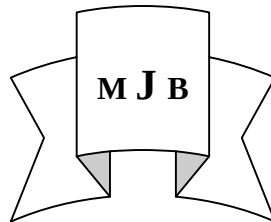


Roles of Malondialdehyde, Glutathione Peroxidase and N-Acetyl-β-D-glucosaminidase in Serum of Urolithiasis

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Abstract

The present study is conducted to determine the level of malondialdehyde (MDA) as an index of free radical induced lipid peroxidation, glutathione peroxidase (GPx) and the role of N-Acetyl-β-D-glucosaminidase in 60 confirmed cases of urolithiasis. Significantly high level of MDA and NAG ($p < 0.05$) with significantly low level of GPx ($p < 0.05$) have been observed in serum of urolithiasis patients as compared to normal controls. There have been no correlation between the level of MDA and neither GPx nor NAG in serum of urolithiasis patients, and no correlation between serum NAG and GPx ($p > 0.05$). There has been no difference between male and female in the levels of serum MDA, GPx and NAG. In conclusion, it appears that a role of lipid peroxidation and oxidative function exist in the pathogenesis of urolithiasis, also the serum NAG has a role in urolithiasis, but the exact mechanism is unknown.

الخلاصة :

لقد وضع هذا البحث لدراسة مستوى المالدون داي ألدهايد (MDA) كدالة على تفاعلات أكسدة الدهون و أنزيم الكلوثنائون بيروكسيداز (GPx) و دور أنزيم ان استايل كلوكوز امنيديز (NAG) في مصل مرضى حصي الجهاز البولي البالغ عددهم ستين مريضاً أما مجموعة السيطرة فقد كانت ثمانية و عشرين شخصاً. لقد بينت الدراسة عن وجود ارتفاع معنوي في مستوى (MDA) و (NAG) ($p < 0.05$) مع انخفاض معنوي في مستوى (GPx) ($p < 0.05$) عن مستواه في مصل مجموعة السيطرة. كما أظهر مستوى (MDA) عن عدم وجود ارتباط معنوي مع مستوى (GPx) او مستوى (NAG) و كذلك لم يظهر ارتباط بين (NAG) و (GPx) في مصل مرضى الحصى الكلوي اما بالنسبة لتأثير جنس المرضى فلم يظهر أي فرق معنوي في مستويات (MDA) و (GPx) و (NAG) في مصل المرضى. نستنتج من ذلك إن هنالك دوراً لعمليات أكسدة الدهون و مضادات الاكسدة في عملية نشوء الحصى و كذلك هنالك دور ل (NAG) في مصل مرضى حصي الجهاز البولي و لكن آلية هذه التغيرات مجهولة.

Introduction

Malondialdehyde is a major end product of lipid peroxidation reaction on membrane fatty acids [1]. Lipid peroxidation represents oxidative tissue damage caused by hydrogen

peroxide, superoxide anion and hydroxyl radicals, resulting in structural alteration of membrane with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products [2]. Although

the cell is endowed with several antioxidant systems to limit the extent of lipid peroxidation, under certain conditions protective mechanism can be overwhelmed, leading to elevated tissue levels of peroxidation products [3]. Our first line of defense against oxidative damage is sequestration or chelation of redox-active metal ions. These chelators include a number of metal binding proteins that sequester iron and copper in inactive form, such as transferrin and ferritin. Despite efficient chelation of metals, reactive oxygen species are formed in the body and do cause chemical damage. In these cases, there are a group of enzymes that act to detoxify the precursors of hydroxyl radicals [4]. Glutathione peroxidase (EC 1.11.1.9) is a preventive antioxidant enzyme (reduce the rate of chain initiation) that catalyze the reduction of lipid peroxides as well as of hydrogen peroxides [5]. It has been suggested that glutathione peroxidase may be able to break the autocatalytic chain reaction of lipid peroxidation protecting the membrane from oxidative damage [6].

