patients with breast cancer. In addition, the correlations between IL-18 and IFN-γ levels with different prognostic factors were studied before and after *in vitro* treatment by IL-12.

2. Methods

2.1. Subjects

This study was conducted on 40 female patients with histologically proved breast cancer. They were divided into two-groups (metastatic and non-metastatic).

Patients were recruited from cancer management and research department, Medical Research Institute, Alexandria University. The study also included 20 healthy age matched females were taken as a control group.

Between January 2006 and September 2006, 40 patients with breast cancer presenting and treated in cancer management and research department, Medical Research Institute were included in the study and followed for 4 years. Eligibility criteria were histologically proved breast cancer, adequate hematologic parameters, normal electrocardiogram, no history of cardiac problems and no previous chemotherapy, complete hematologic and biochemical studies, assessment of performance status according to Karnofsky scale, radiological investigation including X-ray chest and CT abdomen and pelvis, bone scan. Assessment of estrogen and progesterone receptor as well as HER_2 neu was performed by immunohistochemistry.

2.2. Immunological studies

2.2.1. Separation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from heparinized freshly drawn blood of each subject under study before treatment by Ficoll-hypaque density gradient centrifugation.[21]

2.2.2. Stimulation with IL-12

The separated cells were cultured either alone or with the optimum dose of recombinant IL-12 r (IL-12) for 72 h at 37 °C and 5% CO_2 atmosphere. The culture supernatants isolated and stored at −70 °C until cytokines assay.

2.2.3. IL-18 and IFN-γ immunoassay

IL-18 and IFN-γ levels in culture supernatant of each sample were assayed before and after the addition of IL-12 using enzyme linked immune absorbent assay (ELISA).[22]

2.3. Survival analysis

Actuarial curves of overall survival were obtained according to Kaplan–Meier method. Comparison between curves were made using log rank test.

3. Results

3.1. Clinical assessment

Patient's characteristics are summarized in Table 1.

Table 1 Clinicopathological characteristics of patients.

Clinicopathological characteristics	Frequency			
	Non-metastatic		Metastatic	
	No.	%	No.	%
Age (years)				
30−	2	10	2	10
40−	10	50	8	40
50+	8	40	10	40
χ^2, p	1.85, 0.56			
Menstrual status				
Pre-menopausal	12	60	10	50
Post-menopausal	8	40	10	50
χ^2, p	0.98, 0.71			
Tumor grade				
Grade II	18	90	14	70
Grade III	2	10	6	30
χ^2, p	1.250, 0.26			
Tumor size				
T_1 (< 2 cm)	2	10	0	0
T_2 (2–5 cm)	14	70	12	60
T_3 (> 5 cm)	4	20	8	40
χ^2, p	1.744, 0.418			
Lymph node status				
+ve	16	80.0	20	100.0
−ve	4	20.0	0	0.0
χ^2, p	2.22, 0.13			
Number of Lymph node involvement				
1–3	2	10.0	10	50.0
4–10	14	70.0	8	40.0
> 10	0	0.0	2	10.0
χ^2, p	6.48, 0.09			
Hormonal receptor status				
ER				
−ve	2	10.0	4	20.0
+ (Mild)	10	50.0	4	20.0
+ + (Moderate)	4	20.0	8	40.0
+ + + (Strong)	4	20.0	4	20.0
χ^2, p	2.29, 0.51			
PR				
−ve	2	20.0	4	10.0
+ (Mild)	6	30.0	6	30.0
+ + (Moderate)	8	40.0	8	40.0
+ + + (Strong)	4	10.0	2	20.0
χ^2, p	0.67, 0.82			
HER_2 neu				
Positive	4	20	7	35
Negative	16	80	13	65
χ^2, p	0.57, 0.72			

3.2. Immunological results

3.2.1. IL-18 assay

The mean value of IL-18 in culture supernatants of the normal controls before IL-12 supplementation was 1.44 ± 0.16 ng/ml while it was 0.80 ± 0.10 and 0.61 ± 0.07 ng/ml in non-metastatic and metastatic, respectively (Table 2).

