Clinical and Biochemical Study in urinary stone patients in Babylon Province

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Abstract

Background: Evaluate the oxidative stress in patient with different types of urinary stone by measuring the total antioxidant capacity, study the oxidative stress in cystinuria patients, study the changes in superoxide dismutase activity and Cu, Zn level in sera of different types of urinary stone patient.

Patient and method: 0ne hundred patients (67males and 33 females) in the age group ranging from 5-75 years old,and forty apparently healthy individual(20 male and 20 female) in the age group ranging from (6-70). admitted to Al-Hila Teaching Hospital, Urology Department from the 1st of January 2013 till 30th of June 2013. All patients underwent full history and physical examination including: age, gender, family history of urolithiasis, past history of recurrent stone and any current medical diseases. the antioxidant determination by add 100 µl Cu2+ working solution to all standard and sample wells and cover the plate and incubate at room temperature for 1.5 hours, Read the absorbance at 570 nm using the plate reader.the superoxide dismutase determination by take 50 ml of serum and 1 ml of tris base, 1 ml of pyrogallole . After the addition of pyrogallol ,immediately read the absorbance spectrophotometriclly at 420 nm against blank .

Results : Serum concentration of Total antioxidant capacity(SOD) was decrease significantly in patients with urinary stone when compared with control group ($p<0.001$) in uric acid stone, ($p<0.05$) in calcium oxalate stone,($p<0.05$) in calcium phosphate stone ,The concentration ofsuperoxide dismutase (SOD) was decrease significantly in patient with urinary stone compere to control group, The result between male and female of the patients show significant decrease when compare with the male and female of the control.

Conclusion: The study show lowerlevel Superoxide dismutase activity ,Increased levels of Total antioxidant indicate to absorption uric acid and the activation of antioxidant enzymes as an adaptation to the oxidative stress,The antioxidant strength is further decreased by lower activity of SOD.

key words: (SOD) superoxide dismutase, (TAC) total antioxidante

الخلاصة

الاهداف : تقييم جهد الاكسدة في المرضى وأنواع مختلفة من الحصى البولية عن طريق قياس قابلية مضادة للأكسدة الكلية، دراسة الاكسدة في مرضى البول السيستيني، دراسة التغيرات في النشاطSODومستوى النحاس والزنك في مصول أنواع مختلفة من الحصى البولية للمريض.

الطريقة : تضمنت الدراسة مائة مريض بحصى الكلى من الفئة العمرية (٥-٧٥) سنة, واربعين شخص ظاهريا اصحاء من الفئة العمرية (٦-٧٠). واجريت الدراسة في مستشفى الحلة التعليمي في قسم المسالك البولية من الفترة ١ كانون الثاني ٢٠١٣ وحتى ٣٠حزيران ٢٠١٣ . وخضع جميع المرضى الى تاريخ المرضى العائلي والفحص البدني بما في ذلك العمر, الجنس , التاريخ العائلي للتحصي البولي والتاريخ الماضي من الحصى المتكرر واي الامراض الطبية الحالية .تقدر مضادات الاكسدة بواسطة اضافة ١٠٠ميكروليتر Cuلعمل محلول قياسي ويحضر في درجة حارة الغرفة لمدة ١.٥ سعة وقراءة الامصاصية عند ٥٧٠ نانوميتر. اما الSOD تقدر بواسطة اخذ ٥٠ مل من السيرم و ١ مل من البايروغولول وبعد الاضافة يتم قراءة الامتصاصية في ٤٢٠ نانوميتر.

النتائج : نلاحظ في تركيز المصل من إجمالي القدرة المضادة للأكسدة (SOD) انخفاض معنوي في المرضى الذين يعانون من حصى البولية بالمقارنة مع مجموعة السيطرة($P<0.001$) في حصى حامض اليوريك،($P<0.05$) في حصى أوكزالات الكالسيوم، ($p<0.05$) في حصى فوسفات الكالسيوم ،ولاحظ في تركيز (SOD) انخفاض معنوي في مرضى حصى الكلى مقارنة مع مجموعة السيطرة، والنتيجة بين الذكور والإناث من المرضى تظهر انخفاضا كبيرا عند مقارنتها مع الذكور والإناث من مجموعة السيطرة .

الاستنتاج:اظهرت الدراسة انخفاض فعالية SOD,زيادة مستويات المضادات للاكسدة تشير إلى امتصاص حامض اليوريك وتفعيل الانزيمات المضادة للأكسدة باعتبارها تتأثر بالإجهاد التأكسدي، انخفاض قوة مضادة للأكسدة بواسطة انخفاض فعالية ال SOD.

مفاتيح الكلمات : ثنائي اوكسيد الديسموتاز, مضادات الاكسدة الكلية.

