ASSESSMENT OF THE CLINICAL SIGANIFICNCE OF LIPID PEROXIDATION ANTIOXIDANT ENZYMES AND *N*-ACETYL-β-D-GLUCOSAMINIDASE IN SERA OF PATIENTS WITH UROLITHIASIS

A Thesis

Submitted to the Collage of Medicine-Babylon University as a Fulfillment of the Requirements for Master Degree of Science in Clinical Biochemistry

By

Roula Hamid Mahmoud

M B Ch B (Babylon University)

2009 A.D 1430 A.H

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Summary

The current study has been conducted to assess the clinical significance of lipid peroxidation (malondialdehyde (MDA) concentration), antioxidant enzymes (glutathione peroxidase (GPx) and catalase (CAT) activities) and *N*-acetyl-*ß*-D-glucosaminidase (NAG activity) in sera of patients with urolithiasis. Sixty male and female patients (aged 21-74 years) have been involved in the current study during their admission to Al-Hilla Teaching Hospital, Urology Department during the period November 2007 till October 2008, who have been diagnosed to have radio-opaque urinary stones. The control group has been 28 apparently healthy persons of both genders.

The study have revealed a significant increase in the concentration of MDA and NAG activity, and a significant decrease in the activities of GPx and CAT in sera of urolithiasis patients in comparison to their levels in sera of controls. No correlation has been detected between MDA concentration and activities of GPx, CAT and NAG, and between activity of NAG and activities of GPx and CAT in sera of urolithiasis patients.

A higher occurrence of radio-opaque stone has been in the age group 31-40 years old. Neither age nor gender have correlated to serum changes of MDA concentration and activities of GPx, CAT and NAG.

The studied patients have had one stone in 68.3%, two stones in 18.3%, and multiple in 13.3%. The size of the urinary stone in 6.67% has been $\leq$4mm, in 22.22% has been 4-7mm, and 71.11% has been >7mm. The duration of the disease symptoms has been 2 days as a minimum and 36 months as a maximum. No correlations of serum MDA concentration

and activities of GPx, CAT and NAG to stone number, stone size and duration of the disease symptoms have been detected.

The studied patients have got recurrent attack of the disease in 63.33%, while in 36.67% have got first episode of the disease. Positive family history of urolithiasis has presented in 26.67%. Neither recurrence of the disease nor family history of urolithiasis have shown a significant difference in serum changes of MDA concentration and activities of GPx, CAT and NAG.

The anatomical distribution of urinary stone has revealed 64.91% unilateral stone, 5.26% bilateral stones, 19.30% uretric stone, 7.02% renal and uretric stones, and 3.51% vesical stone. No significant difference in serum changes of MDA concentration and activities of CAT and NAG among the anatomical distribution of urinary stone has been detected, while serum activity of GPx has shown a significant difference among anatomical distribution of urinary stones. This difference has been presented between ureteric and renal calyceal stone, ureteric and renal pelvic stones, vesical and renal pelvic stones, and vesical and renal calyceal stones.

The smokers among patients have been 21.7%, no significant difference has been detected in serum concentration of MDA and activities of GPx, CAT, and NAG between smokers and non smokers urolithiasis patients.

In conclusion, urolithiasis disease has affected sera concentration of MDA and activities of GPx, CAT and NAG. Age, gender, smoking, number of stones, size of stones, duration of the disease, recurrence of the disease and family history of the disease have had no effect on sera concentration of MDA and activities of GPx, CAT and NAG, but the

anatomical site of the stone has had a significant effect on serum activity of GPx.

Abbreviations

No.	Abbreviation	Details
1	ANOVA	Analysis of variance
2	CaP	Calcium phosphate
3	CaOx	Calcium oxalate
4	CAT	Catalase
5	CoQ	Coenzyme Q
6	CT	Computed tomography
7	DNA	Deoxyribo nucleic acid
8	DTPA	Diethylene triaminepentaacetate
9	EDTA	Ethylenediaminetetra acetate
10	GPx	Glutathione peroxidase
11	GSH	Glutathione (reduced form)
12	GSSG	Oxidized glutathione
13	GUE	General urine examination
14	IVU	Intravenous urography
15	k_{sp}	Thermodynamic solubility product
16	K_{fp}	Formation product
17	KUB	Kidney, ureter and bladder X-ray film
18	LSD	Least significant difference test
19	LLC-PK1	Renal epithelial cell line originally derived from porcine kidneys
20	Lyso-PC	Lysophosphatidylcholine
21	MAP	Magnesium, ammonium, and phosphate
22	MDA	Malondialdehyde
23	MNP-GlcNAc	2-Methoxy-4-(2'-nitrovinyl)-phenyl2-acetoamido-2-deoxy-ß-D- glucopyrinoside
24	MCP-1	Monocyte chemoattractant protein-1
25	NAD	Nicotinamide adenine dinucleotide (oxidized form)
26	NADPH	Nicotinamide adenine dinucleotide phosphate

