Importance of Serum Il-8 and Rantes as Markers for Breast Cancer Progression

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ABSTRACT

Breast cancer is one of the most feared human illnesses. RANTES (regulated upon activation normal T cells expressed and secreted), is a chemokine which is produced by several tumor cells including breast cancer cells. IL-8 is a member of the CXC chemokine family. A tumor promoting role of IL-8 has been proposed in a wide variety of human solid tumors including breast cancer. The aim of the present work is to estimate levels of RANTES and IL-8 in breast cancer patients with and without distant metastasis and to correlate their levels in liver and other organs with metastasis to those without metastasis and healthy controls. The study was carried out on 70 subjects, they were divided into 3 groups: group A (25 breast cancer patients without distant metastasis), group B (25 breast cancer patients with distant metastasis) and group C (20 healthy controls). All subjects were submitted to full history taking, clinical, radiological examinations and measurements of serum RANTES and IL-8. Regarding RANTES, the results showed, a significant difference between control group and group A and also between control group and group B (P< 0.001), while there was no significant difference between group A and group B (P>0.05). In group A and B, RANTES showed no significant difference between those with loco regional lymph nodes and liver metastasis when compared with those without loco regional lymph nodes and liver metastasis respectively. On the other hand, IL-8 showed a significant difference between control group and group A, between control group and group B and also between group A and group B (P<0.001). In group B, the mean value of IL-8 among those with liver metastasis was significantly higher than those without liver metastasis. Similarly, in group A, in patients with loco regional lymph nodes metastasis when compared to patients without loco regional lymph node metastasis. Conclusion: IL-8 may be a useful marker in monitoring metastatic breast cancer and with other tumor markers as CEA and CA15-3 it can be a useful indicator of metastatic breast cancer, while RANTES can't be used in monitoring breast cancer progression and degree of metastasis, possibly because of its increase in other malignancies and inflammatory diseases.

INTRODUCTION

Breast cancer is the most common cancer among Egyptian women representing 18.9% of total cancer cases[(1)] of its high incidence[(2)], with a rate more than twice that of colorectal cancer and cervical cancer[(3)]

The contribution of inflammation and inflammatory cells to the process of tumor progression is being recognized [(4)]. Several reports have elaborated the involvement of chemokines in tumor growth, invasion and metastasis [(5)]

RANTES (regulated upon activation normal T-cells expressed and secreted) belongs to a family of CC chemokine[(6)], generated by circulatory lymphocytes and certain kinds of tissue cell monocytes[(7)]. It is a potent chemoattractant for various important inflammatory cells such as esinophils[(8)], memory T cells, monocytes and basophiles[(9)].

RANTES is thought to play an important role in a variety of disease states including allergic inflammatory diseases as asthma, allergic rhinitis and atopic dermatitis [(10)] through their ability to cause direct migration of leucocytes[(11)]. RANTES is the major determinant of macrophage and lymphocyte infiltration in the carcinomas of the breast, ovary and cervix [(12)] which has a potential role in breast cancer progression [(13)].

IL-8 is a member of the CXC chemokine family of related proinflammatory cytokines, it acts as a chemoattractant for neutrophils [(14&15)]. It is considered as an angiogenic chemokine,[(16)], as it bears glutamic acid, leucine, arginine positive motif[(17)] and it is secreted by stromal cells[(18)]. A tumor promoting role for IL-8 has been proposed in a wide variety of human solid tumors including breast cancer [(19)].

The aim of the current work is to estimate levels of RANTES and IL-8 in breast cancer patients with and without distant metastasis and to correlate their levels in liver and other organ metastasis with those without metastasis and healthy controls.

SUBJECTS & METHODS

The study was carried out on 70 females, they were divided into 3 groups:-

- **Group A:** Included 25 breast cancer females without distant metastasis and included 9 patients with loco regional lymph nodes involvement with mean age 40.2±9.1 years.
- **Group B:** Included 25 breast cancer females with distant metastasis, with mean age 45.17±8.12 years.
- **Group C:** Included 20 age matched healthy females with mean age 40.33±9.9 years.

All breast cancer patients were recruited from Surgery and Oncology departments while healthy volunteers were picked up when tested at the blood banks, at Menoufiya University Hospital, Faculty of Medicine, from March 2010 to December 2010.

