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CHANGES OF ARGINASE ACTIVITY AND ARGINASE ISOENZYME SPECTRUM IN HUMAN SERUM DURING DIFFERENT STAGES OF BREAST CANCER BEFORE SURGERY AND AFTER CHEMOTHERAPY

Introduction

It is well established that during development of malignancies metabolic changes occur, including alternations of enzyme activities and isoenzyme expression. At this point arginase is one of those enzymes considered to be involved in tumorigenesis. Arginase (EC 3.5.3.1) is a ureohydrolase , which catalyses the hydrolysis of L-arginine into ornithine and urea. There are at least 2 isoforms of arginase which are encoded by distinct genes and there are significant differences between this two isoforms regarding their subcellular location, Km values, isoelectric points and inhibition characteristics [1, 2]. Arginase I has cytosolic location and mainly is found in liver (sometimes referred as liver isoform), where it is included in urea biosynthesis and plays key role in the process of ammonia detoxification. Arginase II which has mitochondrial sublocation (sometimes referred as kidney isoform) is more abundant isoform and takes part in metabolic pathways other than urea biosynthesis. Arginase II provides ornithine for polyamines, proline and glutamic acid biosynthesis [3, 4]. Particularly inasmuch as arginase II supplies polyamines biosynthetic pathway with ornithine it is implied to be involved in growth and development of different forms of malignancies [5].

Breast cancer is the major cause of death among woman population therefore huge efforts are made to develop novel and more efficient tools for early diagnosis and treatment. The hypothesis that arginase activity is elevated during breast cancer has been supported by numerous papers, but it is still unclear whether that elevation is correlated with disease stage [6, 7]. Studies performed to reveal correlation of disease stage with arginase activity changes has indicated that although arginase activity elevations are coincided with disease progression, statistically significant elevations take place only during more advanced stages[8].

However little is known about arginase isoenzyme spectrum pattern in human serum during malignant neoplasms. Particularly for breast cancer it is not mentioned how arginase isoenzymes are expressed in human serum and are there any differences compared with healthy individuals.

The purpose of current study was to assay arginase activity in breast cancer patients before surgery and after chemotherapy and compare arginase activity changes with control group. The second point was the investigation of human serum arginase isoenzyme spectrums and assessment of changes during breast cancer.

Patients and Methods

Patients: In this study we essayed arginase activity in the 41 breast cancer patients' serum before therapy and 8 patients' after chemotherapy. All patients were hospitalized in the National Center of Oncology after V.A. Fanarjyan. 11 cases had stage I (57±8, $T_1N_0M_0$), 16 cases had stage II (52±10, $T_{1-2}N_1M_0$), 14 cases had stage III (55±11, $T_{2-3}N_{1-3}M_0$)). Control group included 8 volunteers (49±15).

Arginase activity assay. Arginase activity was determined by colorimetric measurement of urea amounts. In test tubes was added 0.5 ml of serum diluted with 1.5 mL of glycine buffer (0.2 M, pH 9.7), 0.4 mL L-arginine (50 μM), and 0.2 mL $MnCl_2 \cdot 4H_2O$ as an activator. Control tubes were prepared with the same content except L-arginine. Mixtures were incubated at 37°C for 1 hour. After incubation reaction was terminated by adding 1 mL of 20% trichloracetic acid in each tube, followed by centrifugation for 10 min under speed of 5000 rpm. Urea determination was performed according Archibald method with some modifications. 1 mL of supernatant was added to the 2.5 mL of acidic mixture and 0.4 mL of DAMO (diacetilmonoxime) and heated at 100°C for 45 min in the dark room. Color intensity was measured with spectrophotometer at 487 nm. Urea concentrations were calculated as urea amount (μM) in 1 mL serum and expressed as a mean $\pm$ SD (standard deviation).

Arginase isoenzyme separation by gel filtration chromatography. In order to reveal arginase isoenzyme spectrum by gel chromatography, Sephadex G-150 column was used (2.5x50cm) equilibrated with 0.2 M phosphate buffer (pH 7.2). Diluted serum specimens were applied to the column and eluted with phosphate buffer. Total 40 fractions were collected, 4 ml each.

