

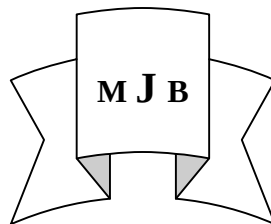
Roles of Malondialdehyde and Catalase in Serum of Urolithiasis

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Abstract

The current study is conducted to determine the level of malondialdehyde (MDA) as an index of free radicals induced lipid peroxidation and catalase as an index of antioxidant enzyme in the serum of patients with urolithiasis. Significantly high level of MDA ($p < 0.05$) and significantly low level of catalase ($p < 0.05$) were observed in the serum of urolithiasis patients as compared to normal controls. There was no difference in serum level of MDA and CAT between male and female neither in patients nor controls. Also, there were no correlation between the serum MDA and serum CAT in both patients and controls. Among patients, 21.7% were smokers. There was no significant difference between smokers and non smokers patients regarding serum level of MDA and CAT. In conclusion, it appears that a role of lipid peroxidation and oxidative function exists in the pathogenesis of urolithiasis, but its mechanism is unknown.

الخلاصة

لقد وضع هذا البحث لدراسة دور المالون داي الأديهايد (MDA) كدالة على حدوث تفاعلات أكسدة الدهون و أنزيم الكاتاليز (CAT) كمضاد للأكسدة في مصل مرضى حصى الجهاز البولي . و لقد اظهر البحث ارتفاعا معنويا في متوسط مستوى ال (MDA) مع انخفاض معنوي في متوسط مستوى ال (CAT) عند مقارنتها بمستوياتها في مصل مجموعة السيطرة ($p < 0.05$). و لم يكن هنالك فرق معنوي في مستويات تلك المؤشرات بين الإناث و الذكور في كل من المرضى و مجموعة السيطرة ($p > 0.05$). كما لم تظهر علاقة معنوية بين ال (MDA) و (CAT) ($p > 0.05$). كانت نسبة المدخنين 21.7% بين المرضى و لم يكن هنالك فرق معنوي بين المدخنين و غير المدخنين نسبة الى مستويات MDA و CAT في مصل مرضى حصى الجهاز البولي. يمكن الاستنتاج أن هنالك دورا لتفاعلات أكسدة الدهون و مضادات الأكسدة في عملية نشوء حصى الجهاز البولي ولكن آلية الحدوث مجهولة.

Introduction

Urolithiasis is calculus formation at any level in the urinary collecting system, but most often the calculi arise in the kidney [1]. Patients with urolithiasis constitute an important part of everyday urological practice [2]. Urinary calculi are the third most

common affliction of the urinary tract, exceeded only by urinary tract infections and pathologic conditions of the prostate. Stones are composed primarily of a crystalline component [3]. The great majority of stones, 70 to 80 percent, are composed of calcium oxalate crystals [4]. Renal cellular exposure to oxalate (Ox)

and/or CaOx crystals leads to the production of reactive oxygen species, development of oxidative stress followed by injury and inflammation [5]. Damaged epithelia might act as nuclei for stone formation aggravating calcium oxalate precipitation during hyperoxaluria [6]. Renal injury and inflammation appear to play a significant role in stone formation. ROS are produced from many sources and involve a variety of signaling pathways [5]. Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation [7]. Lipid peroxidation is initiated by a hydroxyl or other radical that extracts a hydrogen atom from a polyunsaturated lipid, thereby forming a lipid radical [8]. Lipid peroxidation and consequence degradation products such as malondialdehyde (CHO-CH₂-CHO) are seen in biological fluids [9]. Malondialdehyde appears in the blood and urine and is used as an indicator of free radical damage [8]. There is evidence that the process of calcium stone formation starts as a precipitation of calcium phosphate either in the loop of Henle or in the distal part of the distal tubule [10]. Although the urine at these levels of the nephron might be critically supersaturated with calcium oxalate in patients with hyperoxaluria and in experimental animals following administration of ethylene glycol, the ion-activity product of calcium oxalate is usually too low to result in calcium oxalate crystal formation [11]. Any crystallization that occurs in this part of the nephron most certainly is facilitated by promoters and it has been suggested that

lipoprotein membranes from the brush border of proximal tubular cells might serve this purpose [12]. The brush border membrane might be injured by free radicals formed as the result of toxic effects on the cell [11]. This might lead to lipid peroxidation and cell death [13]. The released membrane fragments that are transported down the nephron thereby can supply a suitable surface for deposition of both calcium oxalate and calcium phosphate [11]. Catalase and peroxidases are preventive antioxidants (reduce the rate of chain initiation) that react with ROOH and chelators of metal ions. Catalase (EC 1.11.1.6) is a hemoprotein containing four heme groups [7]. Catalase is used to remove the H₂O₂ when it is generated in large quantity [9].