Group A included 18 cases with stage II, and 7 cases with stage III. Group B included 25 cases with stage IV according to TNM classification **(AJCC, TNM classification, 7th edn.)** (table 1).

Breast cancer was diagnosed by histopathological studies and metastasis was proven by clinical examination, imaging studies and tumor markers. Females with inflammatory or allergic diseases were excluded from the study.

Sample collection and methods:

Samples were collected prior to the start of cytotoxic chemotherapy as it alters the cytokine expression

profiles [20]. Five ml of blood were withdrawn from each subject into a plain tube, left to stand for 15 minutes at room temperature, then centrifuged at 4000 rpm for 5 minutes and serum was then collected and placed into 2 tubes and stored at -80°C until analysis of IL-8 by competitive enzyme immunoassay (EIA) [21]. RANTES was assayed by enzymatic sandwich type immunoassay using antibodies with high specificity for RANTES [22].

Statistical analysis:

Results were collected, tabulated, statistically analyzed by IBM personal computer and statistical package SPSS version 16 (SPSS Inc. Chicago, Illinois, USA). Student t-test was used for comparison between two groups having quantitative variables. ANOVA (F) test was used for comparison between three groups having quantitative variables. A P-value of <0.05 was considered statistically significant.

RESULTS

Table (1): Patients characteristics

variable		Group A n= 25	Group B n=25	Group C n=20
Pathology	IDC	25	25	NA
Stage	II	18	NA	NA
	III	7	NA	NA
	IV	NA	25	NA
Grade	II	17	18	NA
	III	8	7	NA
ER	Negative	15	16	NA
	Positive	10	9	NA
Metastasis	Liver	NA	4	NA
	Liver +others	NA	9	NA
	Others	NA	12	NA
LN	Negative	16	NA	NA
	Positive	9	NA	NA

NA = not applicable. ER= Estrogen receptors**. LN= Lymph node IDC =**

Among the patients in group A, 18 cases were stage II and 7 cases were stage III. Most of the breast cancer cases were of Grade II (17 in group A and 18 in group B). Estrogen receptors were positive in 10 cases in group A and 9 cases in group B. There were no statistical significant differences as regard these histopathological parameters between both groups. Liver was the only site of metastasis in 4 cases while in 9 cases it was associated with other metastatic sites. Twelve cases have other metastasis (bone and lung) without liver involvement.

Table (2): Serum levels of RANTES & IL-8 in controls and patients with cancer breast (**Mean ±SD**).

	Group A No (25)	**Group B No (25)**	**Controls No (20)**	**P**
RANTES (pg/ml)	60035.63±3076.59	61193.64±2938.36	16899.41±1403.4	P_1<0.001 P_2<0.001 P_3> 0.05
IL-8 (pg/ml)	12.05 ± 0.34	38.73 ± 1.41	5.21 ± 0.31	P_1<0.001 P_2<0.001 P_3<0.001

P_1 = between controls & breast cancer patients without metastasis
P_2 = between controls & breast cancer patients with metastasis
P_3 = between breast cancer patients without metastasis & with metastasis.
There is a significant increase in group A on comparing to the control group and also in group B compared to the control group, while there is no significant difference between group A and group B regarding RANTES. On the other hand, IL-8 shows a significant increase in group A on comparing to the control group and in group B when compared to the control group, and also between group B and group A.

Table (3): Statistical comparison of RANTES & IL-8 in metastatic breast cancer patients having liver metastasis, liver and other organs metastasis and other organs metastasis.

	Liver metastasis No(4)	**Liver & other organ metastasis, No(9)**	**other organ metastasis, No(12)**	**P**
RANTES (pg/ml)	60864.25±4099.19	61603.22±1905.99	60996±3375.47	>0.05
IL-8, (pg/ml)	39.35 ±0.52	40.2 ±0.21	39.36± 1.64	>0.05

There was no significant difference among group B with liver metastasis, with liver and other organ metastasis and with other organ metastasis regarding RANTES and IL-8.