Arginase isoenzyme separation by ion-exchange chromatography. For ion-exchange chromatography CM-cellulose column (1.5x35cm) was used. The elution of the samples was performed using a linear KCl gradient (0.05-0.25 M). 32 fractions were collected total, 4 ml each.

Both after gel chromatography and ion-exchange chromatography amounts of protein were measured spectrophotometrically, at 280 nm. Arginase activity essay has been performed to confirm the presence of arginase isoenzymes in fractions.

Results and Discussion

The results of arginase activity assay are summarized in **Fig.1**. As the results show, arginase activity increase is correlated with disease stage advances. Compared with control group arginase activity was increased for 39.91%, 53.37%, 79.42% and 30.12% in stages I,II, III and after chemotherapy respectively.

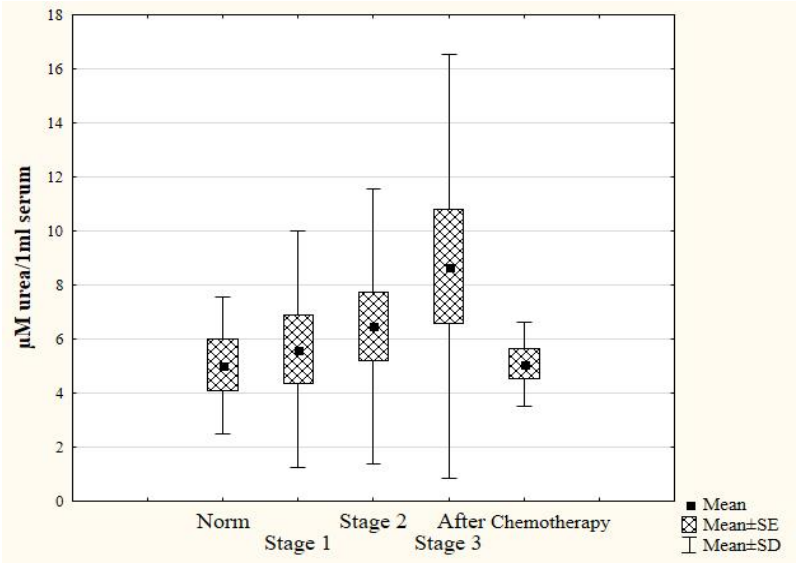

Fig. 1. The arginase activity changes in serum at different stages of breast cancer and after chemotherapy expressed in amounts of urea.
Norm 3.75 ± 1.91 µM/ml (n=8); Stage I 5.62 ± 4.37 µM/ml (n=11); Stage II 6.48± 5.09µM/ml (n=16); Stage III 8.69 ± 7.85µM/ml (n=14); After chemotherapy 5.08 ± 1.55 µM/ml (n=8), p<0.001

This is an interesting result, since our data showed statistically significant correlation for all stages in contrast to other published data, where correlations were found only in advanced stages. Also this results have approved the results from previous studies that arginase activity increase was corresponded to breast cancer progression [9]. Special attention is worth giving to decrease of arginase activity after chemotherapy. In post-chemotherapy group arginase activity showed 26.7% decrease compared with breast cancer patients before surgery indicating that arginase activity after chemotherapy has been closer values to the stage I, however didn't reach to the control group value. This insinuates that arginase may have some role in promoting tumor growth and development and its activity can

be considered as an important marker to determine disease progression or regression.

Arginase isoenzyme spectrum after gel filtration chromatography is represented in **Fig. 2.**

