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ARGINASE ACTIVITY IN BREAST CANCER: IS IT A SIGNIFICANT BIOMARKER?

Amal A. Mahmoud[1], Sahar E.M. El-Said[1], Manal A.M. Mandour[1], Madeha M. Zakhary[1] and Doaa Wadie Maximous[2]

[1]*Department of Medical Biochemistry, Faculty of Medicine, Assiut University, Assiut, Egypt*
[2]*Department of Surgical Oncology, South Egypt Cancer Institute, Assiut University, Assiut, Egypt*

نظراً لاهمية إنزيم الأرجينيزفي مختلف الامراض السرطانية، فقد إستهدفت هذه الدراسة تقدير ومقارنة نشاط إنزيم الأرجينيز في الخلايا السرطانية ونظائرها من الخلايا الطبيعية والأورام الحميدة. تم تعيين مستوى نشاط إنزيم الأرجينيز النسيجي في ثلاثين مريضة بسرطان الثدي، ستة مريضات بورم ثدي حميد وتسعة سيدات أصحاء. لقد وجد أن مستوى نشاط إنزيم الأرجينيز يزداد زيادة ذات دلالة إحصائية في أنسجة أورام الثدي مقارنة بنظائرها من الأنسجة الطبيعية ولكن الإختلاف لم يكن ذو دلالة إحصائية مقارنة بأنسجة أورام الثدي الحميدة. وبالنظر إلي مرحلة تقدم الورم، فقد وجد أن إنزيم الأرجينيز يزداد في المراحل المتقدمة من الورم مقارنة بالمراحل المبكرة، و لكن الاختلاف لم يكن ذو دلالة إحصائية. إضافة إلي ذلك، فإن الأورام سلبية مستقبلات الإستروجين أظهرت معدلات مرتفعة من مستويات نشاط إنزيم الأرجينيز ولكن ايضاً هذا الإرتفاع لم يكن ذو دلالة إحصائية مقارنة بنظائرها من الأورام إيجابية مستقبلات الإستروجين. عندما تؤخذ حالة الإياس في الإعتبار، فإن الأنسجة المأخوذة من المريضات قبل سن الإياس أظهرت مستوي منخفض من إنزيم الأرجينيز مقارنة بنظائرها من المريضات بعد سن الإياس. علي الصعيد الآخر، لقد أظهر المرضي ذوي مدلول صحي متدني معدلات مرتفعة من إنزيم الأرجينيز ولكنها ليست ذو دلالة إحصائية مقارنة بالمرضي ذوي مدلول مرضي حسن. نستنتج مما سبق أن نشاط إنزيم الأرجينيز له علاقة بالسلوك البيولوجي لسرطان الثدي وأن تحديده في الأنسجة السرطانية من الممكن أن يتنبأ بمصيرها.

Due to the importance of arginase enzyme in different malignant disorders, the purpose of the present study was to determine and compare the arginase activity in cancerous cells and their normal and benign counterparts. The tissue arginase activity level was evaluated in 30 females with breast cancer, in 6 females with benign breast disease and in 9 healthy control subjects. The arginase activity levels were significantly increased in malignant breast tissues in comparison to healthy ones, while the difference did not reach the level of significance in comparison to benign breast diseased tissues. Patients with advanced stage showed insignificantly higher arginase activity compared to those with early stage. In addition, estrogen receptor negative tumors showed insignificant higher arginase activ6ity levels compared to estrogen receptor positive tumors. Moreover, tissues of premenopausal patients showed lower activity levels of arginase compared with those of postmenopausal ones.Meanwhile, patients with bad prognosis revealed insignificantly higher activity levels of arginase compared to those with good prognosis. It could be concluded that tissue arginase activity seems to be involved in the biological behaviour of breast cancer and its determination in cancerous tissues could predict its outcome.

INTRODUCTION

Breast cancer is one of the most common types of cancer accounting for 19% of all cancer-related mortality in women[1].The pattern of enzymatic alterations may have an intimate correlation with the malignant state and the progression of cancerous cells in the tumor. Cancer cells may differ from their normal counterparts in the activities or concentration of certain enzymes, including arginase. That difference may act as a useful biological marker of malignancy and/or aggressiveness in particular tumors[2]**.** The increase in the

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activities of certain enzymes is considered an indicator of the prominence or abeyance of particular biochemical reactions or metabolic pathways. Accordingly, the application of measures correlating the activities of such enzymes may lead to elucidation of therapeutic approaches to cancer[3].