After IL-12 supplementation, the mean value of IL-18 in normal controls was 2.72 ± 0.28 ng/ml while, it was 1.4 ± 0.21 and 0.95 ± 0.14 ng/ml in non-metastatic and metastatic patients, respectively (Table 2).

The percent of change (stimulation%) in IL-18 levels induced by IL-12 supplementations in the culture supernatants of the normal controls (88.89%), non-metastatic patients (76.25%) and metastatic group (55.7%) showed a significant increase in the IL-18 levels after IL-12 supplementation (Fig. 1).

3.2.2. INF-γ assay

Regarding IFN-γ levels, it was observed that its mean value in the culture supernatants of normal controls was

Table 2 IL-18 levels (ng/ml) in culture supernatant of normal controls, non-metastatic and metastatic patients with breast cancer before and after stimulation with IL-12.

	Control "gp. I"	Non-metastatic "gp. III"	Metastatic "gp. II"
Before			
Range	1.16–1.64	0.69–0.95	0.5–0.7
Mean	1.44	0.80	0.61
SD	0.16	0.10	0.07
F	137.78		
p	0.0001		
After			
Range	2.3–3.10	1.2–1.9	0.75–1.20
Mean	2.72	1.41	0.95
SD	0.28	0.21	0.14
F	176.733		
p	0.0001		

Table 3 IFN-γ levels (pg/ml) in culture supernatant of normal controls, non-metastatic and metastatic patients with breast cancer before and after stimulation with IL-12.

	Control	Non-metastatic	Metastatic
Before			
Range	12–22	3.0–8.0	2.0–6.0
Mean	15.70	5.00	3.70
SD	3.09	1.49	1.34
F	95.811		
p	0.00001		
After			
Range	22.0–45.0	6.0–15.0	4.0–8.0
Mean	31.80	9.30	6.30
SD	8.85	2.91	1.34
F	65.740		
p	0.0001		

15.70 ± 3.09 pg/ml whereas it was 5.00 ± 7.44 and 0.70 ± 1.34 pg/ml in non-metastatic and metastatic patients, respectively. However after IL-12 supplementation the mean value of IFN-γ was 31.8 ± 8.85 pg/ml while it was 9.30 ± 2.91 and 6.3 ± 1.34 pg/ml in non-metastatic and metastatic patients, respectively (Table 3).

Concerning the percent of change (stimulation %) in the IFN-γ levels, it was observed that the % change in the normal controls was 102.55% and in non-metastatic patients was 86.00%. Whereas, in the metastatic patients it was 70.70% indicating a significant increase ($p = 0.00001$) in the IFN-γ levels induced by IL-12 supplementation (Fig. 2).

3.2.3. IL-18 and IFN-γ and clinico-pathological findings

Statistical analysis of our data revealed a significant decrease in the mean values of IL-18 as well as of IFN-γ in the different clinicopathological parameters (large tumor size, negative hormone receptor status, positive HER_2 neu, and hight grade tumor in both metastatic and non-metastatic.

3.2.4. Correlation analysis in non-metastatic patients

Our data showed, significant positive correlations between IL-18 levels before and after supplementations with IL-12 ($r = 0.85$, $p = 0.001$) and also between IL-18 before supplementation and the level of IFN-γ after supplementation with IL-12 ($r = 0.63$, $p = 0.023$). Besides, after IL-12 supplementation there was a significant positive correlation between the level of IL-18 and IFN-γ ($r = 0.58$, $p = 0.031$). Moreover, there was a significant positive correlation was indicated between IFN-γ before and after supplementation with IL-12 ($r = 0.64$, $p = 0.01$) (Table 4).

As regards, correlation analysis in metastatic patients with breast cancer, it showed a significant positive correlation between IL-18 levels before and after supplementation with IL-12 ($r = 0.79$, $p = 0.002$). Also, there was a significant positive correlation between the levels of IL-18 before supplementation and IFN-γ levels after supplementation ($r = 0.70$, $p = 0.011$). After IL-12 supplementation, there was a significant positive correlation between IL-18 and IFN-γ levels ($r = 0.61$, $p = 0.025$). Also, there was a significant positive correlation between IFN-γ before and after supplementation with IL-12 ($r = 0.74$, $p = 0.005$). In addition, there were non-significant correlations were observed between the other parameters of the metastatic patients (Table 5).

