Evaluation of Enzymatic and Non Enzyme Antioxidants From Sera of Asthmatic Patients in Hilla City

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Abstract

The study was conducted on healthy subjects [control (n=39), male=19, females =20] and asthmatic patients who were treated with inhaled salbutamol [patients (n=41), male=21,females=20] and asthmatic patients who were treated with Montelukast drugs [patients (n=37), male = 19 ,females=18].The samples of patients were obtained from Asthma and Allergy Center in Hilla city. The study was performed at the laboratory of biochemistry department in college of medicine, University of Babylon. Measurements the total antioxidant capacity (TAC) , total protein and Superoxide dismutase (SOD)enzyme activities , also Se, Zn and Cu levels were measured by flame atomic absorption spectrophotometry (FAAS), in sera.The results revealed that highly significant decreasing in the TAC and SOD activity in sera of asthmatic patients ($p<0.01$) in comparison with that of controls subjects. Serum levels of Zn, Cu, and Se were significantly different ($p<0.001$ for all) in patients when compared with healthy subjects.

الخلاصة

أجريت الدراسة على أشخاص أصحاء (مجموعة سيطرة عددهم 39(الذكور ١٩والاناث ٢٠)) ومرضى الربو الذين يتعاطون المستنشق السالبيوتامول (المرضى عددهم ٤١ (الذكور ٢١,الإناث ٢٠) و ومرضى الربو الذين عولجوا بعقار مونتيلوكاست [المرضى (ن = ٣٧)، الذكور = ١٩، الإناث = ١٨] .

تم الحصول على عينات من المرضى من مركز الحساسية و الربو في مدينة الحلة . وقد أجريت الدراسة في مختبر قسم الكيمياء الحياتية في كلية الطب ، جامعة بابل. تم قياس مضادات الأكسدة الكلية والبروتين الكلي وفعالية أنزيم النحاس والزنك سوبراوكساید ديسموتاز وكذالك قياس مستويات السيلينيوم والخارصين والنحاس بواسطة مطياف الامتصاص الذري أللهبي (FAAS) في مصل الدم . النتائج أظهرت وجود انخفاض ملحوظ في مضادات الأكسدة الكلية وفعالية أنزيم النحاس والزنك سوبراوكساید ديسموتازفي مصول مرضى الربو ($p<0.01$) بالمقارنة مع مجموعة السيطرة . واختلاف ملحوظ في مستويات الزنك و النحاس والسيلينيوم ($p<0.01$) للكل بالمقارنة مع مجموعة السيطرة.

Introduction

Asthma is characterized by the excess of oxygen free radicals production and/or the decrease of antioxidant defense capacity. The intensity of oxidative stress is correlated with the appearance of complications of asthma . Asthma is a chronic inflammatory disease associated with oxidative and antioxidative disequilibrium. High oxygen levels of the pulmonary environment render it susceptible to oxidative stress reactions**[1].** Products generated in this condition, such as reactive oxidative species (ROS) and

reactive nitrogen species (RNS), are biomarkers for disease progression **[2,3]**.The combination of these facts, including increase in ROS and RNS, oxidative stress and nonenzymatic production of inflammatory mediators, give us indication of the oxidative imbalance importance in asthma**[4]** .

Another feature found in asthma is the loss of antioxidant defenses **[5]**. Lungs and blood have many enzymatic and nonenzymatic antioxidants, like glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), heme oxygenase, glutaredoxin, thioredoxin, peroxiredoxin, glutathione, carotenoids, vitamins E, A and C all with the purpose of counteracting oxidants toxicity. Increased ROS and RNS lead to structural and functional protein modification that are biologically relevant for inflammation initiation and maintenance **[6,7]**.

Minerals are only found in trace quantities in animals and are a small proportion of dietary antioxidants, but play important roles in their metabolism. Regarding antioxidant activity, the most important minerals are selenium and zinc. Selenium can be found in both organic (selenocysteine and selenomethionine) and inorganic (selenite and selenite) forms in the human body. It does not act directly on free radicals but is an indispensable part of most antioxidant enzymes (metalloenzymes, glutathione peroxidase, thioredoxin reductase) that would have no effect without it **[8]**.

Many studies pointed to the biological role of these elements in many physiological and pathological conditions as they play an important role in protection the body from free radicals and toxic minerals, and decreased levels of these elements has its effect on antioxidant systems and lead to hyperactivity and inflammation in respiratory system **[9,10]**. In this work study the relationship of oxidative stress associated with asthmatic patients who were treated with inhaled salbutamol and asthmatic patients who were treated with montelukast drugs, evaluation of the antioxidant enzymes (TAC , SOD),and also Se, and study the relationship between antioxidant with Zn , Cu levels in sera of control and asthmatic patients.