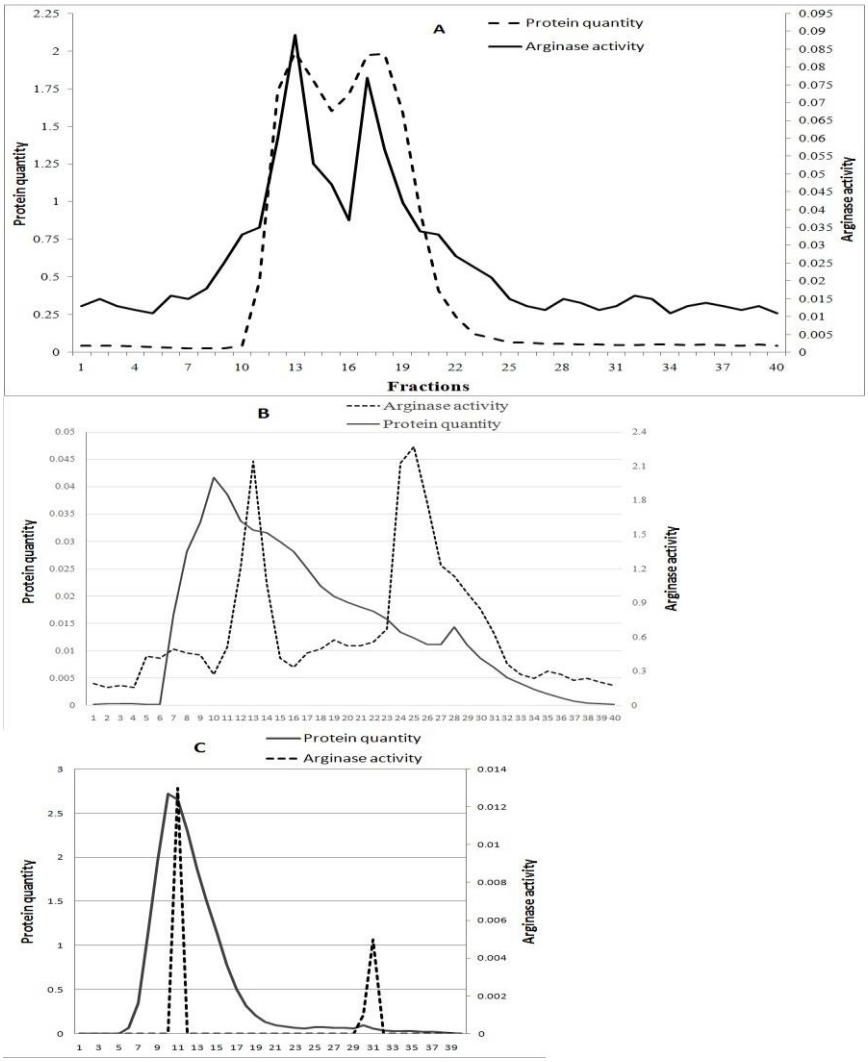

Fig. 2. Gel-chromatography of arginase from serum of healthy people and cancer patients. Control group B. Breast cancer group before surgery C. Breast cancer group after chemotherapy

Arginase isoenzyme spectrum after ion-exchange chromatography is shown in **Fig. 3.**

