

## Importance of Serum IL-18 and RANTES as Markers for Breast Carcinoma Progression

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### ABSTRACT

**Background:** Interleukin-18 (IL-18), a cytokine that plays an important role in the T-cell-helper response, acts as an angiogenic factor and a tumor suppressor. RANTES (regulated upon activation normal T-cells expressed and secreted) is a member of the C-C chemokine family with chemoattractant activity for a variety of cell types. High incidence and intensity of RANTES were noted in advanced breast carcinoma.

**Aim of the Study:** To correlate the levels of RANTES and IL-18 in serum of breast cancer patients with bone or other organ metastasis compared to breast cancer patients without metastasis and healthy controls and to estimate the role of each of them as a prognostic marker for the progression of the disease.

**Patients and Methods:** The study was conducted on 60 breast cancer patients (25 cases with no metastasis and 35 cases with metastasis) who were admitted to the outpatient clinic of the NCI, Cairo University during the period from March 2004 to September 2004 and 30 apparently healthy controls who were volunteers at the blood bank of the NCI, Cairo University.

**Results:** Showed that there was a statistically significant difference between the level of IL-18 in breast cancer patients without metastasis and the control group ( $p < 0.05$ ) while there was a highly significant difference between the metastatic group and the control group ( $p < 0.001$ ). There was a significant increase in IL-18 levels between metastatic and non-metastatic cases ( $p < 0.01$ ). RANTES showed a significant increase in breast cancer cases with no metastasis and the control group ( $p < 0.05$ ) and it showed a highly significant increase in metastatic patients compared to controls ( $p < 0.001$ ). There was no significant increase in the level of RANTES in metastatic compared to non-metastatic patients ( $p > 0.05$ ).

**Conclusions:** IL-18 is an important non invasive marker suspecting metastasis. Even though RANTES levels were higher in cancer patients compared to controls, its role in staging of breast cancer was not clear in this study.

**Key Words:** Breast cancer - IL 18 - RANTES.

### INTRODUCTION

RANTES (regulated upon activation normal T- cells expressed and secreted), which is one of the  $\beta$  (C-C) chemokines [1] that are chemo attractant for a variety of cells particularly mononuclear cells, basophils, and eosinophils is thought to be released by activated T lymphocytes and monocytes/macrophage [2] epithelial and bronchial epithelium [3,4], dermal fibroblasts [5] and renal tubular epithelium [6]. RANTES-induced chemotaxis is associated with receptor dimerization and activation, with the redistribution of adhesion factors and cytoskeleton rearrangement [7]. RANTES is also thought to play an important role in a variety of disease states, including allergic inflammatory diseases as asthma, allergic rhinitis and atopic dermatitis [8].

Recently, it has been reported that platelets are also an important source of RANTES and that platelet-released RANTES plays an important role in allergic conditions. Many studies had reported the correlation between breast cancer progression and monocyte-infiltration-site RANTES (a major monocyte chemo attractant by breast tumor cells) which has a potential role in breast cancer progression [9,10].

Interleukin-18 (IL-18) previously known as interferon  $\gamma$  (IFN  $\gamma$ )- inducing factor, is an 18.3 kilo Dalton cytokine produced by activated macrophage keratinocytes kupffer cells, intestinal epithelial cells, and osteoblast [11]. In 1996, human IL-18 was cloned by expression of the cDNA in Escherchia coli [12]. IL-18 enhanced the immune defense against tumor cells by

activating and inducing the production of IFN  $\gamma$ - enhancing capacity. IL-18 also augments the cytotoxic activity of Natural Killer (NK) and T cells and enhances their production of other pro-inflammatory mediators such as Tumor necrosis factor  $\alpha$  (TNF $\alpha$ ), IL-1 $\beta$ , IL-8 and nitric oxide [13]. IL-18 elicits antitumor immunity in the murine system by inhibiting tumor angiogenesis, reducing tumorigenesis [14], and enhancing apoptosis in tumor cells [15]. Some clinical studies showed that the level of serum IL-18 may be a prognostic factor in patients with gastric carcinoma, hematological malignancies [16] and metastatic breast cancer [17].

The aim of this study was to correlate the levels of RANTES and IL-18 in serum of breast cancer patients with bone or other organ metastasis with those of breast cancer patients without metastasis and with healthy controls and to estimate the role of each of them as a prognostic marker for the progression of the disease.

