

Association of the Arginase I with Bronchial Asthma

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Abstract

Objective The aim of this study was to detect the arginase I (ARG I) enzyme in asthma patients, clarify its role, in addition to examining the relationship of this enzyme with bronchial asthma.

Methods:

Blood samples were collected from 100 patients from Department of Medicine in Rizgary Hospital in Erbil City, in addition to intact 100 volunteers; the introduced questionnaire was filled out on the basis of type-induced asthma, duration of the disease, age of the patients, gender, family history, and allergy condition. Serum was separated to perform **Enzyme-linked immunosorbent assay (ELISA)** in Medical Research Center to examine the association of ARG I with bronchial asthma. By ARG activity we can measure the conversion of arginine to ornithine and urea. By using a quantitative colorimetric assay at 490 nm, employing a QuantiChrom arginase assay kit (Bioassay Systems).

Results: Our results depicted the association between ARG I and bronchial asthma: based on their age, significant elevation of serum arginase level was observed in the patients with ≥ 81 years old, which mean value ($100.16 \pm 19.77^\circ$), p value (0.000); also the duration of asthma ≥ 20 years ($82.48 \pm 38.81^\circ$), p value (0.01) were remarkably affected; this significance was found in those with types of induced asthma and with allergy condition. But non-significant difference in frequency of abnormal serum arginase level was observed in those patients that have family history of asthma disease and gender of the patients. This finding demonstrated a remarkable association of ARG I in the development of asthma at $p < 0.05$.

Conclusion: The ELISA results indicated that the ARG I and bronchial asthma are remarkably correlated. Patients with asthmatic symptoms have the high level of ARG I (> 40 IU/L).

1- Introduction

Asthma is chronic lung disease affects 300 million people worldwide [1]. Allergic asthma is a disease of airway inflammation and hyperresponsiveness. Asthma occur by activating IgE, receptor which is bearing mast cells and basophils, and triggering factors for this, is inhalation of allergen when it bind to complementary IgE. And this is lead to lung infiltration by eosinophils, neutrophils, then activated (M2) macrophages and (T_H2) lymphocytes, which are inflammatory triggers and all these increase mucus production, which is leading to mucosal edema, smooth-muscle hyperresponsiveness, and airway hyperresponsiveness (AHR) due to exposure to e.g. histamine and methacholine [2], [3].

Arginase (ARG) is an essential enzyme that can be detected in mammals and plants. Particularly in humans, being a partaker in the last phase of urine cycle, ARG accelerates conversion of arginine to ornithine and urea. Moreover, ARG level remarkably escalates in those whose body suffers from asthmatic symptoms [4].

The ARG according to Vercelli and Meurs has rule in asthma through different mechanisms which is leading to remodeling of airway through polyamine and proline synthesis by inhibiting nitric oxide, (NO) synthesis, reduction of arginine bioavailability, and increasing ornithine production [5], [6].

Cederbaum et al. (2004) showed that the enzyme has two types; ARG I and ARG II [7]. Also, both are, "encoded by a different genes" [8]. Furthermore "ARG I may be present inside the cytoplasm and expressed in the liver as a part of the urea cycle. ARG I also present in the airway and lung, which they do not have urea cycle [7]. Some studies reported that increase in the level of arginine is an autosomal recessive disease which is caused by a defect in the ARG I enzyme [9].

There is expanding proof that ARG is moreover imperative in the pathophysiology of human asthma [10]. Morris et al (2004), and other researchers reported that decreasing ARG activity and increasing L-arginine concentrations will occur in some patients which have improvement of asthma symptoms [11].

Moreover, immunohistochemical studies about expanded ARG I protein articulation in the aviation route epithelium and aviation route smooth muscle layer of smoking asthmatics contrasted with nonsmoking patients, which may be initiated by nicotine [12].

With respect to the different opinion about the importance of ARG I in asthma, the aim of this research was to detect ARG I enzyme in asthma patients, clarify its role, in addition to examining the relationship of this enzyme with bronchial asthma.

2- Materials and Methods

2 -1 Participants

We performed this study on one hundred subjects with clinical signs of asthma (patients group), and one hundred subjects without clinical signs of asthma (control group), during September 2017 up to May 2018. The ages of the patients ranged from 40-103 years and categorized into three groups: ≤ 60 , 61-80, and ≥ 81 .