Materials and Methods

Patients and Controls

A total of sixty urolithiasis patients in age ranging from 21 to 74 years old, who were introduced to Al-Hilla Teaching Hospital, Department of Urology, and were proved to have radio-opaque urinary stones which had been diagnosed by plane X-ray film of kidney, ureter and bladder, intravenous urography and abdominal ultrasound. Patients with hypertension, diabetes mellitus and rheumatologic diseases had been excluded. Twenty eight healthy subjects, age and gender matched from public free from any history of smoking, alcoholism, and coexistence of any medical disease which can also lead to similar changes such as diabetes mellitus and hypertension. All tests were performed on serum in Biochemistry Department of College of Medicine in Babylon University.

Chemicals

All the chemical compounds, which have been used in this work, are produced from BDH, Sigma, Gill Man, Fluka and Hopkin Williams.

Methods

Determination of Serum Malondialdehyde

The principle of serum MDA determination is based on the spectrophotometric measurement of the colour which is produced during the reaction of the thiobarbitric acid (TBA) and MDA. The used reagents are 17% Trichloroacetic acid (TCA), 0.6% Thiobarbitric acid (TBA) and 70% (TCA). The procedure involves addition of 17.5% TCA (1ml) and 0.6% TBA (1ml) into the serum (0.15ml), the tubes are mixed well, incubated in boiling water bath for 15 minutes and after cooling 70% TCA (1ml) is added. The mixture is let to stand at room temperature (20 minutes), centrifuged at 450Xg for 15 minutes and the supernatant is read at 532 nm [14].

Assay the activity of serum catalase

Catalase activity is determined by the decrease in absorbance due to hydrogen peroxide (H_2O_2) consumption. The needed reagents are phosphate buffer solution (50 mM, pH 7.0) and hydrogen peroxide (H_2O_2) (30mM) which is prepared freshly by diluting 0.34 ml of 30% H_2O_2 with phosphate buffer to 100

ml. The serum is diluted with 5 ml of phosphate buffer solution immediately prior to assay. Hydrogen peroxide (1ml) is added into the diluted serum (2ml), mixed immediately and the absorbance was read after 15 and 30 seconds at 240 nm [15].

Statistics

Student's *t*-test had been used to determine whether there had been a significant difference between the two groups at $p=0.05$ level. While the correlation between two variables had been estimated by Pearson's correlation coefficients at the 0.05 level.

Results

Sixty patients with urolithiasis (30 males and 30 females) and 28 healthy individuals (16 male and 12 female) were included in the present study. The mean age of urolithiasis patients was 44.06 years (44.88 years of control group). There was no significant difference in age of patients and control group ($P>0.05$).

Serum MDA in urolithiasis

The mean serum level of MDA had shown an increase in its level in patients with urolithiasis in comparison to that of the control group and it revealed significant difference with serum MDA in control group patients ($P<0.05$) (table 1).

Table 1 The mean serum MDA of urolithiasis patients in comparison to control group.

Group	Mean MDA (μmol/L)	S.D (μmol/L)	P value
Patients	7.01	2.51	0.0000*
Control	3.25	1.08	
*Correlation is significant at 0.05 level (2-tailed) P value =4×10 ⁻¹¹ .			

Serum catalase in urolithiasis patients

The mean serum level of catalase had shown a decrease in its level in patients with urolithiasis in

Table 2 Serum catalase of urolithiasis patients.

comparison to that of the control group and it revealed a significant difference between both groups ($P < 0.05$) table 2.

Group	Mean catalase (K/ml)	S.D (K/ml)	P value
Patients	0.13	0.07	0.0000*
Control	0.28	0.08	
*Correlation is significant at 0.05 level, P value =5×10 ⁻¹³ .			

The relation between serum MDA and serum CAT. The current study had shown that the serum MDA had

no correlation to serum CAT ($P > 0.05$) (table 3).

Table 3 The correlation between serum MDA and serum CAT in urolithiasis patients and control groups.

The correlated parameter to MDA ($\mu\text{mol/L}$)	Patient		Control	
	Correlation coefficient	P value	Correlation coefficient	P value
Catalase(K/ml)	0.03	0.78	0.11	0.56

The effect of gender on serum MDA and CAT in urolithiasis patients and control.