## PATIENTS AND METHODS

The study was conducted on a total of 60 females (66.7%) with breast cancer who were admitted to the outpatient clinic of the National Cancer Institute (NCI), Cairo University during the period from 18<sup>th</sup> March 2004 to 14<sup>th</sup> September 2004 %); Patients with inflammatory conditions as allergy and infections were excluded from the study and 30 healthy controls (33.3%) who were volunteers of blood bank at NCI, Cairo University. Their age ranged between (34-65 years) with a mean of 51.35 $\pm$ 12.6 years. The patients were staged into four stages according to the 1997 TNM classification: 50 cases were stage 2 (83.3%) and 10 cases were stage 3 (16.7%). The diagnosis was made by biopsy, imaging studies and corresponding tumor markers. The patients were divided into two groups according to the presence or absence of metastasis: 25 cases had non-metastatic breast cancer (41.7%) and 35 cases were metastatic (58.3%). Metastatic cases were further subdivided into: patients having bone metastasis (16 cases) (45.7%), patients having bone and other organ metastasis (9 cases) (25.7%), and patients having only organ metastasis (10 cases) (28.6%).

Five ml of blood was collected from each patient and left to clot at 37°C for 45 minutes. Blood was then centrifuged at 3000 rpm for 10

minutes. The collected serum was then divided into two aliquots and stored at until analyzed.

All patients and controls were subjected to estimation of RANTES (Quantikine-Catalog number DRNOOB) and IL-18(MBL: CODE NO 7620) in their serum using ELISA technique according to manufacturers instructions.

*Statistical analysis:* Descriptive statistics were presented as means, standard deviations and percentages. Analysis of variance (F test) or corresponding non parametric test (Kruskal-Wallis test) were used for comparing more than 2 groups. When test was significant we used the post hoc test for comparing each two groups.

*Significance level:* Significance level of 0.05 was used throughout all statistical tests within this study.

## RESULTS

In the present study, IL-18 level in serum of control cases ranged between (50-400 pg/ml) with a mean of 253 $\pm$ 105.87 pg/ml. It was increased in serum of non-metastatic breast cancer patient with a range between (120-750 pg/ml) and a mean of 318.6 $\pm$ 132.73 pg/ml. A further increase was found in metastatic cases ranging between (110-800 pg/ml) with a mean of 441.57 $\pm$ 192.85 pg/ml. A significant difference was found between the level of IL-18 in the control group and the breast cancer group with a *p* of 0.049. A highly significant difference was found between the control group and the metastatic group with a *p* value of 0.001 (Table 1). A statistically significant increase in the level of IL-18 was found between metastatic and non-metastatic groups with a *p*-value of 0.003 (Table 1). Distribution of serum IL-18 levels of patients with breast cancer with and without metastasis and healthy controls was demonstrated in fig. (1).

No significant difference was found between cases having bone metastasis and cases having bone and other organ metastasis. No significant difference was found between patients having bone metastasis and patients having only organ metastasis (Table 2).

RANTES level in the control group ranged between (5000-90000 pg/ml) with a mean of 60000 $\pm$ 27437.7 pg/ml. The level showed an increase in non-metastatic patients ranging

between (32000-120000 pg/ml) with a mean of  $84960 \pm 25397.6$  pg/ml. The level of RANTES in breast cancer patients with metastasis ranged between (26000-127000pg/ml) with a mean of  $91542.8 \pm 24868.3$  pg/ml. A significant difference was found between the levels of RANTES in the control and breast cancer groups with a *p* value of 0.001. A highly significant difference was found between the levels of RANTES in the control and metastatic groups with a *p* value of 0.001 (Table 1). No significant difference

was found between metastatic and non metastatic cases. Also no significant difference was found between RANTES levels in patients having bone metastasis and patients having bone with other organ metastasis. A non-significant difference was also found between patients having bone metastasis and patients having only organ metastasis (Table 2). Distribution of serum Rantes levels of patients with breast cancer with and without metastasis and healthy controls was demonstrated in fig. (2).

Table (1): IL-18 and RANTES levels in controls compared to non-metastatic and metastatic breast cancer patients.

	Controls N=30	Non-metastatic breast cancer N=25	Metastatic breast cancer N=35	F	<i>p</i>
IL-18 (pg/ml)	253±106	318.6±133	441.6±193	12.944	<0.001
RANTES (pg/ml)	60.000±27.438	84.960±25.398*	91.543±24.868*	12.840	<0.001

\* The two groups are insignificant.

Table (2): IL-18 and RANTES levels in metastasis breast cancer patients having bone metastasis, bone and other organ metastasis and only organ metastasis.