Table (4): Statistical comparison between breast cancer patients with liver metastasis and those without metastasis among group B regarding mean value of RANTES & IL-8(**Mean ± SD)**

Parameter	**group B with liver metastasis, No.(13)**	**group B without liver metastasis No.(12)**	**P-value**
RANTES(pg/ml) **IL-8 (pg/ml)**	61375.85 ± 2597.83 39.94 ± 0.51	60996.25 ± 3375.47 37.42 ± 0.65	> 0.05 < 0.001

The mean value of IL-8 among those with liver metastasis is significantly higher than those without liver metastasis, while RANTES shows no significant difference.

Table (5): Statistical comparison between patients with loco regional LN metastasis & those without Loco regional LN metastasis among group A regarding RANTES & IL-8 (Mean ± SD)

Parameter	group A with LN metastasis, (n = 9)	group A without LN metastasis (n = 16)	P-value
RANTES(pg/ml)	61603.22 ± 1905.98	60963.25 ± 3423.39	> 0.05
IL-8 (pg/ml)	40.2 ± 0.21	37.9 ± 1.05	< 0.001

The mean value of IL-8 between those with LN metastasis is significantly higher than those without LN metastasis, while RANTES shows no significant difference.

DISCUSSION

Breast cancer is the second leading cause of cancer deaths in women world-wide[23], members of the chemokine family have been observed to contribute to both growth and progression of different types of human cancers[24]**.** The aim of the present work was to estimate levels of RANTES and IL-8 in breast cancer patients with and without distant metastasis and to correlate their levels in liver and other organ metastasis with those without metastasis and healthy controls.

The present study showed that there was a significant increase in the level of IL-8 in serum of breast cancer patients without metastasis when compared to the controls and a highly significant increase in breast cancer patients with metastasis was found when compared to the control. Also, a significant increase was noted in metastatic group when compared to non metastatic group.

These results are in accordance with those of **Smith et al., & Duan et al.**[25&26] who found that blocking of IL-8 with antibodies in non small cell lung cancer cells in vivo decreased tumor growth by approximately 40%, and also in accordance with the results of **Green et al.**[27]**,** who evaluated difference in mRNA transcripts for 13 different cytokines between normal and neoplastic breast tissues. They found that the only correlation in their study was a higher IL-8 mRNA level in neoplastic breast tissue.

The exact mechanism by which IL-8 might promote tumor growth remains to be elucidated. An autocrine role of IL-8 modulating survival and proliferation of tumor has been suggested [17]**.**

Huang *et al.*, and Yuan *et al.*, [28&29] also reported a tumor promoting role for IL-8 in a wide variety of human solid tumors, one of them is breast cancer. **Delarco *et al.*, and Delorco *et al.*,** [30&31] also showed a strong correlation between the metastatic potential of breast carcinoma cell lines and their ectopic expression of IL-8 and showed that increased IL-8 expression in metastatic prone cell lines is possibly caused by atypical epigenetics, whereby upstream CPG methylation rather than promoter methylation results in increased IL-8 production.

Razmkhah *et al*., [32], found that IGF-1 and IL-8 RNA in breast cancer tissues had 28.6 and 56 fold more expression in high stage compared to low stage patients respectively.

Chen *et al*., [15] found that estrogen receptor beta (ER beta) and PEA3 play an important role in tumor invasion by positive regulation of ER beta and PEA3- IL-8 pathway.

Vegran *et al*., [16] found that the lactate/NF–kappa B–IL-8 pathway is an important link between tumor metabolism and angiogenesis.

The current study showed that there was no significant difference between liver, liver with other organ metastasis and other organ metastasis in breast cancer patients with metastasis regarding IL-8, while there was a significant increase in patients with liver metastasis when compared to those without liver metastasis and also in patients with loco regional LN metastasis when compared to patients with no loco regional LN metastasis.

These results correlated with **Benoy *et al*., [20]** who explained that the increase of IL-8 in liver metastasis might be due to decrease clearance of IL-8 by liver cells and metastatic tumor cells continue to produce IL-8, and explained that the increase in nodal metastasis speculate that IL-8 contribute to early metastasis.