Urolithiasis is calculus formation at any level in the urinary collecting system, but most often the calculi arise in the kidney [7]. Stones are composed primarily of a crystalline component [8]. The great majority of stones, 70 to 80 percent, are composed of calcium oxalate crystals [9]. 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]. 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 [11]. The brush border membrane might be injured by free radicals formed as the result of toxic effects on the cell [12]. 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 [12]. However the oxidant and antioxidant imbalance may be one of the major factors leading to the process of crystal deposition in renal tissues [14].

The enzyme N-acetylglucosaminidase (EC 3.2.1.30) (130000-140000 D) is a lysosomal enzyme widely distributed in human tissue [15]. The lysosome in the renal proximal tubular cells contains high amount of NAG, so it is secreted in urine when kidney is damaged [16]. It is released into serum from cells by extracellular secretion of this lysosomal enzyme (exocytosis) or from the breakdown of cells [17]. Serum NAG activity is reported to be influenced by oxidative stress [18, 19].

Materials and Methods

Patients and controls

A total of sixty urolithiasis patients in the age ranging from 21-74 years old, who have had a radio-opaque stone(s) demonstrable on plane film of kidney, ureter and bladder X-ray (KUB) and intravenous urography (IVU). Twenty eight healthy subjects, age and gender have been matched from public who have been 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. Pregnant patients, diabetic, hypertensive and patients with rheumatologic diseases have been excluded from the study group.

Chemicals

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

Methods

All tests have been performed on serum in Biochemistry Department of Collage of Medicine in Babylon University. Blood samples have been collected from patients and controls. Clean and sterile vials without any anticoagulant have been used to collect 10 ml of blood in each vial. The blood has been separated to serum to be used for detection of MDA, GPx and NAG.

Serum malondialdehyde (MDA) has been determined manually according to Burtis and Ashwood method [20].

Serum glutathione peroxidase (GPx) has been determined manually according to Rotrouck method [21].

Assay the activity of N-Acetyl- β -D-glucosaminidase activity (expressed in micromoles of substrate converted

per hour) has been measured using the PPR NAG test kit (PPR Diagnostics, Ltd., London, UK).

Statistics

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

Results

Distribution of urolithiasis patients according to age and gender.

Sixty patients with urolthiasis (30 males and 30 females) and 28 healthy individuals (16 male and 12 female) have been included in the present study. The mean age of urolithiasis patients has been 44.06 years (44.88 years of control group). There has been no significant difference in age of patients and controls ($P>0.05$). Analysis of age groups has been shown a higher occurrence of urolithiasis among the age group between 31-40 (30%) as shown in figure (1).

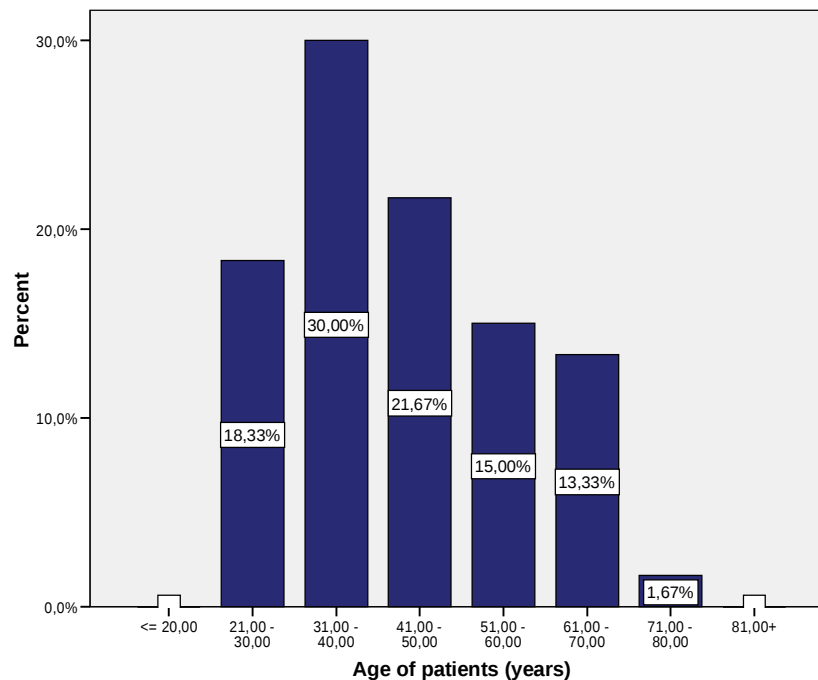


Figure 1 Distribution of 60 patients with urolithiasis according to their years of age and the percentage of each age group.