3.2.5. Survival and IL-18

0.8 ng/ml was considered as cut off value for non-metastatic and 0.6 ng/ml for metastatic breast cancer patients.

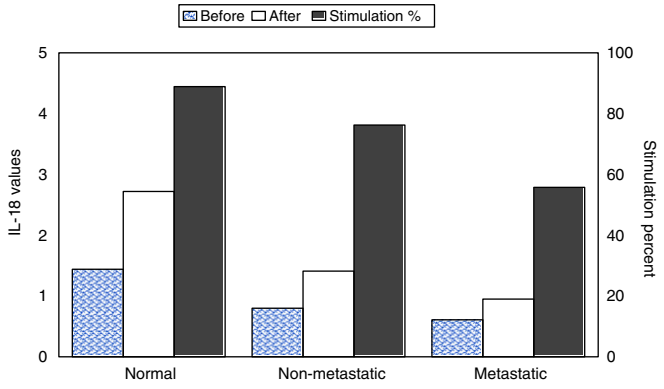

Figure 1 The mean values and the percent change (stimulation %) of IL-18 (ng/ml) in the culture supernatants of normal controls, non-metastatic and metastatic patients with breast cancer.

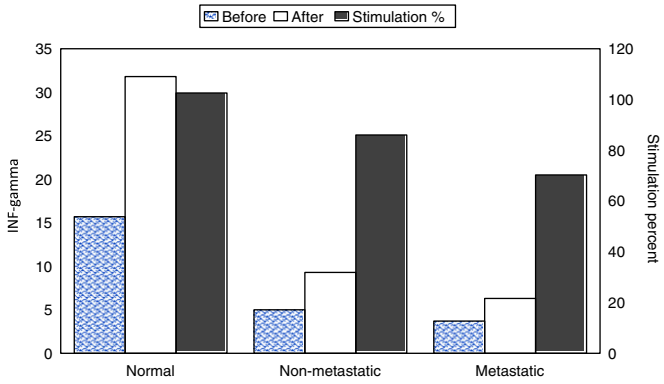

Figure 2 The mean values and the percent change (stimulation %) of INF-γ (pg/ml) in the culture supernatants of normal controls, non-metastatic and metastatic patients with breast cancer.

Table 4 Correlation analysis between IL-18 and IFN-γ levels in culture supernatants of non-metastatic patients with breast cancer before and after stimulation with IL-12.

	IL-18 before stimulation	IL-18 after stimulation	IFN-γ before stimulation
IL-18 after stimulation			
r	0.85		
p	0.001*		
IFN-γ before stimulation			
r	0.25	0.33	
p	0.25	0.19	
IFN-γ after stimulation			
r	0.63	0.58	0.64
p	0.01*	0.038*	0.046*

* Significant at $p < 0.05$.

Table 5 Correlation analysis between IL-18 and IFN-γ levels in culture supernatants of metastatic patients with breast cancer before and after stimulation with IL-12.

	IL-18 before stimulation	IL-18 after stimulation	IFN-γ before stimulation
IL-18 after stimulation			
r	0.79		
p	0.002*		
IFN-γ before stimulation			
r	0.19	0.41	
p	0.45	0.11	
IFN-γ after stimulation			
r	0.70	0.61	0.74
p	0.011*	0.025*	0.005*

* Significant at $p < 0.05$.

At the end of 4 years, the overall survival of patients with IL-18 level higher than 0.8 ng/ml was 70% versus 45% for those having IL-18 concentration less than 0.8 ng/ml (Fig. 3).

In metastatic breast cancer the overall survival of patients with IL-18 level higher than 0.6 ng/ml was 30% versus 10% for those having IL-18 concentration less than 0.6 ng/ml (Fig. 4).

In univariate analysis by age, tumor size, tumor grade, number of lymph nodes, ER-PR, IL-18 level, and HER_2 neu. It was found that patients with IL-18 level higher than 8 ng/ml at the time of diagnosis had higher survival than those with lower IL-18 concentration (70% versus 45%) in non-metastatic and (30% and 10%) for metastatic breast cancer patients.