Regarding RANTES, the present study found a significant increase in breast cancer patients without metastasis when compared to the control group, and a highly significant increase in breast cancer patients with metastasis when compared to the control group, while there was no significant difference between metastatic and non metastatic groups. These results are in agreement with those of **Luboshits et al., Wigler et al., and Eissa et al., [33,13,34].**

Soria *et al*., [35] who suggested that chemokines RANTES and MCP-1 contribute independently to breast malignancy, however co-expression of RANTES and MCP-1 in the same tumor was associated with more advanced stages of disease. **Forst *et al*., [36]** studied the inter play between tumor cell derived cytokines RANTES and Sl00A4, they found that altogether, the data presented strongly validate the prometastatic function of SlooA4 in the tumor microenvironment and define how cell derived RNATES act as a critical regulator of SlooA4 dependent tumor cell dissemination.

On the other hand, **Niwa *et al*.,[37]** reported a significant increase in RANTES level in breast cancer patients when compared with the control group and also reported a highly significant increase in metastatic group when compared to non metastatic group.

The current study showed that, there was no significant difference between liver, liver with other organ metastasis and other organ metastasis in breast cancer patients group with metastasis and also no significant difference was found on comparing patients with liver and loco regional LN metastasis when compared with those without liver and loco regional LN metastasis respectively. These results are matched with [34]

CONCLUSION

IL-8 may be a useful marker in monitoring metastatic breast cancer

and with other tumor markers as CEA and CA15-3 it may be a useful indicator of metastatic breast cancer, while RANTES can't be used in monitoring breast cancer progression, possibly because of its increase in other malignancies and inflammatory diseases.

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أهمية الرانتيس والإنترلوكين- ٨ كمؤشرات لتطور سرطان الثدي

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سرطان الثدي من الأمراض المخيفة للإنسان. يعتبر رانتيس هو المؤثر الكيميائى الذى ينتج من العديد من الأورام ومنها خلايا سرطان الثدي والإنترلوكين - ٨ هوأيضا مؤثر الكيميائى وقد وجد أن لة دور في إحداث العديد من الأورام ومنها سرطان الثدي.
ويهدف البحث إلي قياس مستوي الرانتيس والإنترلوكين ٨ في مرضي سرطان الثدي المصحوب وغير المصحوب بالانتشار وتقييم أداورهم كمؤشر لتطور المرض.
وقد أجريت هذة الدراسة علي سبعين شخصا وقد تم تقسيمهم إلي ثلاث مجموعات:
المجموعة أ – تشمل ٢٥ مصاب بسرطان الثدي بدون انتشار
المجموعة ب – تشمل ٢٥ مصاب بسرطان الثدي مصحوبا بانتشار
المجموعة ج – تشمل ٢٠ شخصا من الأصحاء (المجموعة الضابطة)
وكل هؤلاء الأشخاص قد تم أخذ تاريخهم المرضى وتم عمل اللازم لهم من الأشعة المطلوبة وقياس مستوي الرانتيس وانترلوكين ٨ وقد وجد أنة بالنسبة للرانتيس :
توجد اختلاف ذو دلالة إحصائية بين المجموعة أ و المجموعة الضابطة وكذلك بين المجموعة ب والمجموعة الضابطة بينما لا توجد اختلافات ذو دلالة إحصائية عند مقارنة المجموعة أ،ب وكذلك عند مقارنة المجموعة التي تم انتشار المرض إلي الكبد والغدد الليمفاوية القريبة بالمجموعة التي لم يتم انتشار المرض إلي الكبد والغدد الليمفاوية القريبة
علي الجانب الآخر بالنسبة للإنترلوكين – ٨:
توجد اختلافات ذو دلالة إحصائية عند مقارنة المجموعة أ والمجموعة الضابطة وكذلك عند مقارنة المجموعة ب والمجموعة الضابطة وأيضا عند مقارنة المجموعتين أ،ب .
يوجد أيضا هذا الاختلاف عند مقارنة المجموعة التي تم انتشار المرض إلي الكبد والغدد الليمفاوية القريبة بالمجموعة التي لم يتم انتشار المرض إلي الكبد والغدد الليمفاوية القريبة
وقد خلص البحث إلي أن:إنترلوكين – ٨ قد يلعب دورا في تطور المرض في المراحل المتقدمة والمراحل المتأخرة لذلك يمكن استخدامه كمؤشر مفيد في متابعة انتشار مرض سرطان الثدي بينما الرانتيس لايمكن أن يكون له هذه الأهمية وربما يكون ذلك لأنة يزيد في أمراض أخري مثل الالتهابات و السرطانات الأخرى.