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Serum Uric acid Level in the Blood of Asthmatic patients in Basrah Governorate-Iraq.

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Abstract

The study included the measurement of the mean serum uric acid level in asthmatic patients. In this study, a total number of (50) asthmatic patients (24 males, 26 Females) visiting Allergic and Asthma Medical Center in Basrah city were involved, classified according to age, sex, severity, smoking and family history. From each patient blood samples were collected to estimate uric acid concentration. In addition, (50) healthy subjects were investigated as a control group.

It was found that a highly significant increase ($P < 0.01$) is in uric acid serum level in all asthmatic patients as compared to healthy control. In accordance with age and severity, serum uric acid level increases significantly ($P < 0.05$) while with smoking patients, and positive family history a highly significant increase is ($P < 0.01$). There were no significant changes in the uric acid level between males and females.

In conclusion, asthmatic patients suffer a high degree of reactive oxygen species (ROS) formation causing considerable oxidative stress indicated by a high level of the serum uric acid.

Key words*:* MDA: Malondialdhyde, ROS: Reactive Oxygen Species, RNS: Reactive Nitrogen Species, SOD: Superoxide Dismutase ,UA:uric acid .

Introduction

Uric acid is a naturally occurring of purine metabolism [1]. Urates are the ionized form of uric acid predominate in plasma extracellular fluid. Uric acid production varies with purine content of the diet and the rates of purine biosynthesis and degradation. Normally two-thirds to three-fourths of uric acid are excreted by kidneys and most of the remainder is eliminated through the intestines [2]. Uric acid is also found within cell and in all body fluids at lower concentrations than in plasma where it exists mostly in its ionized form urate at physiological pH [3]. Humans have no enzyme for further oxidation of uric acid , so an access of uric acid is excreted by kidney. In normal condition the rate of synthesis of uric acid equals the rate of its consumption and excretion [4].

At physiologic pH, urate is a strong reducing substance, electron doner, and antioxidant, contributing half of the plasma

antioxidative capacity. Urate binds pro-oxidant metals iron and copper (fenton reaction), thus preventing their interaction with hydrogen peroxide (H_2O_2) to produce hydroxyl radical ($OH^{\cdot}$). Also, it reacts with reactive oxygen species (ROS) and inhibits lipid peroxidation [5]. Increased plasma urate levels may be a marker of oxidative stress [6].

Reaction of uric acid with peroxynitrite ($ONOO^{-}$) which is one of the most reactive species in human body. Uric acid acts as a specific inhibitor of radicals resulting from the breakdown of peroxynitrite [7]. Peroxynitrite can erode the structure and function of proteins by causing nitration of their tyrosine residues [8].

Uric acid repairs the oxidiative damage to DNA bases [9]. It protects erythrocytes from peroxidation damage that lead to lysis. Urates are much more easily oxidized than deoxynucleotides by singlet oxygen ($O^{\cdot}_2$) [10].

Furthermore, uric acid inhibits the degradation and deactivation of extracellular superoxide dismutase (SOD), an enzyme with significant antioxidant activity in vascular endothelium [11].

It is suggested that uric acid functions as an antioxidant when present at normal concentrations, but works as a pro-oxidant at higher concentration, accerlerates atherosclerosis[12]. Increased levels of uric acid (hyperuricemia) are also associated with diseases such as gout, renal and cardiovascular disease[13,14].

Asthma is a chronic inflammatory disease of the respiratory tract of unknown etiology. According to the world health organization, asthma is now a serious public health problem with over 100 million sufferers worldwide, death from this condition has reached over 180,000 annually [15,16].

Asthma remains the most common chronic illness of childhood, it is regarded as the fifth cause of death in children [17].

Low uric acid levels (hypouricemia) is much less common than hyperuricemia[18]. The range of uric acid levels in males (3.5-7.5)mg/dL (208-428)µmol/L, and in females is (2.5-6.0)mg/dL (155-357)µmol/L.

The present study was undertaken to evaluate the uric acid concentration in the serum of asthmatic patients with several parameters (age, sex, severity, smoking, and family history) in Basrah Governorate-Iraq.

Material and Methods

Samples

Fifty patients (24 males, 26 females) with asthma clinically admitted to allergic center from the first of October 2012 to the end of March 2013 in Basrah city-Iraq whose age ranged between (15-60) years for males and females,are divided into three groups (15-35) years, (36-50)years, (>50) years.

The following information was recorded for asthmatic patients: age, sex, smoking, date of admission, family history of allergy, sign and symptom and severity of the acute attack.