In multivariate analysis IL-18 did not have an independent influence on the survival.

4. Discussion

Breast cancer like other malignancies results from stepwise genetic alteration of host cells and non-genetic changes in the behavior of not only malignant but also host cells that interact with the tumor such as immune alterations.[7]

Previous reports have indicated that outcome of malignant neoplasia in human is often accompanied by defective cellular immunity. To induce more effective antitumor immune-reaction, increasing the production of Th1-cytokines is needed. T-helper type I (Th-1) cells promote cellular immunity through increased interferon-γ production. Therefore, cytokine kinetics may represent a mirror of the immunologic phenomena occurring in the tumor micro-environment, where immune and malignant cells interact. Yet, cytokines are currently used in a clinical setting to polarize the immune response against cancer.

Despite the large amount of information available on immune system physiology, little is known about the role of cytokines in modulating the effectiveness of immunotherapy, and the clinical trials aimed the treatment of patients with cancer.[15,16]

Interleukin-12 is an important cytokine that induce the activation of natural killer (NK) cells, and T cells. It induces naïve CD4+ T helper cells to develop into Th1 cells *in vivo* and *in vitro*. Pretreatment of T cells and B cells with IL-12 rendered them responsive to IL-18.[13]

Interferon-γ production by T- and NK cells is central to the scenario of the IL-18 induced anti-tumor cascade reaction.[16] Thus assessing the capacity of PBMCs in cancer patients to produce IFN-γ as well as IL-18 in response to IL-12, would be important for investigating the impaired cellular immunity

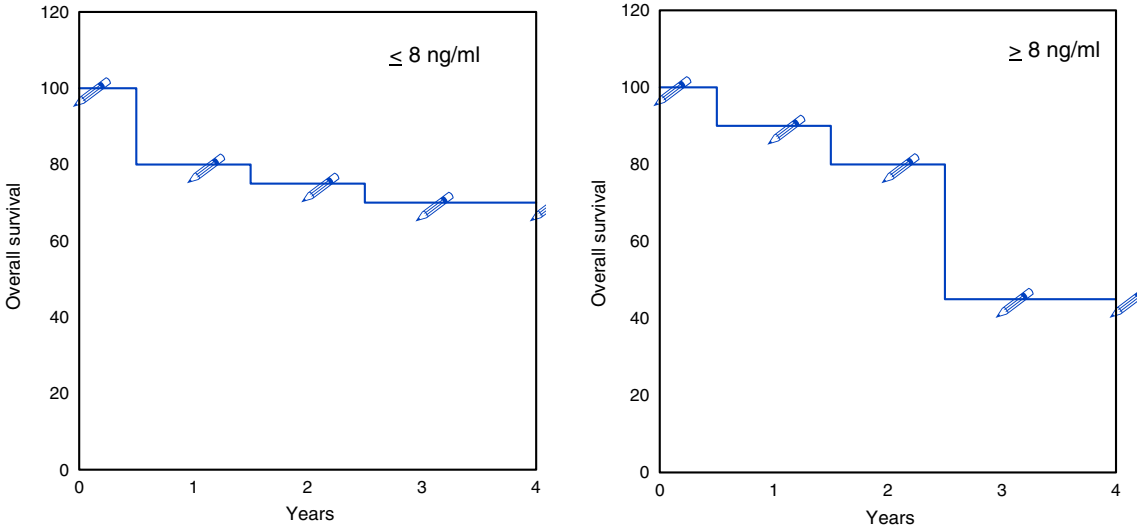

Figure 3 Overall survival in non-metastatic breast cancer patients.