All work involving human subjects selected from patients attending the Department of Medicine in Rizgary Hospital, in addition to intact volunteers; the introduced questionnaire was filled out on the basis of type-induced asthma, duration of the disease, age of the patients, gender, family history of allergy, and allergy condition, consensually, of course: 79(79%) out of 100 asthma subjects had an abnormal level of ARG I, and 21(21%) had a normal level. The ethics approval of this study was given by the Ethics Committee of Medical Research Center /Hawler Medical University.

2-2 Enzyme-linked immunosorbent assay (ELISA)

ELISA (ELx800-BioTeK80, U.S.A) in Medical research center was performed for all subjects to examine the association of ARG I with bronchial asthma. Two hundred blood samples were taken from asthma and non-asthma participants. Serum was separated to perform ELISA. By ARG activity, we can measure the conversion of arginine to ornithine and urea, by using a quantitative colorimetric assay at 490 nm, employing a QuantiChrom arginase assay kit (Bioassay Systems). (40 μ l) of serum sample was combined with 10 μ l of the substrate buffer, then added to a 96-well flat-bottomed plate with the appropriate blank controls and 50 μ l 1mM Urea reagent (supplied with the kit) then added to wells to stop the reaction of arginase and incubated at RT for two hours. Then measuring the absorbance at 490 nm and enzyme activity (in IU), calculated according to the kit instructions.

The test result was considered negative (normal value), when the arginase level was ≤ 40 IU and positive (abnormal level) at > 40 IU.

2-3 Statistical Analysis

Statistical Package for Social Science (SPSS) software version 19 was used for statistical analysis. Qualitative parameters were compared between groups by Pearson Chi-square, while quantitative variables were expressed as the mean $\pm$ standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

4-Results and Discussion

Study participants were classified into two groups: asthma (n=100) and non-asthma (n=100) subjects. Table (1) depicts the frequencies of normal and abnormal serum ARGs level in healthy and patient groups; in which normal level of control was (100%), normal level of the patients was (21%), and the patient abnormal level was (79%).

Table 1. The frequency of normal and abnormal serum arginase level in the healthy person and patient groups.

		Control group No. 100	Patient group No. 100	p. value
Arginase IU/L	Normal level	100 (100%)	21 (21%)	0.000
	Abnormal level	0 (0%)	79 (79%)	

According to table (2), the mean age of the study population was 65.73 ± 15 , the control mean age was 39.9 ± 10.59 , and the male to female ratio was 7/93. This table summarizes the characteristics of topics by many clinical parameters: significant differences in age, duration of the disease and serum ARG level were observed between control and patient groups. Gender, types of induced asthma, family history and allergy condition were significantly different between groups. Mean value of arginase was 25.93 ± 7.01 and 61.28 ± 33.93 for healthy donor and asthmatic patients, respectively.

Table 2. Comparison of clinical history between the healthy persons and asthma patients.

		Control group No. 100	Patient group No. 100	P. value
Age (Mean $\pm$ SD)		39.9 $\pm$ 10.59	65.73 $\pm$ 15.08	0.041
Arginase level IU/L (Mean $\pm$ SD)		25.93 $\pm$ 7.01	61.28 $\pm$ 33.93	0.000
Duration year (Mean $\pm$ SD)		0.00 $\pm$ 0.00	10.01 $\pm$ 10.65	0.000
Types of induced asthma	Gender (No.)	Male 6 Female 40	7 93	0.000
	Adulthood induced (No.)	Yes 0 No 100	98 2	0.000
	Exercise -induced (No.)	Yes 0 No 100	93 7	0.000
	Occupational induced (No.)	Yes 0 No 100	68 32	0.000
	Family history (No.)	Yes 0 No 100	39 61	0.000
	Allergy (No.)	Yes 0 No 100	53 47	0.000
	P value <0.05: Significant difference			

As table (3) shows, the age of the patients ranged from 40-103 years and categorized into three groups: 23% of asthma patients which have age ≤ 60 years have a mean of ARG level 53.64 ± 24.26^{ab} ; 69% of asthma patients with 61-80 years old have a mean of ARG level 59.45 ± 32.46^b , and; 8% of them which have ≥ 81 years old have mean of ARG level 100.16 ± 19.77^c . Significant elevation of serum ARG level was found in asthma patients with age group ≥ 81 compared with other groups ($p=0.000$). Interestingly, in our study, the patients who were ≥ 81 years showed the highest mean of ARG level (100.16 ± 19.77), if compared with other group ages. We proposed that the age of patients was related to asthmatic subjects, and can be attributed to increases in ARG I expression and activity. The previous study showed that bronchial asthma is a significant health issue: 5% to 10% of people at different ages suffer from such chronic airway disease [14].