Serum MDA , GPx and NAG in urolithiasis

The mean serum level of MDA has shown an increase in its level in patients with urolithiasis in comparison to that of the control group and it revealed a significant

difference with serum MDA in control group patients ($p < 0.05$). While the mean serum level of glutathione peroxidase has shown decrease in its level and revealed a significant difference with serum GPx in control group ($p < 0.05$) (table 1).

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

Biochemical test	Patients Mean $\pm$ SD	Controls Mean $\pm$ SD	P value
MDA ($\mu\text{mol/L}$)	7.01 $\pm$ 2.51	3.25 $\pm$ 1.08	0.0000*
GPx (U/L)	61.79 $\pm$ 42.64	188.8 $\pm$ 42.13	0.0000**
NAG ($\mu\text{mol/h/L}$)	1152.72 $\pm$ 390.24	356.36 $\pm$ 146.78	0.0000***
* The mean difference is significant at 0.05 level, P value = 4×10^{-11} .			
** The mean difference is significant at 0.05 level, P value = 3×10^{-22} .			
*** The mean difference is significant at 0.05 level, P value = 6×10^{-17} .			

Serum level of MDA has shown no significant correlation neither to GPx nor to NAG. Also, serum NAS has

had no significant correlation neither to serum MDA nor GPx. (table 2,3).

Table 2 The correlation of serum MDA to serum GPx and NAG in urolithiasis patients .

The correlated serum biochemical variable to serum MDA ($\mu\text{mol/L}$)	Correlation coefficient	P value
GPx (U/L)	0.19	0.14
NAG ($\mu\text{mol/h/L}$)	-0.02	0.89

Table 3 The correlation of serum NAG to serum MDA and GPx in urolithiasis patients.

The correlated serum biochemical variable to serum NAG ($\mu\text{mol/h/L}$)	Correlation coefficient	p value
MDA ($\mu\text{mol/L}$)	-0.02	0.89
GPx (U/L)	-0.041	0.77

Serum level of MDA, GPx and NAG have been analysed according to the gender of patients and controls. They have shown no difference between male and female neither in serum of patients nor controls and they

revealed no significant difference ($p > 0.05$). While the difference in the mean of MDA, GPx and NAG remain significant ($p < 0.05$) in serum of patients and controls of the same gender as shown in table (4).

Table 4 The difference in serum MDA, GPx and NAG between male and female in patients and controls.

Serum biochemical test		Male Mean $\pm$ S.D	Female Mean $\pm$ S.D	P value*
MDA ($\mu\text{mol/L}$)	Patient	6.02 $\pm$ 2.54	7.02 $\pm$ 2.54	0.96
	control	3.43 $\pm$ 1.36	3.00 $\pm$ 0.51	0.30
	P value**	0.0000 ^a	0.0000 ^b	
GPx (U/L)	Patient	64.55 $\pm$ 42.72	59.03 $\pm$ 43.11	0.62
	Control	200.93 $\pm$ 45.26	172.66 $\pm$ 32.63	0.08
	P value**	0.0000 ^c	0.0000 ^d	
NAG ($\mu\text{mol/h/L}$)	Patient	1130.2 $\pm$ 425.3	1175.23 $\pm$ 357.65	0.65
	Control	342.1 $\pm$ 170.2	375.26 $\pm$ 112.71	0.56
	P value**	0.0000 ^e	0.0000 ^f	

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

^a The mean difference is significant, $p=4 \times 10^{-6}$.

^b The mean difference is significant, $p=3 \times 10^{-6}$.

^c The mean difference is significant, $p=4 \times 10^{-13}$.

^d The mean difference is significant, $p=4 \times 10^{-10}$.

^e The mean difference is significant, $p=8 \times 10^{-9}$.

^f The mean difference is significant, $p=3 \times 10^{-9}$.