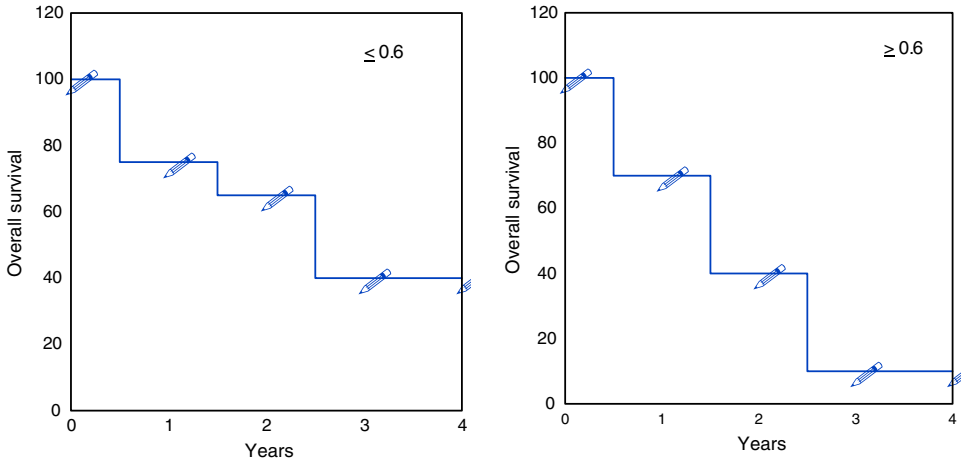

Figure 4 Overall survival in metastatic breast cancer patients.

and the application of immunotherapeutic principles to the treatment of breast cancer.

The results of the present study revealed that the levels of IL-18 were significantly decreased ($p = 0.0001$) in non-metastatic (0.80 ± 0.10 ng/ml) and metastatic (0.61 ± 0.07 ng/ml) patients with breast cancer than normal controls (1.44 ± 0.16 ng/ml) before stimulation with IL-12.

The impairment in the production of this cytokine can be explained by the results of Campoli et al.[8] who clearly demonstrated that, progressively growing tumors impair the adaptive immune response by blocking the maturation and function of antigen presenting cells and causing alterations in T cell signal transduction and function. The antigen presenting cells are the main cells which produce IL-18.

In agreement with our data, Blanck et al.[21] Found that differentiated monocytes from breast cancer patients are defective in IL-18 production.

In addition, the findings of Frydecka et al.[22–24] supported the obtained data as they demonstrated impaired IL-18 responsiveness in peripheral blood mononuclear cells from cancer-bearing patients. The underlying mechanism for this impairment was, at least in part, ascribed to a dramatic decrease of natural killer (NK) cells and cytotoxic T-lymphocytes (CTL) which constitutively and highly express IL-18 receptor (IL-18R) and also attributed to null production of IL-12 which up-regulates IL-18.

The current study indicated that, there is a significant (0.00001) decrease in the mean values of IFN-γ in non-metastatic (5.00 ± 1.49 pg/ml) and metastatic (3.70 ± 3.09 pg/ml) patients with breast cancer compared to normal controls (15.70 ± 3.09 ng/ml).

This suppression of IFN-γ production is indicated by the observation of Satthapom et al.[25] They revealed that the immune-regulatory CD4(+) CD25(+) T cells have a role in the suppression of anti-tumor immunity in cancer patients.

Besides, this impairment of immune response associated with cancer is consistent with the findings of Blanck,[21] who reported that the presence of classical tumor suppressor proteins, which regulate tumor cell immune function genes, as well as tumor cell mutations that eliminate MHC class I expression affect the anti-tumor immune response. In addition, Pockaj et al.[26] found that reduced T cell and dendritic cell (producers

of IFN-γ and IL-18, respectively) function is related to cyclooxygenase-2 over expression and prostaglandin E2 secretion in patients with breast cancer. This adds further explanation to the decreased production of IFN-γ and IL-18 in those patients.

On the other hand, our results demonstrated that when the culture was supplemented with IL-12, which is a major immune-regulatory cytokine and a key component in numerous immune functions, there was a significant increase in the levels of IL-18 as well as IFN-γ production in each group under study.

The obtained results are coincided with many investigators who reported that IL-12 is a critical cytokine that is required for the promotion of Th1 development as well as IFN-γ production.

Other investigators stated that the primary functions of IL-18 included the induction of IFN-γ production in T- and NK-cells, which agree with our results.

Similarly, it was reported that IL-12 administration increased the circulating as well as liver associated levels of IL-18 in wild type mice. IL-12 and IL-18 regulate each other's production. IL-18 contributes to induction of IFN-γ by IL-12.[27] These data are consistent with our findings.