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) is homotrimeric binuclear manganese metalloenzyme that catalyzes the hydrolysis of L-arginine, rendering urea for ammonia elimination, mainly in urotelic animals, and L-ornithine (a non-protein amino acid) for biosynthetic pathways[4]. There are at least two forms of arginase. Arginase I is cytosolic and is most abundant in the liver, primarily responsible for ammonia detoxification as urea. A second isoenzyme, arginase II, is involved in the production of ornithine as a precursor to proline, glutamate or polyamines, such as spermine and putresine, essential for cellular growth[5]. Polyamines are vital for cell proliferation and it is possible that the increased level of ornithine, due to the elevated arginase activity, may be linked to the development of carcinogenesis[6].

A high arginase level in breast cancer was detected to be released into serum. Preoperative values of serum arginase activity in patients with breast carcinoma were up to 4-fold those found in healthy women[7]. Consequently, the more advanced the breast cancer, the higher the serum level of arginase enzyme activity. It has been reported that the mean activity of arginase is high in the early stages and higher in the advanced states of the malignant group compared to those of the normal ones. Therefore, arginase enzyme seemed to be a useful biological marker in breast cancer and as an indicator of breast cancer progression[8].

The activity levels of arginase in malignant breast tissues were reported by Porembska *et al.*[6]; Straus *et al.*[7] and Erbas *et al.*[9] to be increased compared with healthy tissues. However, besides the limited number of these studies, none of authors traced the relation between enzyme activity and biological behavior of tumors. Therefore, the present study was designed not only to determine arginase activity levels in breast cancer tissues but also to correlate them with biological behavior of these tumors.

PATIENTS AND METHODS

A) Patients

The current study was carried out on three different groups:

1. Normal group "Control group": Nine histologically healthy breast tissues were obtained from uninvolved areas of patients with benign pathology or with malignant breast lesions, about 10 cm away from the tumor border as described by Filmus *et al.*[10]; Pollet *et al.*[11] and Podhajcer *et al.*[12]. That group of tissues represents the control group.

2. Benign breast disease group: That group included 6 patients with different benign breast diseases who attended the South Egypt Cancer Institute.

The mean ages of the healthy and benign patients were comparable to the malignant ones.

3. Breast cancer group

That group included 30 patients, 29 were females and one was male, with pathologically proven breast cancer who were hospitalized in the South Egypt Cancer Institute. Their mean ± S.E. age was 50.65±1.94 years. They included 14 post menopausal and 11 pre menopausal women. All cases were infiltrating duct carcinoma. Among these patients, 12 cases was stage I, 6 cases had stage II, 15 cases had stage III and 3 cases had stage IV. Considering prognosis, 8 cases showed bad prognosis whereas, 15 cases showed good prognosis. Patients were considered to have good prognosis if they remain free of recurrence during the study period (2006-2009). Bad prognosis is considered if the patients developed recurrence or died during the study period. Regarding estrogen receptor status, 5 cases were estrogen receptor negative, while 17 cases were estrogen receptor positive. We could not find the data of the rest of the patients because of several reasons such as death or emigration.

Each patient was followed up each 3 months by abdominal U/S, chest X-rays and bone scan for suspected patient of bone metastasis.

Follow up included for detection of local and/or distant metastasis.

For the patients with advanced stage (III and IV), neoadjuvant chemotherapy was applied to downstage the tumor.

Demographic criteria of patients are shown in (Table 1).

Table 1: Demographic characteristics of patients with breast cancer.

Character	Mean ± S.E.	Median	Range
Age (Years)	50.65 ± 1.94	52.0	35.0-70.0
Weight (Kg)	71.47 ± 4.76	70.0	45.0-100.0
Height (Cm)	153.8 ± 3.97	158.0	109.0-174.0

B) Tissue specimens' collection and handling

Cancerous tissue specimens were obtained from the surgically resected tumors of malignant cancer patients. In order to assure adequate diagnostic evaluation of the tissue, each sample was divided into two parts; one was sent for pathology examination and the other for biochemical assessment. The samples were analyzed on a blind basis such that the personnel performing biochemical or pathological studies were unaware of the results of the other study group till completion of the research. Informed consent was obtained from all patients.

Tissue specimens were stripped from adipose tissue, weighed and frozen in a mortar containing TED buffer at -70°C until handled. Tissue samples were pulverized while frozen and then homogenized using a tissue homogenizer. The homogenates were decanted into labeled polycarbonate tubes and centrifuged at 10,000 r.p.m. for 30 minutes. Finally, the resulted supernatants were transferred as small aliquots into labeled eppendorf tubes and frozen at -70°C till assay.

C) Biochemical Measurements

Arginase enzyme activity levels were determined using colorimetric techniques[2]. Pathological data and estrogen receptor status were obtained from the files of the patients.