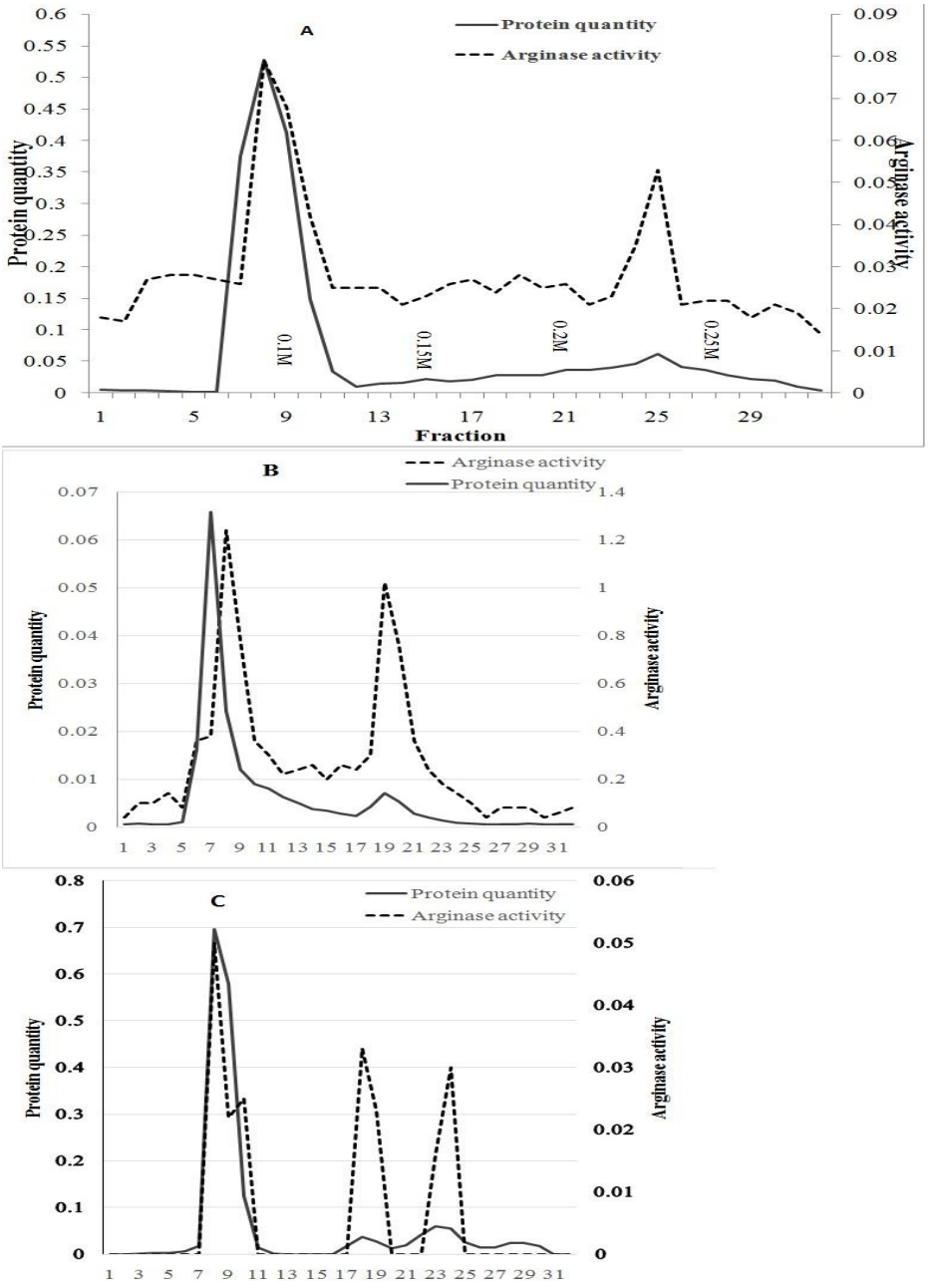

Fig. 3. Ion-Exchange chromatography of arginase from healthy individuals' and cancer patients' serum. Control group B. Breast cancer group before surgery C. Breast cancer group after chemotherapy

In control group gel filtration reveals 1 peak with split into 2 minor peaks. Breast cancer group as well as group after chemotherapy arginase spectrum is

represented with 2 peaks. It is early to judge which isoenzymes exactly are present in healthy and diseased human serum, however relying on available seldom information and previous studies performed in our chair it can be inferred ,that both peaks represent arginase II. It should be mentioned that after chemotherapy on the plot there is a decrease in second peak activity, suggesting that arginase activity decreases after chemotherapy may be driven exactly by changes in this form of arginase.

In ion-exchange chromatography again arginase spectrum is presented with 2 peaks, except post-chemotherapy group where 3 peaks are visible. At this point, as it was mentioned before, very few papers are available regarding isoenzyme spectrums in serum. However for brcast cancer it was revealed that the only isoenzyme expressed in serum is cationic form [10] (arginase II), thus it can be suggested that in breast cancer arginase II is expressed in two forms which differ from each other at least by charge. Arginase isoenzyme spectrum pattern requires further detailed studies, which may lead to the discovery of new isoenzyme of arginase playing key role in development of malignant breast neoplasms.

Conclusion

In current study for the first time was showed that arginase activity decreases after chemotherapy ,as well as the suggestion was supported, that arginase activity is elevated in case of breast malignancies. On the other hand isoenzyme spectrums revealed the possibility of new arginase isoform expression during breast cancer.

So we can draw a conclusion that arginase is an important partaker of cancerogenesis and can be considered as an important diagnostic tool for breast cancer early diagnosis.