Furthermore, our findings are parallel to the study of Tominga et al.[28] who demonstrated that IL-18 by itself can not stimulate human T cells to produce IFN-γ. However, once human T cells are activated with IL-12, they expressed IL-18 Rα and produce IFN-γ in response to IL-18. It was documented that IL-12 can induce the production of IL-18 and has a synergistic effect with IL-18 on the activation of natural killer (NK) and cytotoxic T-lymphocytes.

Our work also found that there is non-significant difference between the mean values of IL-18 and the different clinicopathological characteristics in non-metastatic patients with breast cancer before stimulation with IL-12 as well as in metastatic patients after stimulation with IL-12.

However, after supplementation with IL-12 there is a significant decrease ($p = 0.041$) in the mean values of IL-18 in non-metastatic patients in grade III than those in grade II due to disease regression.

Regarding IFN-γ levels in different clinicopathological parameters, our study revealed a significant decrease ($p = 0.21$) in the mean values of IFN-γ in non-metastatic patients with age $\geqslant 50$ years than those < 50 years and also in patients with tumor size > 5 cm than those with tumor size < 5 cm before supplementation with IL-12. Whereas, a significant increase ($p = 0.01$) in the mean values of IFN-γ was observed in patients with (+ + +) ER/PR compared to those with (+ +), (+), and (−) ER/PR after IL-12 supplementation.

As for the metastatic patients our results indicated a significant decrease ($p = 0.041$) in the mean values of IFN-γ in patients with grade III than those in grade II as well as in patients with tumor size > 5 cm than those with tumor size < 5 cm. However, the mean values of IFN-γ were significantly increased ($p = 0.021$ and 0.031) in patients with (+ + +) ER/PR compared to patients with (+ +), (+), and (−) ER/PR after supplementation with IL-12.

These data clearly indicated that levels of IL-18 and IFN-γ were significantly decreased in breast cancer patients who have a biologically aggressive disease.

The correlation studies in the present work showed significant positive correlation was existed between the mean values of IL-18 and IFN-γ production before and after supplementation with IL-12 in all subjects under study denoting that the significant increase in IL-18 levels is associated with significant increase in IFN-γ production.

In addition, our correlations also revealed that the supplementation of cultures with IL-12 significantly increases the mean values of both IL-18 and IFN-γ in all studied groups, confirming the previously mentioned data.

In this context, Segel et al.[29] reported that IL-12 has a role in the rejection of established tumors, which dependent on the induction of a Th1 response producing IFN-γ that acts on host cells.

Other study by Boehm et al.[30] demonstrated that IL-12 and IL-18 synergize to enhance IFN-γ production in human T cells and NK cells.

In addition, it was documented that IL-12 directly upregulated the expression of Fas (CD95, APO-1), a cell surface molecule capable of inducing ligand-mediated apoptosis, on human breast cancer cells which plays a role in the elimination of metastatic tumor activity. The mechanism underlying this anti-tumor activity may be related to its ability to inhibit angiogenesis. Other investigators showed that IL-12 primes macrophages for nitric oxide production which is involved in mechanisms that control tumor growth in animal models.

Moreover, it was found that IL-12 treatment of a murine model breast cancer lead to tumor regression and systemic anti-tumor immunity.[31–33]

From our findings we can conclude that: Interleukin-12 plays a crucial role in the improvement of impaired immunity by induction of Th1 response in patients with breast cancer. It is a potent stimulant of the immune response by enhancing the production of IL-18 and IFN-γ. Consequently, we can suggest that, IL-12 can be used as a therapeutic agent for correction of the immunodeficiency associated with malignancy. It can be an effective weapon against cancer, but it can also cause toxic complications that interfere with its use. Therefore, its clinical safety and usefulness have to be proven in additional studies.