The serum level of MDA and CAT had been analysed according to the gender in patients and controls. The

serum MDA and CAT had shown no difference between male and female neither in the serum of patients nor controls and they revealed no significant difference ($p > 0.05$) (table 4).

Table 4 The difference in mean serum MDA and CAT between male and female urolithiasis patient and control group.

Serum biochemical test		Male Mean±S.D	Female Mean±S.D	P value*
MDA (μmol/L)	Patient	6.02±2.54	7.02±2.54	0.96
	control	3.43±1.36	3.00±0.51	0.30
	P value**	0.0000 ^a	0.0000 ^b	
Catalase (K/ml)	Patient	0.12±0.05	0.13±0.09	0.57
	Control	0.26±0.04	0.31±0.12	0.19
	P value**	0.0000 ^c	0.0000 ^d	

*P value of the difference in mean of serum biochemical variable between male and female at p=0.05.

** P value of the difference in mean of serum biochemical variable between patient and control groups of the same gender at p=0.05.

^a $p = 4 \times 10^{-6}$ ^b $p = 3 \times 10^{-6}$

^c $p = 1 \times 10^{-12}$ ^d $p = 1 \times 10^{-5}$

Effect of smoking on serum MDA and CAT in urolithiasis patients.

The percentage of smokers among urolithiasis patients in this study had been 21.7% and 78.3% had been non

smokers. There had been no difference in serum MDA and serum CAT between smokers and non smokers patients with urolithiasis ($p>0.05$) (table 5).

Table 5 The difference in mean serum MDA and CAT between smoker and non smoker patients with urolithiasis.

Biochemical variable	Smokers	Non smokers	P value
MDA ($\mu\text{mol/L}$)*	7,3 $\pm$ 2,3	6,9 $\pm$ 2,6	0,6
CAT (K/ml)*	0,13 $\pm$ 0,05	0,13 $\pm$ 0,08	0,9

*The values are expressed as mean $\pm$ SD and the p value examined at 0.05 level of significance.

Discussion

The result of significant increase in serum level of MDA is coherent with previous two studies [16,17]. Other workers had detected a significant increase in MDA level in the plasma (18,3), erythrocytes [18] and urine [18,19] of urolithiasis patients. However, this significant elevation of MDA can be supported by experimental rat studies which had

been reported an elevation in lipid peroxidation in induced calcium oxalate nephrolithiasis rats administered sodium oxalate [20]. It has been reported that the conditions which enhance peroxidation and depletion of thiol content increase the oxalate binding activity, which in turn promotes nucleation and aggregation property of stone matrix protein fractions. This behavior is also

associated with peroxidized mitochondria and nuclei, suggesting that the peroxidation can be a causative factor for the initial stage of stone formation [21]. A study had been published in 2005 had been demonstrated *in-vivo* evidence that hyperoxaluria-induced peroxidative injury induced individual calcium oxalate crystal attachment in the renal tubules [22].

The current study had revealed a significant decrease in serum CAT. One study had been done on humans in India, it had revealed a significant increase in CAT which had been determined in haemolysate of urolithiasis patients [23], while the studies which had been done on experimental rats had been shown significant increase in CAT level in kidney homogenates [24], also the renal epithelial cells (LLC-PK1) had showed decrease in its cellular contents of CAT after exposure to oxalate [25]. The mechanism of induction of lipid peroxidation by oxalate may involve inhibition of catalase activity since *in vitro* studies have revealed progressive inhibition of catalase activity and increase in lipid peroxidation with increasing oxalate concentration [26]. Reduction in serum level of CAT here is due to the consumption of the antioxidant enzyme by increasing the lipid peroxidation in urolithiasis but there is no correlation between serum MDA and CAT which is coherent with previous study [27].

The current study had revealed no difference in serum MDA and CAT between male and female neither in urolithiasis patients nor controls. The difference in MDA level between male and female had been studied in

normal subjects and shown no difference in its mean in the plasma between healthy male and female voluntaries [28]. While the CAT had shown variation in different studies, some workers had shown no difference [29] and some had shown difference where females had higher level [30], while other had shown higher level in male [31]. This can be explained by environmental and nutritional effect.

The current study had revealed no effect of smoking on neither serum MDA nor serum CAT in urolithiasis patients. This result with the exclusion of hypertensive patients, diabetic patients and patients with rheumatoid diseases from the study group can give the conclusion of the changes in serum MDA and CAT are due to urolithiasis as a cause or as a result.

It can be concluded from this study that the roles of lipid peroxidation and antioxidant enzymes exist in the pathogenesis of urolithiasis and its mechanism need to be elucidated.

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