D) Statistical analysis

Student t-test was used for normally distributed data and Mann-Whitney was used for kurtotic and skewed data. The significance level was set at $P \leq 0.05$. The data was analyzed using the SPSS for Windows version 11.0. Graphics were performed using Prism version 3.0, whereas the cut off point, sensitivity and specificity were made using MedCalc program (Trial version).

RESULTS AND DISCUSSION

Results

Patients with advanced stage (III and IV) who received new adjuvant chemotherapy showed downsizing of the tumor and modified radical mastectomy was done for those patients.

Follow up of the patients revealed that six patients developed distant metastases (bone, liver or lung) and two patients developed local recurrence.

The results of the present work are presented in (Tables 2 and 3) and illustrated in (Figures I to VI). As evident in (Table 2), arginase activity levels were significantly increased in malignant breast tissues in comparison to healthy breast tissues. However, the levels exhibited an increase in arginase activity levels in malignant breast tissues compared to benign breast diseased tissues, but the difference did not reach the level of significance. These differences are further shown in (Figure I). Table 3 shows the relationship between arginase activity levels and tumor as well as host characteristics. Patients with advanced stage (III and IV), showed higher arginase activity compared to those with early stage (I and II). However, the difference did not reach the level of significance. This is shown in (Figure II). When estrogen receptor status is considered, estrogen receptor negative tumors showed insignificantly higher arginase activity levels compared to estrogen receptor positive tumors. The differences are also further shown in (Figure III). Tissues of pre- menopausal patients showed lower activity levels of arginase compared with tissues of postmenopausal patients (Table 3 and Figure IV). It is noted that patients with left breast tumors exhibited insignificantly higher arginase activity levels compared to patients with right breast tumors (Table 3 and Figure V). Patients with bad prognosis showed insignificantly higher activity levels of arginase compared with patients with good prognosis (Table 3 and Figure VI).

Table 2: Arginase activity levels in malignant breast tissues compared to benign and control tissues.

Parameter		Malignant (n = 28)	Benign (n = 6)	Normal (n = 9)
Arginase (U/mg protein/g. tissue weight)	Mean ± S.E.	35.61 ± 6.54	20.69 ± 5.46	20.15 ± 2.73
	Median	25.98	18.04	20.80
	Range	5.67-154.54	5.24-42.17	11.29-30.88
	Significance	*P < 0.05 **N.S.	*N.S.	

* Versus normal
** Versus benign

Table 3: Arginase levels in breast cancer tissues as function of host and tumor characteristics.

Host and tumor characteristics		Arginase (U/mg protein/g. tissue weight)
Menopausal status		
Pre menopausal (n = 11)	Mean ± S.E.	26.31 ± 5.29
	Median	23.61
	Range	5.67-61.94
Post menopausal (n = 14)	Mean ± S.E.	37.52 ± 8.51
	Median	29.37
	Range	8.30-126.1
Site of tumor		
Right (n = 15)	Mean ± S.E.	30.61 ± 7.5
	Median	23.61
	Range	5.93-126.1
Left (n = 12)	Mean ± S.E.	43.96 ± 11.91
	Median	33.29
	Range	5.67-154.54
Stage of tumor		
Early (n = 18) (stage I and II)	Mean ± S.E.	31.80 ± 8.21
	Median	21.07
	Range	5.67-154.54
Advanced (n = 8) (stage III and IV)		
	Mean ± S.E.	48.14 ± 13.02
	Median	31.57
	Range	15.43-126.1
Estrogen receptor status (ER)		
ER negative (n = 5)	Mean ± S.E.	52.34 ± 21.18
	Median	43.93
	Range	9.0-126.1
ER positive (n = 17)	Mean ± S.E.	31.86 ± 8.52
	Median	21.50
	Range	5.67-154.54
Prognosis		
Bad (n = 8) Patients developed local and/or distant metastasis	Mean ± S.E.	38.59 ± 13.86
	Median	27.07
	Range	9.0-126.1
Good (n = 15) Patients do not develop local and/or distant metastasis	Mean ± S.E.	25.01 ± 4.67
	Median	20.63
	Range	5.67-69.26

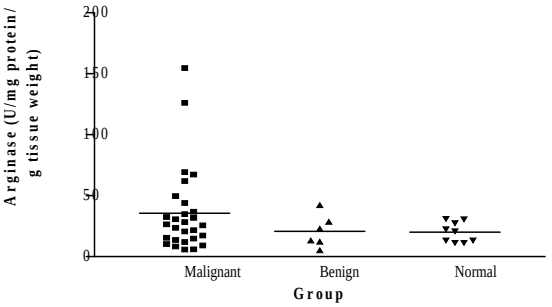

Fig. I: Scatter diagram of arginase.