Materials and Methods

1–Samples

Three groups of samples were included in this study. Group (1) contained (39) healthy subjects (19 males,20 females).Group (2) consisted of (41) asthmatic patients who were treated with inhaled salbutamol (21males,20 females). Group (3) consisted of (37) asthmatic patients who were treated with Montelukast drugs were included in this study. (19males,18 females) . The study was carried out from the first of January 2013 to the day 31 of June 2013. The blood samples of patients were obtained from, Asthma and Allergy Center in Hilla city. The study was performed at the laboratory of Biochemistry Department in College of Medicine, University of Babylon. Serum used to assay antioxidant enzymes activities, Se, Zn and Cu concentration.

2- Determination of plasma Total Antioxidant capacity (TAC)

Total Antioxidant capacity was determined by Bio Vision TAC Assay Kit. Bio Vision developed the TAC Assay Kit, which can measure either the combination of both small molecule antioxidants and proteins or small molecules alone in the presence of our proprietary Protein Mask**[11]**.

3- Determination of total serum protein concentration .

The protein content of all samples was determined by total protein kit **[12]**using Bovine Albumin 6 g/dL as standard protein for all samples.

4- Determination of Superoxide Dismutase(SOD) Activity by inhibit the autoxidation of pyrogallol.The principle of this method is based on the competition between the pyrogallol autoxidation by $O_2^{\cdot -}$ and the dismutation of this radical by SOD **[13]**.

5-Determination of trace elements .

Zn, Se & Cu ions levels were measured in sera of patients and control groups by atomic absorption spectroscopy and Hydride generation atomic absorption spectroscopy for Se , values expressed in microgram per deciliter µg /dl for Se and mg/dl for Zn & Cu **[14]**.

6-Statistical Analysis:

All results were given as the mean ± standard deviation value and data analysis were performed by SPSS 18.0 statistical program. The data coded in to a data base and analyzed by SPSS. Differences between studied groups were tested by t test and P< 0.05 value was accepted as statistically significant**[15]**.

Results

All patients were diagnosed as asthmatics. The frequency for less than 10 years duration of asthma was 45%, 10-19 years duration was 33% and 20 years or more was 22%. They all had a history of intermittent wheezing, shortness of breath, chest tightness and were taking different asthma medications. Serum Parameters level was found significantly different in patients compared to controls, (, P value was 0.001 (table 1).

Table (1) TAC,total protein , and SOD level in the studied groups.

Parameters	G1 Mean± SD	G2 Mean± SD	G3 Mean± SD	P
TAC nmol/µl	4.00 ± 0.32	2.38 ± 0.55	2.35 ± 0.62	P<0.001
Cu-Zn SOD U/ml	23.04 ± 2.05	16.87 ± 0.74	16.82 ± 0.79	P<0.001
Total protein g/dl	7.03 ± 0.26	7.79 ± 0.38	7.73 ± 0.34	P<0.001
Sp .Act U/ g	3.35±0.33	2.17±0.13	2.15±0.14	P<0.001

Table (2) Zn, Se & Cu concentration in the studied groups.

Parameters	G1 Mean± SD	G2 Mean± SD	G3 Mean± SD	P
Selenium µg /L	72.74 ± 8.52	43.28 ± 8.61	49.57± 11.12	P<0.001
Zinc mg /dl	0.94±0.11	1.08 ±0.14	1.08±0.13	P<0.001

Copper mg /dl	1.62 ±0.22	0.94 ± 0.13	0.89 ±0.15	P<0.001
Cu/Zn ratio	1.74 ± 0.32	0.87±0.14	0.83 ±0.19	P<0.001

Table (3-13) The correlation between parameters level in G2.