Introduction

Kidney stones are poly crystaline aggregations composed of varying amount of crystalloid and organic matrix[1]. Urinary stones are typically classified by their location in the kidney (nephrolithiasis), ureter (ureterolithiasis), or bladder (cystolithiasis), or by their chemical composition (calcium-containing, struvite ,uric acid, or other compounds)[2]. the type of kidney stone is Calcium stones account for 75% of renal calculi. Recent data suggest that a low-protein, low-salt diet may be preferable to a low-calcium diet in hypercalciuric stone formers for preventing stone recurrences[3].Struvite stone is account for 15% of renal calculi[4]. They are associated with chronic urinary tract infection (UTI) with gram-negative rods capable of splitting urea into ammonium, which combines with phosphate and magnesium[5].Uric acid stones account for 6% of renal calculi. These are associated with urine pH less than 5.5, high purine intake (e.g. organ meats, legumes, fish, meat extracts, gravies), or malignancy (rapid cell turnover). Approximately 25% of patients with uric acid stone have gout[6]Cystine stones account for 1% of renal calculi. They arise because of an intrinsic metabolic defect resulting in failure of renal tubular reabsorption of cystine, ornithine, lysine, and arginine. Urine becomes supersaturated with cystine, with resultant crystal deposition[7]Xanthine stone are absolutely rare. They are caused by an inborn defect of xanthine oxidase . xanthine cannot be oxidized to uric acid, so that the excretion of hypoxanthine and xanthine increased[8] the Indinavirstone is Protease inhibitors are a popular and effective treatment in patients with acquired immunodeficiency syndrome . Indinavir is the most common protease inhibitor that results in radiolucent stone in up to 60% of patient who are prescribed this medication[9].

Cystinuria

Cystinuria is an autosomal recessive disease caused by defective trans-epithelial transporters for the dibasic amino acids cystine, ornithine, lysine, and arginine within the renal proximal tubules and intestinal tract[10]. Although uncommon, cystinuria can account for 10% of pediatric stone disease[11]. Patients with cystinuria experience frequent nephrolithiasis recurrence, forming stones, on average, every 3 years[12].

Oxidative Stress

Oxidative Stress is defined as an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage[13].in oxidative stress, total antioxidant capacity (TAC) decreases. Free radicals initiate a cascade, causing lipid peroxidation, DNA damage, cell death, and neurological problems. Total antioxidant capacity is measured as an indicator of oxidative stress [14].

Free Radicals

Free radicals are defined as an atoms or molecules one or more unpaired electrons, making them unstable and highly

reactive[15]. They became stable by acquiring electrons from nucleic acid, proteins, carbohydrates or any nearby molecule causing a cascade of chain reactions resulting in cellular damage and disease[16].

Antioxidant

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols[17].

Superoxide dismutase

Superoxide dismutase are enzymes that catalyze the dismutation of superoxide (O_2^-) into oxygen and hydrogen peroxide, then H_2O_2 hydrolyzed by catalase to H_2O and molecular oxygen. In human there are three forms of SOD:

1. Cu/ZnSOD: it is found in the cytoplasm and organelles of virtually all mammalian calls.
2. MnSOD:-it is found in the mitochondria of almost all calls.
3. FeSOD extracellular:- it is synthesized by only a few call types including fibroblasts and endothelial calls.

These three type of SOD can be identified by their differential sensitivity to cyanide and H_2O_2. Cu/ZnSOD is characterized as being sensitive to both H_2O_2 and cyanide. FeSOD is sensitive to H_2O_2 only and MnSOD is resistant to bothinhibitors[18].

Materials and Methods

One hundred patients (67males and 33 females) in the age group ranging from 5-75 years old, admitted to Al-Hilla Teaching Hospital, Urology, Forty apparently healthy individuals were taken as a control group. This group comprises of (20 males and 20 females) their age ranging from 6-70 years. Blood samples have been collected from patients. Blood samples were withdrawn without the use of tourniquet.All patients underwent full history and physical examination including: age, gender, family history of urolithiasis, past history of recurrent stone and any current medical diseases. The patient sent for routine investigation (Uric acid, Blood urea, Sugar urea, Complete blood picture) and radiological investigation (ultrasonography (US), plain abdominal X ray film of kidney, ureter and bladder (KUB), and (CT) scan), the specific biochemical test include following[Total antioxidant (TAC), Superoxide dismutase (SOD)]

Determination of serum the Antioxidants

Trolox standard curve: Add 0, 4, 8, 12, 16, 20 µl of the Trolox standard to individual wells. Adjust the total volume to 100 µl with ddH_2O to give 0, 4, 8, 12, 16, 20 nmol of Trolox standard.

Preparation of sample: The kit has been tested with serum. If only small molecule TAC is desired, samples should be diluted1:1 with protein mask. Sample volumes between 0 - 100 µl can be assayed per well and should be done in duplicate.

Preparation of working solutions: Dilute one part Cu^{2+} reagent with 49 parts of Assay diluent. Dilute enough working solution for the number of assays. Each well requires 100 µl of Cu^{2+}working solution.

Assay procedure

1) Add 100 µl Cu^{2+} working solution to all standard and sample wells.

2) Cover the plate and incubate at room temperature for 1.5 hours.
3) Read the absorbance at 570 nm using the plate reader.