Appendix A. Treatment protocol

A.1. Chemotherapy

All non-metastatic patients were planned to receive six cycles of FAC regimen. Flourouracil 600 mg/m^2 IV/D1, Adriamycin 60 mg/m^2 IV/D1, Endoxan 600 mg/m^2 IV/D1. Cycles were repeated every 21 days and all patients received the total of 6 courses of treatment. Docetaxel and Cisplatin as first line treatment in metastatic patients previously treated with Adriamycin, Cisplatin 75 mg/m^2 IV 2 h infusion, Docetaxel 75 mg/m^2 IV 1 h infusion. Cycles were repeated every 3 weeks-carboplatin was substituted for Cisplatin in the event of renal toxicity.

Patients received pre medication with Dexamethasone (20 mg IV) cimetidine (200 mg IV) and other antihistaminic drugs just before Docetaxel administration. Patients with stable disease, partial response or complete response receive the combination until they developed progressive disease.

A.2. Hormonal treatment

Nolvadex: All patients with positive ER and PR receptor received Nolvadex (10 mg) 2 tablets per day for 5 year.

References

1. Reeder JC, Vogel VG. Breast cancer risk management. *Clin Breast Cancer* 2007;**7**:833–40.
2. Hery J, Felay J, Boniol M, Autier P. Changes in breast cancer incidence and mortality in middle-aged and elderly women in 28 countries with Caucasian majority populations. *Ann Oncol* 2008;**19**:1009–18.
3. Sabel MS, Skitzki J, Stoolman L, Egilmez NK, Bailey N, Chang AE. Intramural and TNF-alpha loaded microspheres lead to regression of breast cancer and systemic anti- tumor immunity. *Ann Surg Oncol* 2004;**11**:147–56.
4. Schreiber RD, Farrar MA, Luna J. Interlukin-18 increase metastasis and immune escape via down regulation of CD70 and maintenance of CD44. *Carcinogenesis* 2009;**30**(12):1987–96. doi:10.1093/Carcin/bgp158.
5. Son YI, Dallal RM, Mailliord RB. IL-18 synergizes with IL-12 to enhances cytotoxicity, interferon-γ production and expansion of natural killer cells. *Cancer Res* 2006;**61**:884–8.
6. Günel N, Coskun U, Sancak B, Hasdemir O, Sare M, Bayram O, Celenkoglu G, Ozkan S. Prognostic value of serum IL- 18 and nitric oxide activity in breast cancer patients at operable stage. *Am J Clin Oncol* 2003;**26**:416–21.
7. Okamura H, Tsutsui H, Kamatsu T, Yutsudo Y, Akira K, Kusimoto MC. Loning of a new cytokine that induces IFN-7 production by T-cells. *Nature* 2005;**378**:3285–94.
8. Campoli M, Ferrsone S, Zea AH, Rodriguez PC, Ochoa AC. Mechanisms of tumor invasion. In: Khleif SN, editor. Tumor Immunology and Cancer Vaccines. *Cancer Treat Res* 2005; **123**:61-88.
9. Pockaj BA, Basu GD, Gray RJ, Gendler SJ, Hernandez JL, Pathangey LB. Reduced T-cell and dendritic cell function is related to cyclooxygenase-2 over expression and prostaglandin E2 secretion in patients with breast cancer. *Ann Sur Oncol* 2004;**11**:328–39.
10. Carbone JE, Ohm DP. Immune dysfunction in cancer patients. *Oncology (Huntingt)* 2002;**16**:1–8.
11. Oguma A, Yoshikai Y, Masuda A, Matsuguchi T. Interleukin 15 induce interleukin 12 Receptor B1 gene expressions. *Blood* 2005;**105**:711–20.
12. Goldspy RA, Kindt TJ, Osborne BA. Cytokines. In: Kupy immunology. 5th ed. New York: W.H. Freeman and Company; 2004. p. 276–98.
13. Eapen S, Yamuda KI, Hurwitz D, Dowgiert R. Expression of interlukin-18 and Caspase-1 in cutaneous T cell lymphoma. *Clinical Caner Res* 2006;**12**:376–82.
14. Papadakis KA, Landers C, Hans Q, Luo X, Wei P. TLR1A-synergizes with IL-12 and IL-18 to enhance IFN-γ production in human T cells and NK cells. *J Immunol* 2004;**172**:7002–7.