Acknowledgments

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Մարիամ Սարգսյան, Հերմինե Գևորգյան, Նիկոլայ Ավթանդիլյան,
Սուսաննա Կարապետյան

ԱՐԳԻՆԱԶԻ ԱԿՏԻՎՈՒԹՅԱՆ ԵՎ ԻԶՈՖԵՐՄԵՆՏԱՅԻՆ ՍՊԵԿՏՐԻ ՓՈՓՈԽՈՒԹՅՈՒՆՆԵՐԸ ՄԱՐԴՈՒ ՇԻՃՈՒԿՈՒՄ ԿՐԾՔԱԳԵՂԻ ՔԱՂՑԿԵՂԻ ՏԱՐԲԵՐ ՍՏԱԴԻԱՆԵՐՈՒՄ ՄԻՆՉ ՎԻՐԱՀԱՏՈՒԹՅՈՒՆԸ ԵՎ ՔԻՄԻՈԹԵՐԱՊԻԱՅԻՑ ՀԵՏՈ

Բանալի բառեր. Արգինազ, կրծքագեղձի քաղցկեղ, իզոֆերմենտային սպեկտր, արյան շիճուկ

Ամփոփում

Արգինազի ակտիվության և իզոֆերմենտային սպեկտրի փոփոխությունները կրծքագեղձի քաղցկեղի ժամանակ հայտնաբերելու համար ուսումնասիրվել են հիվանդների արյան շիճուկները: Արդյունքները ցույց են տվել կորելացիա արգինազի ակտիվության բարձրացման և հիվանդության ստադիաների միջև, ինչպես նաև արգինազի ակտիվության նվազում քիմիոթերապիայից հետո: Իզոֆերմենտային սպեկտրը կրում է որոշ փոփոխություններ քաղցկեղով հիվանդ խմբի մոտ: Հիմնվելով ստացված տվյալների վրա, արգինազը կարող է առաջարկվել որպես բիոմարկեր ուռուցքի առաջ զարգացման/հետզարգացման գնահատման համար:

Мариам Саргсян, Эрмине Геворгян, Николай Автандилян, Сусанна Карапетян

ИЗМЕНЕНИЯ АКТИВНОСТИ И ИЗВФЕРМЕНТНОГО СПЕКТРА АРГИНАЗЫ В СЫВОРОТКЕ ЧЕЛОВЕКА ПРИ РАЗНЫХ СТАДИЯХ РАКА МОЛОЧНОЙ ЖЕЛЕЗЫ ДО ОПЕРАЦИИ И ПОСЛЕ ХИМИОТЕРАПИИ

Ключевые слова: аргиназа, рак молочной железы, изоферментный спектр, сыворотка крови

Аннотация

Для выявления изменений активности и изоферментного спектра аргиназы при раке молочной железы нами была изучена сыворотка крови определенного количества больных. Результаты исследования показали корреляцию между повышенной активностью аргиназы и стадиями болезни, а также понижение ее активности после химиотерапии. Нами было установлено, что изоферментный спектр подвергается изменениям в группе больных раком. В статье мы показали, что на основе полученных данных аргиназа может быть предъявлена в качестве биомаркера для оценивания прогрессии/регрессии опухоли.

Mariam Sargsyan, Hermine Gevorgyan, Nikolay Avtandilyan, Susanna Karapetyan

CHANGES OF ARGINASE ACTIVITY AND ARGINASE ISOENZYME SPECTRUM IN HUMAN SERUM DURING DIFFERENT STAGES OF BREAST CANCER BEFORE SURGERY AND AFTER CHEMOTHERAPY

Key words: arginase, breast cancer, isoenzyme spectrum, blood serum

Summary

Blood serum was examined to reveal changes of arginase activity and serum arginase isoenzyme spectrum in case of breast cancer. The results showed correlation between arginase activity increase and the disease stage, as well the decrease in activity after chemotherapy. Isoenzyme spectrum has undergone some changes in the cancer group. Based on the received data arginase activity may be suggested as a biomarker for evaluation of tumor progression/regression.