Determination of serum the Superoxide dismutase

1. Tris-buffer mM, pH 8.2 : This solution contains:

- Tris-base: Dissolve 0.285g of Tris-base in small amount of DW.
- EDTA: Dissolve 0.111g of EDTA in small amount of DW.

2. Pyrogallol : This solution must be prepared freshly. Pyrogallol solution was prepared as described below and the materials should be added sequentially 100 ml of DW,60 ml of HCL and 0.0252 g of pyrogallol.

Take 50 ml of serum and 1 ml of tris base, 1 ml of pyrogallole .After the addition of pyrogallol ,immediately read the absorbance spectrophotometriclly at 420 nm against blank.

The results were statistically analysed by the help of SPSS version 15 software statistical package using P value at level of significance equal or less than(0.05)

Results

Measurement of the Total antioxidant Capacity (TCA)

Serum concentration of (TAC) was decrease significantly in patients with urinary stone when compared with control group ($p<0.001$) in uric acid, ($p<0.05$) in calcium oxalate,($p<0.05$) in calcium phosphate, As illustrated in table (1.1).in theSuperoxide dismutase(SOD), The principal chain breaking antioxidant is superoxide dismutase (SOD), which acts in the aqueous phase to trap superoxide free radicals. Concentration of superoxide dismutase (SOD) was decrease significantly in patient with urinary stone compere to control group, as shown in the table (1.2).

Table(1.1): Characteristic of total patients group and total control groups related with the total antioxidant in different groups

	Groups	Number	TAC(u/ml) Mean& SD	P value
Patients	Uric acid	35	6.32 ±3.02	P< 0.001
	Calcium oxalate	39	9.99 ± 2.98	P<0.05
	Calcium phosphate	14	8.54 ± 3.09	P<0.05
	Uric acid+ Carbonyl	1	10.58	—
	Calcium oxalate +Calcium phosphate	2	3.47	—
	Uric acid +Calcium phosphate	2	7.97	—
	Cystine	3	3.12 ±1.07	—

	Tri phosphate	**4**	**9.54 ±1.47**	**__**
Control		**40**	**16.68 ± 2.29**	**__**

Table (1.2): Characteristic of patients groups total and control groups total related with the superoxide dismutase in the different groups.

Patients	**Uric acid**	**35**	**0.53 ± 0.14**	**P< 0.05**
	Calcium oxalate	**39**	**0.54 ± 0.11**	**P< 0.05**
	Calcium phosphate	**14**	**0.56 ± 0.17**	**P<0.05**
	Uric acid+ Carbonyl	**1**	**0.707**	**__**
	Calcium oxalate +Calcium phosphate	**2**	**0.59 ± 0.06**	**__**
	Uric acid +Calcium phosphate	**2**	**0.64**	**__**
	Cystine	**3**	**0.59 ± 0.19**	**__**
	Tri phosphate	**4**	**0.68± 0.16**	**__**
Control		**40**	**0.81± 0.35**	**__**

Discussion

The result of total antioxidant show highly significant different ($p<0.001$) in uric acid when compare with control group. SureshC.R *et al*., (2008)[19] suggested that the statistically decrease in total antioxidant in kidney stone patients as compared to controls . Total antioxidant capacity assay is considered as a useful indicator of the system's ability to regulate the damage due to ROS and thus, a novel method of components may not fully reflect the protective efficiency of blood, probably because of interactions that occur in vivo among different antioxidant compounds[20].Increased levels of Total antioxidant indicate absorption of uric acid and the activation of antioxidant enzymes as an adaptation to the oxidative stress, but at a later phase of oxidative stress, thetotal antioxidant falls due to depletion of antioxidants [21]. In addition, high concentration of a number of metabolites, including uric acid can lead to pro-oxidant effects, introducing a further decrease of the antioxidant capacity [22].Thus anti-oxidants such as vitamin E, have been reported to protect against CaOx monohydrate-mediated oxidative stress in renal cells[23]. Oxalate induces free radical production for kidney stone formation by activation of NAD(P)H oxidase[24]. The result of superoxide

dismutase of patients show significant decrease when compare with the control. Elif D, *et al*.. (2007)[25]suggested that the Superoxide dismutase is a significant in control group when compere with patients group. lower SOD activity was [26]. More importantly SOD showed significant relationship with age and a progressive decline after the age of 50 years which suggests forincreased oxidant stress with age.The antioxidant strength is further decreased by lower activity of superoxide dismutase, which is the only enzyme to dismutate superoxide radical, It is logical to believe that low RBC-SOD levels represent a generalised deficiency of this enzyme leading to increased flux of O2[27] superoxide dismutase (SOD) is a new approach to identify association with urolithiasis. Oxidative stress may be involved in the development of stone formation in the renal system.superoxide dismutase is enzymes that directly scavenges potential harmful oxidizing species**.** SOD determination may provide a tool to identify individuals who are at risk of urolithiasis. also provides data about antioxidant status and stone formation[28].

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