Discussion

The result of significant increase in serum level of MDA is coherent with previous two studies [22,23]. Other workers have detected a significant increase in MDA level in plasma [4,24], erythrocytes [24] and urine [24,25] of urolithiasis patients. However, this significant elevation of MDA can be supported by experimental rat studies which has been reported an elevation in lipid peroxidation in induced calcium oxalate nephrolithiasis rats administered sodium oxalate [26]. 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 [27]. A study has been published in 2005 demonstrated *in-vivo* evidence that hyperoxaluria-induced peroxidative injury induced individual calcium oxalate crystal attachment in the renal tubules [28].

The significant decrease in serum level of GPx is coherent with another study which has measured GPx level in the erythrocyte of urolithiasis patients [24], but other study has revealed non significant changes in the level of GPx in the erythrocyte of urolithiasis patients [29], while a rat model of calcium oxalate urolithiasis study in Germany has been revealed that the crystalluria can led to oxidative stress manifested as decreased cytosolic and mitochondrial glutathione and

increased activity of the antioxidant enzymes glutathione reductase and – peroxidase in the mitochondria [30]. Glutathione peroxidase is one of the most important line of defence against the oxidative damage by hydrogen peroxide or lipid peroxide produced in various cells of the body. It has been suggested that glutathione peroxidase may be able to break the autocatalytic chain reaction of lipid peroxidation protecting the cell membrane from oxidative damage [31] so, that we can explain the reduction in serum level of GPx due to the consumption of the antioxidant enzyme by increasing the lipid peroxidation.

The current study has evaluated serum NAG status in urolithiasis. N-Acetyl-β-D-glucosaminidase (lysosomal enzyme) is released into serum from cells by exocytosis or from the breakdown of cells [17]. It has been revealed that NAG activity has been influenced by oxidative stress [18,19], but in this study no correlation has been found neither between NAG and MDA nor NAG and GPx. However, another mechanism can explain the high level of serum NAG in urolithiasis is the role of endothelial and lysosomal cells. It has been revealed that adherent crystals may be endocytosed into kidney cells, where they may either be dissolved in lysosomes or released at the basolateral surface of the cell where they form sites of interstitial crystal growth [32]. In rat aortic muscle cells the lysophosphatidylcholine (Lyso-PC) increases the expression of monocyte chemo-attractant protein-1 (MCP-1). MCP-1 is induced in renal cells following exposure to oxalate ions or to calcium

oxalate crystals [33] and its expression has been associated with inflammatory responses in a variety of kidney diseases [34] including perhaps, the inflammation produced by crystal deposition in stone disease [33, 35].

There has been no difference in mean level of neither MDA nor GPx in serum of urolithiasis patients. We have not found a previous study had assessed the level of MDA and GPx in urolithiasis patients according to their gender, but the difference in MDA level between male and female have been studied in normal subjects and shown no difference in its mean in plasma between healthy male and female voluntaries [36]. While GPx has been studied by some workers and shown a significantly higher GPx activity in women than in men [37, 38] (these difference could be due to chance or to gender-related differences in life-style such as intake of dietary supplements [27]), but other studies have shown no difference between male and female regarding the level of GPx [39]. It has been known that there is no difference in NAG level between male and female and this has been proved by several studies [40].

The mean age (44.06 years) and the incidence of urolithiasis among age groups (30% in 31-40 years age group) are differ from a study has been done in Baghdad in 2004 [41]. The environmental, geographical, occupational and nutritional factors have been known to affect epidemiology of urolithiasis [42]. Age group of early twenties to late forties is physically most active period in life. Increased physical activities have been shown to induce

a several fold increase in plasma xanthine oxidase that could induce oxidative stress to the filtrating renal tissue [41]. Another possible mechanism may be due to increased level of serum testosterone in age group of 21 – 40 years, which resulted in increased production of oxalate by liver from its endogenous precursors [43]. Oxalate the major stone forming constituent has been reported to induce free radical generation, which results in peroxidative injury to renal epithelial cells [24].

From this study, it appears that a role of lipid peroxidation and antioxidant enzyme defense exist in the pathogenesis of urolithiasis , but the exact mechanism need to be elucidated. Also, the role of serum NAG exists in urolithiasis but further elucidation is needed.

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