Table 3. Serum level of arginase in asthma patients according to age groups.

	≤ 60 No. 23	61-80 No. 69	≥ 81 No. 8	P. value
Arginase level IU/L (Mean$\pm$SD)	53.64 $\pm$ 24.26 ^{ab}	59.45 $\pm$ 32.46 ^b	100.16 $\pm$ 19.77 ^c	0.000
Different letters: mean significant differences, P value <0.05: Significant difference				

Table (4) depicts the duration-based asthma disease. The mean of ARG level was 61.35 $\pm$ 38.15^{ab} for 46% of patients who were suffering from asthma for 9 years and above. For 38% of asthma patients who were living with the disease for 10 to 19 years, had the 52.28 $\pm$ 20.53^b mean of ARG level. And 16% of asthma patients with ≥ 20 disease interval scored 82.48 $\pm$ 38.81^c. Significant elevation of serum ARG level was observed in patients suffering asthma for more than 20 years ($p=0.01$). The results revealed that the duration of disease was related to the level of ARG I. Patients with symptoms of asthma (≤ 9 and ≥ 20 years) have abnormal levels of ARG I, but the highest ARG level (82.48 $\pm$ 38.81^c) was observed in 16% of asthma patients who have the disease for more than 20 years. Moreover, the duration of disease is one of the important factors in increasing the activity of ARG I and decreasing L-arginine concentration. In a study done by North et al. It became evident that the increase of ARG I levels in chronic asthma is more significant, compared with acute cases [13].

Table 4. Serum level of arginase in asthma patients according to disease duration.

	≤ 9 years No. 46	10-19 years No. 38	≥ 20 years No. 16	P. value
Arginase level IU/L (Mean$\pm$SD)	61.35 $\pm$ 38.15 ^{ab}	52.28 $\pm$ 20.53 ^b	82.48 $\pm$ 38.81 ^c	0.01
Different letters: mean significant differences, P value <0.05: Significant difference				

As table (5) shows, no significant correlation was found between ARG I activity and these variables (gender and family history), except for types of induced asthma and allergy asthma, both which were significant. Based on the results of this study, seventy-eight patients with exercise-induced asthma have high levels of arginase, and fifteen patients have a normal level of the enzyme. According to our knowledge, we could find that exercise-induced asthma was significantly associated with ARG activity. [16], in Japan reported that the mean value of ARG activity of eleven employees, who were daily trained for a week, was 6.3 $\pm$ 0.7. For fifty-three of workers exercised not on a daily basis, the mean value of ARG activity was 4.8 $\pm$ 0.3. One hundred nineteen workers with no exercise had their mean value of ARG activity touched 3.9 $\pm$ 0.2. It means that the group with daily training has highest ARG level compared with other groups.

Regarding the study findings, the significant prevalence of abnormal serum ARG level was found in seventy-nine patients with adulthood induced asthma, who were recruited for this study at Rizgary Teaching Hospital/ Medicine department in Erbil. Morris et al. showed that from twenty-six adult patients that have history of asthma at different stages of exacerbation there is a high ARG activity if compared to normal control subjects without asthma (1.6 $\pm$ 0.8 versus 0.5 $\pm$ 0.3 μ mol/ml/hour, asthma versus control, $p < 0.0001$) and low level of arginine, compared to normal control subjects without asthma (45 $\pm$ 22 versus 94 $\pm$ 29 μ M, $p < 0.0001$) [11].

In this study, occupations considered one of the causes of asthma: seventy-four patients were workers who inflicted occupational asthma by breathing in chemical fumes, dust, gases, or other substances on the job, or could result from exposure to a substance which they're sensitive to. The frequency of high level of ARG (which is more than 40 IU/L) found in the seventy-four patients was statistically significant ($p=0.000$). The previous studies articulated that surrounding particles and ozone layer are the most common causes of urban air pollution, both which are causes of environmental exposures in murine models of airways inflammation due to arginase I up-regulation [17].