15. Cheung H, Chen NJ, Ga GZ, Oshashi PS. Accessory protein-like is essential for IL-18 mediated signaling. *Immunology* 2006;**174**:5351–7.
16. Afkarian M, Sedy JR, Yang J, Cereb N, Yang SY. T-bet is a STATI-induced regulator of IL-12R expression in naïve CD4+ T cells. *Nat Immunol* 2006;**3**:549.
17. Sins JE. IL-1 and IL-18 receptors and their extended family. *Curr Opin Immunol* 2004;**14**:117–27.
18. Cao R, Farnebo J, Kurinoto M. IL-18 acts as an angiogenesis and tumor suppression. *FASEB* 2003;**13**:2195–9.
19. Puren AJ, Fantuzzi G, Dimarello A. IL-18 (IFN-γ inducing factor) via interleukin-α production from non-$CD4^+$ human blood mononuclear cells. *J Clin Invest* 2004;**101**:711–7.
20. Le HN, Lee NC, Tsung K, Norton JA. Pre existing tumor sensitized T cells are essential for eradication of established tumors by IL-12 and cyclophosphamide plus IL-12. *J Immunol* 2004;**167**:6765–72.
21. Merendino RA, Gangemi S, Ruello A, Bene A, Losi E, Lonbardo G, Purello-Dambrosio G. Serum levels of interleukin-18 and sICAM-1 in patients affected by breast cancer: preliminary considerations. *Int J Biol Markers* 2001;**16**:126–9.
22. Frydecka I, Mazur G, Kuliczkowski. Decreased production of interferon-γ by anti CD3 monoclonal antibody and interleukin-2-stimulated peripheral blood mononuclear cells in Hodgkin's disease. *Br J Haematol* 1995;**91**:671–3.
23. Merendio J, Park AY, Hondowiez BD, Scott P. Leishmonia major infection. *J Immunol* 2004;**165**:896–902.
24. Kaboshi K, Yishino T, Morimoto Y, Kodama M. Down regulation of IL-18 receptor in cancer patients its clinical significance. *Anti Cancer Res* 2004;**21**:3285–94.
25. Satthaporn S, Robins A, Vassanasiri W, El-Sheemy M, Jibril JA, Clark D, Valerio D, Eremin O. Dendritic cells are dysfunctional in patients with operable breast cancer. *Cancer Immunol Immunother* 2004;**53**:510–8.
26. Pockaj BA, Basu GD, Gray RJ, Gender SJ. Reduced T-cell and dendritic cell function is related to cyclo oxygenase-2 over expression and prostaglandin E2 secretion in patient with breast cancer. *Ann Surg Oncol* 2004;**22**:329–60.
27. Watford WT, Moriguchi M, Morinobu A. O'shea the biology of IL-12: coordinating innate and adaptive immune responses. *Cytokine Growth Factor Rev* 2003;**14**:361–8.
28. Tominga K, Yoshimoto T, Torogoe K, et al. IL-12 synergizes with IL-18 IL-IB for interferon-γ production from human T cells. *Int Immunol* 2004;**12**:151–60.
29. Segel AJ, Sanau O, Kojima H. HER_2 neu over expression enhance breast metastasis-interlukin-18 inhibit metastasis. *Cancer Res* 2007;**67**:4190–8.
30. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that over expresses HER2. *N Engl J Med* 2001;**344**:783–92.
31. Sabel K, Micallef, Yoshida K, Kawii S, Kohono K, Akais, Tanirnoto T. Interlukin-18 regulates transferrine-induced cell proliferation in breast cancer cell line. J Immunol. 2010. Org/cgi/content/meeting/abstract.
32. Hong B, Ren W, Song XT, Evel-Kabler K, Chen SY, Huang XF. Human suppressor of cytokine signaling 1 controls immunostimulatory activity of monocyte-derived dendritic cells. *Cancer Res* 2009;**69**:8076–84.
33. Frydecka I, Mazur G. Oncolytic adenovirus expressing interlukin-18 induce significant anti tumor effects against breast cancer, melanoma, in mice through inhibition of angiogenesis. *Cancer Gene Ther* 2010;**17**:28–36.