Those patients were classified according to the severity into three groups:

1-Mild 2-Moderate 3- Sever

This classification of severity depends on expert panel report; guidelines for the diagnosis and management of asthma, sign and symptoms were used mainly for the classification of severity [19]

Fifty healthy subjects (24 males, 26 Females) were investigated as a control group from Public Health Center in Basrah city aged between (15-60) years.

Blood samples (5mL) were collected from patients and healthy control by vane puncture using a sterile disposable syringe in plain plastic tube.

The blood was centrifuged at 3000 rpm for 10min; the serum was collected and frozen at -20 $^{\circ}$C for estimation of uric acid.

Instruments

1- Spectrophotometer SP8-100 UV pye Unicom, U.K.
2- Centrifuge, Kokusan, Japan model 2200A.
3- Vortex stirrer, Gallen Kamp, Germany model SGP-301.
4- Water bath, Gallen Kamp, Germany model YCW-01.

Estimation of serum uric acid

Uric acid was assayed enzymatically by uricase method using a kit from France Biomerix (No.02160) depending on enzymatic method of Artctiss and Entwistle (1981)[20] which is based on the following principle.

Principle

Uricase acts on uric acid to produce allantion, hydrogen peroxide and carbon dioxide. Hydrogen peroxide in the prescence of peroxidase reacts with a chromogen (4-amino antipyrine and 3,5-dichoro-2-hydroxybenzene sulfonate) to yield quinoneimine a red coloured complex. The absorbance measured at 520 nm is proportional to the amount of uric acid in the sample.

Figure (1): Chromogen (4-amino antipyrine and 3,5-dichloro-2-hydroxybenzene sulfonate)(1) to form a stable, red coloured N-(antipyryl)-3-chloro-5-sulfonate-*p*-benzoquinone-monoimine (II) .

Reagents

1- Reagent (R1) : Standard uric acid 10mg/dL (595µmol/L).
2- Reagent2 (R2) : Buffer solution which is composed from:
   A. Phosphate buffer pH = 8.0 at 25 ºC (50 mmol/L).
   B. 3-5 dichoro-2-hydroxy benzene sulfonate (2.0 mmol/L).
   C. Surface active agent.
3- Reagent3 (R3) : Enzymes.
   A. Uricase (≥ 120 u/L).
   B. Peroxidase (≥ 450 u/L).
   C. 4-aminoantipyrine (0.150 mmol/L).
   D. Potassium hexacyano ferriate (42µmol/L).
4- Working solution : prepare by mixing 30 mL of reagent 3 into 100

mL of reagent 2 . stable for 4week at 2-8 ºC.

Procedure

To 25 µL of serum sample (T), standard uric acid (S) and distilled water for reagent blank (B), add 1 mL of the working solution were added, mixed and incubated at 25 °C for 5 minutes. Then the absorbance of serum and standard were recorded at 520 nm against the reagent blank (B).

calculation

The concentration of uric acid (mg/dL) = $\frac{AT}{AS}$ X 10

10 : the concentration of the standard in mg/dL.

AT : The absorbance of the sample serum.
AS : The absorbance of the standard

Statistical Analysis

The results were expressed as mean ± stander deviation (SD). The data was analyzed by one-way analysis (ANOVA),while the correlation between the data was tested statistically by simple linear test using computer SPSS program (No.21). P-value of less than 0.05 was considered as statistically significant, P-value less than 0.01 as highly significant.

Result and Discussion

The present study revealed a highly significant increase ($P < 0.01$) in uric acid serum level in all asthmatic patients during acute attack as compared to healthy control. These significant changes increase with age and severity from mild to moderate than severe ($P < 0.05$). Also there were no significant differences between male and female patients as indicated in (Table1,Fig.2).

These imply that patients during acute asthmatic attack are exposed to considerable degree of lipid peroxidation, it has a strong correlation with atopic asthma suggesting that oxidative stress affects simultaneously on lipid peroxidation [21].

Oxidative stress can have effects on airway function, including airway smooth muscle contraction, induction of air way hyper responsiveness, mucus hyper secretion, epithelial shedding and vascular exudation [21].

The inflammatory cell of asthmatics has an increased capability to generate free radicals compared to controls, which further contributes to high concentration of ROS. Excess reactive nitrogen species (RNS) may also be produced by asthmatics [22, 23].

Asthma severity is related to the extent of lipid peroxidation, with a positive association between uric acid concentration and disease severity ($P < 0.05$) [24] ,as indicated in(Table1).