		Tpro	TAC	Cu	SOD	Zn	Se	Cu/Zn
Tpro	r	1.000	0.293	0.344*	0.044-	0.062	0.103	0.234
	pvalue		0.067	0.030	0.786	0.704	0.529	0.146
TAC	r	0.293	1.000	0.331*	0.077-	0.119-	0.013-	0.373*
	pvalue	0.067		0.037	0.637	0.464	0.939	0.018
Cu	r	0.344*	0.331*	1.000	0.284	0.241	0.379*	0.646**
	pvalue	0.030	0.037		0.075	0.134	0.016	0.000
SOD	r	0.044-	0.077-	0.284	1.000	0.105	0.224-	0.137
	pvalue	0.786	0.637	0.075		0.521	0.165	0.399
Zn	r	0.062	0.119-	0.241	0.105	1.000	0.181	0.571-**
	pvalue	0.704	0.464	0.134	0.521		0.263	0.000
Se	r	0.103	0.013-	0.379*	0.224-	0.181	1.000	0.165
	pvalue	0.529	0.939	0.016	0.165	0.263		0.309
Cu/Zn	r	0.234	0.373*	0.64**	0.137	0.571-**	0.165	1.000
	pvalue	0.146	0.018	0.000	0.399	0.000	0.309	

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table (3-14): The correlation between parameters level in G3.

		Tpro	TAC	Cu	SOD	Zn	Se	Cu/Zn
Tpro	r	1.000	0.193	0.295	0.080	0.102	-0.170-	0.170
	pvalue		0.233	0.064	0.622	0.532	0.295	0.294
TAC	r	0.193	1.000	0.224	-0.109-	0.058	-0.135-	0.141
	pvalue	0.233		0.164	0.504	0.721	0.407	0.384
Cu	r	0.295	0.224	1.000	0.249	0.110	0.108	0.732**
	pvalue	0.064	0.164		0.121	0.498	0.507	0.000
SOD	r	0.080	0.109-	0.249	1.000	0.081	-0.091-	0.135
	pvalue	0.622	0.504	0.121		0.621	0.574	0.406
Zn	r	0.102	0.058	0.110	0.081	1.000	0.023	0.580-**
	pvalue	0.532	0.721	0.498	0.621		0.890	0.000
Se	r	0.170-	0.135-	0.108	-0.091-	0.023	1.000	0.080
	pvalue	0.295	0.407	0.507	0.574	0.890		0.624
Cu/Zn	r	0.170	0.141	0.732**	0.135	0.580-**	0.080	1.000
	pvalue	0.294	0.384	0.000	0.406	0.000	0.624	

Table (3-14): The correlation between parameters level in G3.

		Tpro	TAC	Cu	SOD	Zn	Se	Cu/Zn
Tpro	r	1.000	0.193	0.295	0.080	0.102	-0.170-	0.170
	pvalue		0.233	0.064	0.622	0.532	0.295	0.294
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	pvalue	0.532	0.721	0.498	0.621		0.890	0.000
Se	r	0.170-	0.135-	0.108	-0.091-	0.023	1.000	0.080
	pvalue	0.295	0.407	0.507	0.574	0.890		0.624
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	pvalue	0.294	0.384	0.000	0.406	0.000	0.624	

**. Correlation is significant at the 0.01 level (2-tailed).

Discussion

Total Antioxidant Capacity (TAC) values for G1,G2andG3 with the mean ± SD (4.00 ± 0.32) (2.38 ± 0.55) (2.35 ± 0.62) respectively. statistically significant difference was detected between G1 with G2 and G3(P<0.001). But there was no statistically significant difference between G2with G3. TAC could not be altered in asthmatic patients **[5]**. In contrast, a small but significant decrease in plasma TAC was found in asthma patients with chronic evolution **[16]**. This could be explained by the fact that severe asthma is associated with low plasma levels of vitamin C and billirubin, and high plasma levels of cholesterol **[17]**.

Cu-Zn SOD structure changes. (Lower Zn damages to the cell structure and effects the activity of Cu-Zn SOD, concequently reduces the ability of Cu-Zn SOD to scavenge free radicals). When the contents of Cu and Zn decrease Cu-Zn SOD activity also diminishes and free radicals causeincreased lung injury**[18]**.

The free radicals were considered as responsible for hundreds of diseases because of their disturbing effects on the cells and tissues . In order to investigate further link between low selenium status and asthma, a study was undertaken in Babylon. This study showed that asthmatic patients in Babylon had lower whole blood selenium concentrations than the control subjects and was in compatible to the previous New Zeland study, which showed that this result was associated with a reduced whole blood glutathione peroxidase activity.

The Copper content of serum was found to be significantly lower in asthmatic patients than in control individuals **[19]**. Low level of copper may be associated with decrease in Cu-Zn SOD activity **[20]** which leads to remove free radicals in respiratory system. The mechanism by which copper deficiency induces anemia is

based on the requirement of copper for several enzymes involved in iron transport and utilization**[21,22].** Increased ROS and RNS lead to structural and functional protein modification that are biologically relevant for inflammation initiation and maintenance **[5]**. Such alterations include the reduced activity of SOD, CAT and GPx **[23,24].**

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