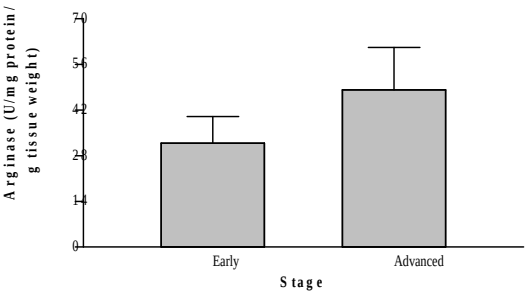

Fig. II: Arginase levels according to stage.

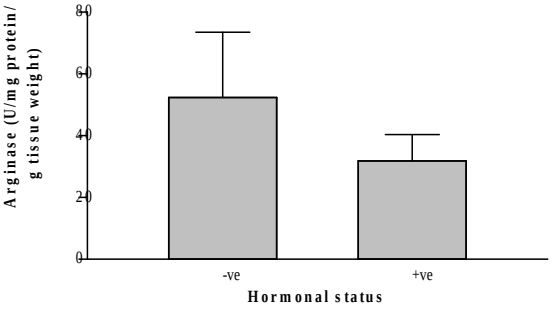

Fig. III: Arginase levels according to hormonal status.

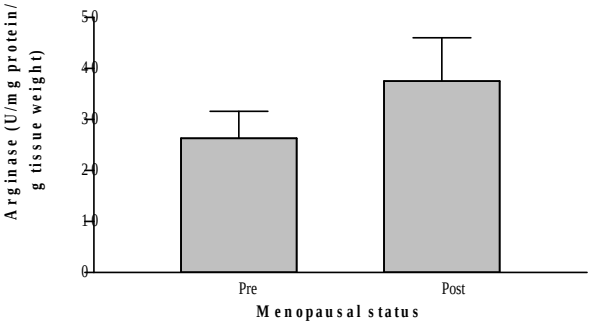

Fig. IV: Arginase levels according to menopausal status.

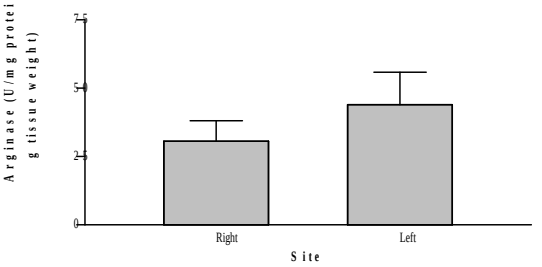

Fig. V: Arginase levels according to site.

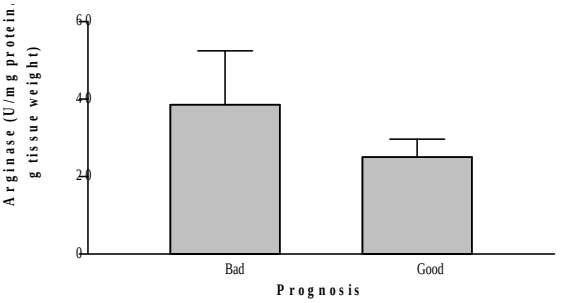

Fig. VI: Arginase levels according to prognosis.

Discussion

Breast cancer is a cancer that starts in the cells of the breast in women and men. Worldwide, breast cancer is the second most common type of cancer after lung cancer (10.4% of all cancer incidence, both sexes counted)[13] and the fifth most common cause of cancer death[14]. In 2004, breast cancer caused 7% of cancer deaths and almost 1% of all deaths[14]**.** Breast cancer is about 100 times as frequent among women as among men, but survival rates are equal in both sexes[15]. Therefore, early detection of the disease is very important. Tumor markers are molecules occurring in blood or tissues that are associated with cancer and whose measurement or identification is useful in patient diagnosis or clinical management.

Investigators observe either enzymes that are native to normal tissue or those that could be associated with changes in metabolism and that are unique to cancer tissue. One of these enzymes is arginase. The enzyme has at least two forms. Arginase I is cytosolic and is most abundant in the liver, primarily responsible for ammonia detoxication as urea. A second isoenzyme, arginase II, is involved in the production of ornithine which acts as a precursor to proline, glutamate or polyamines, such as spermine and putresine, essential for

cellular growth[5]. Polyamines are vital for cell proliferation and it is possible that the increased level of ornithine due to the elevated arginase activity may be linked to the development of carcinogenesis[6].