On the other hand, there was a highly significant difference in uric acid level between asthmatic smokers and non smokers ($P < 0.01$) as indicated in (Table1, Fig.2). Cigarette smoke contains high amount of free radicals such as superoxide anion $(O^{\cdot}_2)^{-}$ and nitric oxide ($NO^{\cdot}$) [25], gas phase can react chemically to form highly reactive free radical peroxynitrite . In addition superoxide anion can react with hydrogen peroxide to form the more active hydroxyl free radical [22].

Many researchers have reported increased level of super oxide anion from circulating neutrophils and increased lipid peroxidation products malondialdhyde (MDA) in the plasma of smokers, supporting the concept of systematic oxidative stress in these individuals [26].

Also family history seems to influence the level of uric acid as compared to positive and negative family history of asthmatic patients, there were a highly significant increase ($P < 0.01$) in the uric

acid serum level as shown in (Table1, Fig2). Some genetic variants may only cause asthma when they are combined with specific environmental exposures, the genetic trait, CD_{14} Single nucleotide polymorphism, and exposure to endotoxin (a bacterial product). Researchers have found that the risk of asthma changes based on a person's genotype at CD_{14} and level of endotoxin exposure [26,27]

Asthma itself may cause physiological changes in serum antioxidants burden associated with disease [28, 29].

Numerous disturbance of antioxidant defense mechanisms have been described in asthma, respiratory epithelial lining fluid contains high concentration of antioxidants: proteins and enzymes, low molecular compounds (predominatly glutathione and urates), ascorbic acid, vitamin E which provide a first line defense against inhaled and endogenously oxidant [30].

Uric acid concentration could play an important role in gene-environment interaction in complex lung disease such as asthma.