	Bone metastasis N=16	Bone and organ metastasis N=9	Organ metastasis N=10	<i>p</i>
IL-18 (pg/ml)	436.89±170.41	433.33±235.64	456.5±205.89	0.875
RANTES (pg/ml)	99312.5±26562.4	86666.7±12257.7	83500±28702.5	0.162

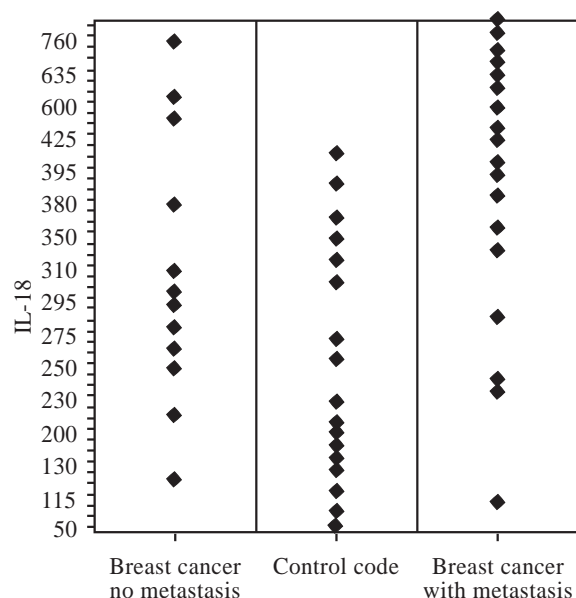


Fig. (1): Distribution of plasma IL-18 levels of patients with breast cancer with and without metastasis and healthy controls.

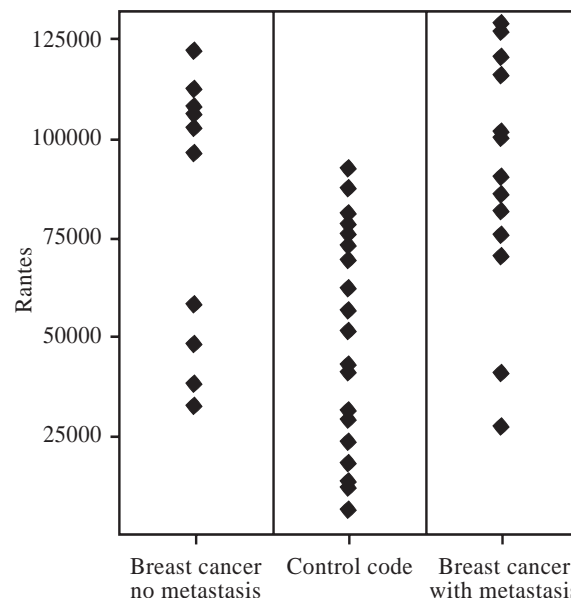


Fig. (2): Distribution of plasma rantes levels of patients with breast cancer with and without metastasis and healthy controls.

## DISCUSSION

The present study showed that there was a significant increase in the level of IL-18 in serum of breast cancer patients as compared to the control group. There was a highly significant difference between metastatic and non metastatic groups. This correlates with a number of previous studies as done by Gunel 2002 [18] who found a highly significant increase in the level of IL-18 in breast cancer group than the control group. They also found a highly significant increase between metastatic and non-metastatic cases. Merendino and coworkers conducted a study in 2002 on 3 groups of breast cancer patients: the first one with no metastasis, the second with lung metastasis and the third with bone metastasis. He compared the level of IL-18 in the serum of the three groups against each other and against the control group [17]. Their results correlated with ours as they found a significant increase in the level of IL-18 in serum of breast cancer patients than the control group. There was also a significant increase in the level of IL-18 between metastatic and non-metastatic groups.

In our study we could not prove the correlation between the increase in level of IL-18 in cases of bone metastasis than any other site of metastasis and this was in disagreement with some of the studies who reported a highly significant increase in level of IL-18 in breast cancer cases with bone metastasis than any other site of metastasis [18]. This may be due to the fact that these studies included a higher number of bone metastatic patients than any other form of metastasis. Also single metastatic site were included and those with multiple metastasis were excluded from these studies while our study had 9 patients (25.7% of metastatic patients) having multiple metastasis, thus a more accurate and precise conclusions was obtained.

RANTES level was found to increase in our study significantly in breast cancer cases than in the control group and a highly significant increase was found between metastatic and non metastatic cases. This was found in accordance with several studies who found an increase in the level of RANTES in breast cancer patients than the controls and between its level in metastatic and non-metastatic cases in relation to controls [10,19]. In 2001, Niwa and coworkers

conducted a study that measured the level of RANTES in plasma of 43 breast cancer patients with various stages of metastasis and found a significant increase in RANTES levels among breast cancer patients than the control group. They also reported a highly significant increase between metastatic and non metastatic patients [20].

From the above we can conclude that IL-18 may be a useful marker in monitoring metastatic breast cancer and together with tumour markers as CA-15.3 can be a useful indicator of metastatic breast cancer and in differentiating between benign and malignant breast lumps. RANTES, on the other hand, although showing significant increase in breast cancer patients, its role in determining breast cancer progression and the degree of metastasis was not proven. Also, its usefulness as a breast cancer prognostic marker is limited by the fact that it is proven to increase in other malignant conditions and inflammatory diseases.

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