The results of our study were strongly related to family history of asthma. Forty-one patients out of 54 had an abnormal level of ARG, while the rest 13 had normal levels ($P=0.414$). Our results demonstrated that a family history of asthma is a significant risk factor for asthma and familial risk assessments also help recognizing an individual's highest risk to get asthma. [18] reported that the results of population-based cohort study indicate that the asthma during the first 27 years of life are usually strongly related to family history of asthma. The results indicate that children of asthmatic mother will develop asthma earlier than those with asthmatic father, which is reflected in the effect estimates. Otherwise study of [19] reported that the familiarly asthma both maternal and paternal asthma had more risk of adult -onset asthma. In the present study, fifty-three patients with asthma have allergies, and this is called allergic asthma

which can be triggered by pet dander, pollen, dust and other allergy stimulators, and forty-seven patients with non-allergic asthma can be spread by stress, medication, air temperature, smoke, and infections of the airway. It means that asthma can be associated with either an allergic or non-allergic conditions. Forty-nine of fifty-three patients with allergy had an abnormal level of arginase. Consistent with the result of Maarsingh et al. [20]. In this study, we revealed the association of arginase I in the development of asthma.

Table 5. The frequency of clinical history parameters among asthma patients according to the normal and abnormal serum level of arginase.

		Arginase level IU/L		P. value
		Normal level No. 21	Abnormal level No. 79	
Gender (No.)	Male (7)	1	6	0.546
	Female (93)	20	73	
Types of induced asthma	Adulthood induced (No.)	Yes (98)	19	0.042
		No (2)	2	
	Exercise induced (No.)	Yes (93)	15	0.000
		No (7)	6	
	Occupational induced (No.)	Yes (68)	5	0.000
		No (32)	16	
	Family history (No.)	Yes (54)	13	0.414
		No (46)	8	
Allergy (No.)	Yes (53)	4	0.000	
	No (47)	17		
P value ≥0.05: Non-Significant difference, P value <0.05: Significant difference				

5-Conclusion

Our findings provide evidence for the key role of ARG I activity in the asthma disease. Therefore, this enzyme might serve as a useful biological marker in asthma disease.

6-Acknowledgment

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Conflict of interests

There are no conflicts of interest.

7- References

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الخلاصة

الهدف: الهدف من هذه الدراسة هو الكشف عن إنزيم أرجيناز I ودوره في مرضى المصابين بالربو و دراسة علاقته بالربو القصبي.

المواد والطرق: تم جمع عينات الدم من ١٠٠ مريض مصابين بمرض الربو اللذين ادخلوا قسم الباطنية في مستشفى زكريا التعليمي و ١٠٠ شخصاً من الأصحاء الذين ليس لديهم علامات سريرية سابقة لأي مرض حاد أو مزمن كمجموعة المقارنة. حيث تم ملء الاستبيان على أساس مرض الربو الناتج عن نوعه ومدة المرض وعمر المرضى والجنس والحالة السريرية للعائلة وظرف الحساسية. في مركز البحث الطبي تم فصل مصل الدم لإجراء فحص ARG1 وإيجاد علاقته مع الربو القصبي باستخدام تقنية مناعي مرتبط بالإنزيم (ELISA). تم تحديد نشاط ARG1 وقياس تحويل الأرجينين إلى أورثين واليوريا باستخدام مقياس قياس الألوان الكمية على طول موجي ٤٩٠ نانومتر.

النتائج: أظهرت النتائج وجود علاقة إيجابية بين ARG1 والربو القصبي. لوحظ ارتفاع ملحوظ في مستوى الأرجيناز في مصل الدم للمرضى الذين تتجاوز أعمارهم عن ٨١ سنة و قيمة متوسط و قيمة p (100.16±19.77^c); (0.000). كما لوحظ علاقة ملحوظ في مستوى الأرجيناز مع مدة الإصابة بالمرض. حيث كان مستوى الأرجيناز عالية في المرضى المصابة بالربو لأكثر من ٢٠ سنة (82.48±38.81^c) و قيمة p (0.01). كذلك لوحظ علاقة ملحوظ بين مستوى الأرجيناز وأولئك الذين يعانون من أنواع الربو المستحث ومع ظرف الحساسية أيضاً. ولكن لوحظ اختلاف غير معنوي في مستوى الأرجيناز للمصل مع الحالة السريرية للعائلة لمرض الربو و جنس المريض.

وأظهرت النتائج وجود ارتباط ملحوظ ل ARG I في تطویر مرض الربو في p < 0.05 .

الاستنتاج: أشارت نتائج ELISA أن ARG I مرتبطة بشكل ملحوظ بالربو القصبي. المرضى الذين يعانون من أعراض الربو لديهم مستوى عال من ARG I (> 40 وحدة دولية / لتر).

الكلمات الدالة: أرجيناز الأول، الربو، L-أرجينين، L-الأورثين، اليوريا.