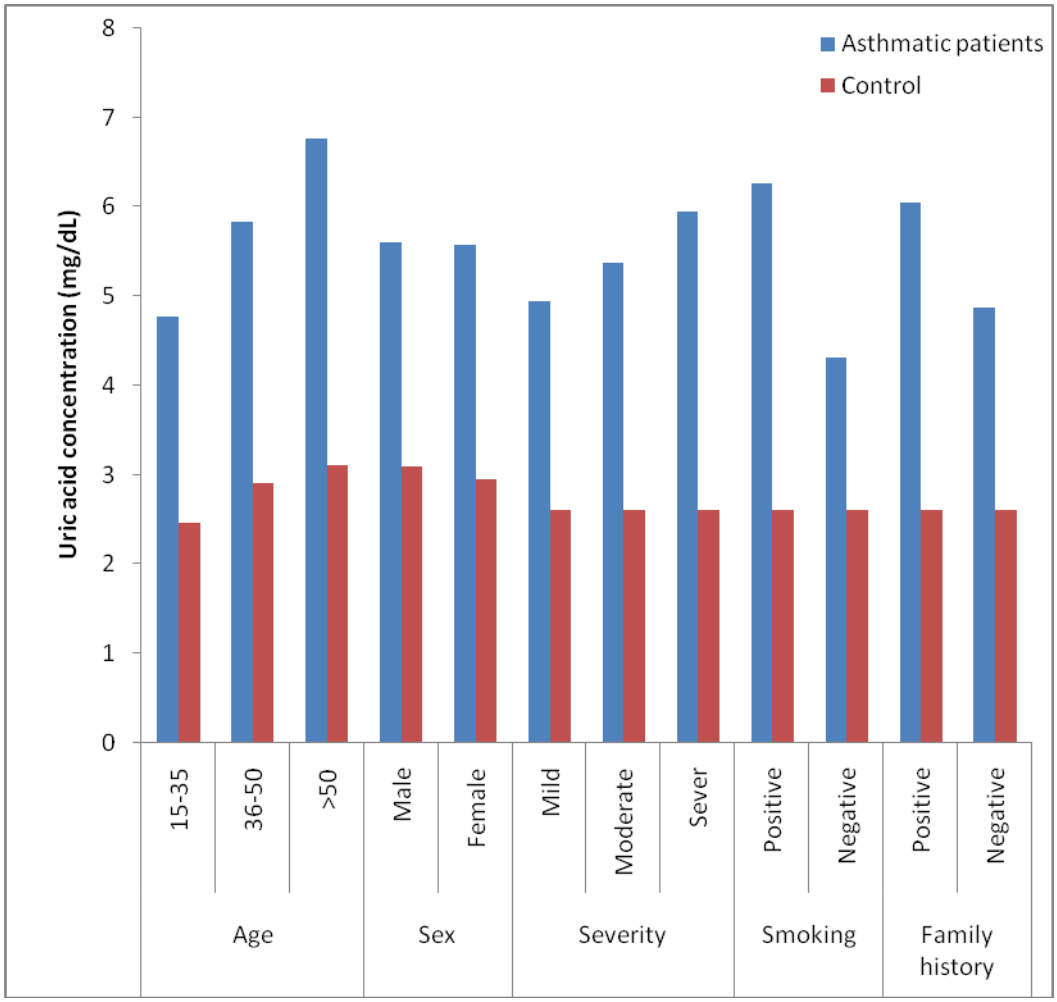

Figure 2. Serum uric acid concentration (mg/dL) in asthmatic patients and healthy control classified according to (Age, Sex, Severity, Smoking and Family history).

Table (1): Serum level of uric acid (mg/dL) in asthmatic patients classified according to (Age, Sex, Severity, Smoking and Family history) and healthy control.

Variable		Uric acid concentration(mg/dL) Asthmatic patients (50)		Uric acid concentration(mg/dL) Control (50)		P - value
		No	Mean ±SD	No	Mean ±SD	
Age/year	15-35	15	4.78 ± 0.20*	15	2.46 ± 0.30	P < 0.05
	36-50	19	5.83 ± 0.40*	19	2.91 ± 0.27	
	>50	16	6.77 ± 0.20*	16	3.11 ± 0.25	
sex	Male	24	5.61 ± 0.30	24	3.10 ± 0.40	P < 0.01
	Female	26	5.58 ± 0.20	26	2.95 ± 0.38	
Severity	Mild	15	4.95 ± 0.42*	50	2.61 ± 0.40	P < 0.05
	Moderate	19	5.38 ± 0.39*			
	sever	16	5.95 ± 0.45*			
Smoking	Positive	27	6.27 ± 0.32**	50	2.61 ± 0.40	P < 0.01
	Negative	23	4.32 ± 0.28			
Family history	Positive	30	6.02 ± 0.35**	50	2.61 ± 0.40	P < 0.01
	Negative	20	4.87 ± 0.27			

*** The mean difference is significant at the 0.05 level**
****The mean difference is significant at the 0.01 level**

Conclusion

Asthmatic patients during acute attack have increased lipid peroxidation level as detected by the increase of serum malondialdehyde (MDA) which is an important marker in increasing the production of free radical that developed bronchoconstriction [22].

As a result of continuous production of ROS the uric acid level was significantly increased especially in severe asthmatic attack, increasing age, and with smoking. There were no significant statistical differences in the mean serum level of uric acid between male and female patients. Also the family history of patients affected on the level of serum uric acid.

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قياس معدل مستوى حامض يوريك المصل لمرضى الربو في محافظة البصرة-العراق

ساهره غريب صياح

قسم الكيمياء– كلية التربية للعلوم الصرفة – جامعة البصرة – بصره – العراق

الخلاصة

تضمنت الدراسة قياس معدل مستوى حامض يوريك المصل لمرضى الربو في محافظة البصرة ، حيث تضمن البحث دراسة (50) حالة مرضية (24 ذكور , 26 إناث) , تم جمع العينات من مركز الحساسية والربو في محافظة البصرة – العراق.

تم تقسيم المرضى على وفق مجموعة متغيرات تشمل العمر ، الجنس ، شدة المرض ، التدخين والتأريخ العائلي مقارنة مع (50) حالة للأصحاء (24 ذكور ، 26 إناث) كمجموعة سيطرة.

أظهرت النتائج ارتفاعاً معنوياً في مستوى حامض يوريك ($P < 0.01$) في مصل دم مرضى الربو مقارنة مع الأصحاء. ويزداد الأرتفاع معنوياً ($P < 0.05$) بتقدم العمر وشدة المرض , بينما مع المرضى المدخنين والذين لديهم تأريخ عائلي ($P < 0.01$) , وكذلك عدم وجود فروقات معنويه بين الذكور والاناث .

ونستنتج من خلال نتائج هذه الدراسة بان لدى المرضى المصابين بالربو درجة اعلى من تكوين الانواع النشطة للاوكسجين و التي تسبب اجهاداً مؤكسداً و زيادة في مستوى حامض يوريك المصل.

الكلمات المفتاحيه : المالون داي اليهايد , الانواع النشطه للاوكسجين , الانواع النشطه للنيتروجين , فوق اوكسيد دسميوتيز , حامض اليوريك .