In the present study, the activity levels of arginase were determined in malignant breast tissues. The study revealed significantly increased levels of arginase in malignant tissues compared to normal tissues ($P < 0.05$). However, no significant differences could be observed between arginase activity in benign breast diseased tissues and healthy tissues, but the activity levels in malignant tissues were higher than in benign tissues (Table 2 and Fig. I). The present data are in agreement with previous studies. Thus, arginase activity levels were reported to be increased in breast cancer by Porembska *et al.*[6], Straus *et al.*[7] and Erbas *et al.*[9]. The sensitivity of tissue arginase in the present study was 39.3% only that of Porembska *et al.*[6] was 74%. These authors have chosen an arbitrary not a calculated cut off value, which gives an explanation to the discrepancy (Table 4).

Table 4: Diagnostic value of arginase.

	Arginase (cut off 30.88)
Sensitivity	39.3%
Specificity	100%

Tracing the literature, no previous results could be found concerning the relationship of arginase activity in breast cancer tissues and clinical criteria. However, Polat *et al.*[8] reported that serum arginase activity was significantly higher in advanced than in early stage. In the present study, it was also observed that arginase tumor tissue activity followed the advance in disease stage despite lack of significance. Patients with bad prognosis showed insignificantly higher arginase activity levels compared with those with good prognosis (Table 3 and Fig. VI). That finding was consistent with a study done by Polat *et al.*[8] who stated that patients who developed recurrence exhibited a higher serum arginase activity level, compared to patients who did not show recurrence.

Some researchers as Polat *et al.*[8] could not find any correlation of arginase serum activity levels with age. Meanwhile, post menopausal women tumor tissues showed higher arginase activity in comparison to premenopausal ones. Estrogen receptor status which is considered important prognostic index was not studied. The present work revealed elevated arginase tissue activity levels in estrogen receptor negative tumors compared to estrogen receptor positive tumors. Estrogen positivity is associated with good prognosis.

Breast tumors may cause an increase in serum enzyme activity. That point has previously been speculated in a study done by Wu *et al.*[16] since they proposed that in gastric cancer, the increased serum arginase enzyme levels may originate from gastric cancer cells. A larger amount of arginase was found in tumor tissues. However, according to Porembska *et al.*[6], the isoforms of arginase in mammary tissues should be considered. The major isoform, the anionic (Arginase I) or near neutral[17], forming 60% of total arginase, was not changed in breast cancer compared to healthy tissues, but it increased significantly during mid-lactation[17]**.** That isoform was never detected in serum. Since the cationic isoform rises in breast cancer but not during lactation, the regulatory mechanisms of mammary gland arginases must be different in pathological and physiological conditions. Thus, only the cationic, extra hepatic arginase (Arginase II) is the form increased in tumor cells and released into extracelllular space being specific for carcinogenesis.

The anionic, arginase I form (hepatic form) is a cytosolic enzyme primarily involved in detoxification of ammonia through urea synthesis, whereas the cationic form, arginase II (extra hepatic form) located in mitochondrial matrix, is involved in biosynthetic functions such as synthesis of ornithine, proline and glutamate[18]**.** Polyamines are subsequently synthesized from ornithine, the second product of arginase reaction. Breast tumor tissues have been reported to have 2-3 fold higher polyamine levels than surrounding normal tissue[19]. The precise mechanism by which the increase in polyamines occurs in breast tumor tissue is not known. It has been suggested that estrogens modulate the growth of certain breast cancer cell lines by increasing the expression of ornithine decarboxylase (ODC)[20], thereby increasing the synthesis of polyamines.

Spermine generally is the most abundant polyamine in human tumors such as breast carcinomas[21] and is synthesized from ornithine via ODC, spermidine synthase and spermine synthase respectively[18]**.** Spermine is rarely found in prokaryotes but is widespread in eukaryotes where its role in cell growth is well established[22]. Polyamines are known to bind DNA and affect gene expression by bringing about structural changes in chromatin and thereby stimulating cell growth[23]. Many studies have suggested the role of arginase as an immune suppressive factor, but that role in tumors is still not clear[16].

In conclusion, determination of arginase enzyme activity in breast cancer tissues would be beneficial in detecting cases with advanced stage, metastatic disease and poor outcome that should be strictly followed. Moreover, therapeutic approaches to decrease enzyme activity and subsequently polyamines would be valuable in cancer therapy. Thus, in a recent study conducted by Erbas *et al.*[9], breast cancer arginase enzyme activity and ornithine levels decreased significantly with carnitine treatment in experimental animals. Carnitine is a metabolic antioxidant acting as a cofactor required for the transport of long chain fatty acyl CoA to the mitochondria. Moreover, mitotic cells were decreased in the group receiving carnitine. That compound could be a future therapy in humans after future studies.

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