		(reduced form)
27	NAG	*N*-acetyl-ß-D-glucosaminidase
28	PHGPx	Phospholipid hydroperoxide glutathione peroxidase
29	PUFA	Poly unsaturated fatty acids
30	RBS	Random blood sugar
31	ROS	Reactive oxygen species
32	SD	Standard deviation
33	SOD	Superoxide dismutase
34	Std	Standard
35	*t*-BuOOH	Tertiary-butyl hydroperoxide
36	TBA	Thiobarbutric acid
37	TCA	Trichloroacetic acid
38	US	Ultrasonography

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التقييم السريري المعنوي للدهون المؤكسدة إنزيمات مضادات الأكسدة و ان-استايل-بيتا-كلوكوزامنديز في مصول دم مرضى حصى الجهاز البولي

رسالة مقدمة إلى

كلية الطب-جامعة بابل كجزء من متطلبات نيل درجة الماجستير
في علم الكيمياء الحياتية السريرية

من قبل الطالبة

رولا حامد محمود

بكالوريوس طب و جراحة عامة-جامعة بابل

١٤٣٠هجرية

٢٠٠٩ميلادية

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَمِنَ النَّاسِ وَالدَّوَابِّ وَالْأَنْعَامِ مُخْتَلِفٌ أَلْوَانُهُ كَذَلِكَ ۗ

إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ

صدق الله العظيم

فاطر ﴿ ٢٨ ﴾

الخلاصة:

لقد وضع هذا البحث لدراسة دور الدهون المؤكسدة (المالون داي ألدهايد (*MDA*)) و انزيمات مضادات الاكسدة (كلوتاثيون بيروكسديز (*GPx*) و كتليز (*CAT*)) و انزيم ان استايل-بيتا-كلوكوز امينيديز (*NAG*) في مصول دم مرضى حصى الجهاز البولي. ستون مريضا مصابون بحصى الجهاز البولي الذين تم إدخالهم وحدة الجهاز البولي في مستشفى الحلة التعليمي في مدينة الحلة لفترة ما بين تشرين الثاني 2007 إلى تشرين الثاني 2008, الذين اثبت امتلاكهم حصى عتمة الأشعة بواسطة تصوير الأشعة السينية و التصوير الملون للجهاز البولي و جهاز السونار. كانت مجموعة المرضى تتكون من 30 ذكر و 30 أنثى و المجموعة الضابطة تتكون من 28 شخصا. لقد نتج عن البحث وجود ارتفاع معنوي لكل من *MDA* و *NAG* و انخفاض معنوي في مستوى كل من *GPx* و*CAT* في مصل المرضى مقارنة بالمستويات لدى المجموعة الضابطة. لم تكن هنالك علاقة معنوية تربط *MDA* بكل من *GPx* و *CAT* و *NAG* و كذلك لم تكن هنالك علاقة معنوية تربط *NAG* بإنزيمات مضادات الأكسدة و المواد المؤكسدة.

كانت أعلى نسبة لحدوث حصى الجهاز البولي في الفئة العمرية 31-40 سنة و بعدها تبدأ النسبة بالانخفاض مع تقدم العمر. أما نسبة الذكور للإناث فكانت 1:1 .لا وجود لتأثير معنوي لكل من العمر و الجنس على مستويات المواد المؤكسدة و إنزيمات مضادات الأكسدة و*NAG* . لقد كانت أعلى نسبة من المرضى يمتلكون حصى واحدة, وحجم تلك الحصى في *71.11%* من المرضى اكبر من 7مليمتر. أما الفترة الزمنية للمرض فكانت الأقل 2 يوم و الأعلى 36 شهرا. لقد كانت النسبة الأعلى من المرضى يعترضهم المرض بصورة متكررة (*63.33%*) و النسبة الأعلى كانوا لا يمتلكون تاريخا عائليا للمرض (*73.33%*) . لم يكن هنالك تأثير لكل من عدد الحصى, حجم الحصاة, تكرار الحصى, التاريخ العائلي للمرض على مستويات المواد المؤكسدة وإنزيمات مضادات الأكسدة و *NAG* في مصل مرضى حصى الجهاز البولي.

التوزيع التشريحي لحصى الجهاز البولي كانت كما يلي: *64.91%* في كلية محايدة , *5.26%* في الكليتين, *19.30*في الحالب,*7.02%* في الكلية و الحالب, *3.51%* في المثانة البولية. و كان هنالك فرق معنوي فقط بالنسبة لمستوى *GPx*في المصل بين المواقع التشريحية للحصى. وكان الفرق معنويا فقط بين الحصى الواقعة في كأس الكلية و الحالب, و بين حصى حوض الكلية الحالب, وكذلك الفرق كان معنويا لحصى المثانة عند مقارنتها لكل من حصى كاس الكلية و حوض الكلية.

كانت نسبة المدخنين بين المرضى 21.7% , و لم يكن هنالك فرق معنوي بين مستويات مستويات الدهون المؤكسدة و إنزيمات مضادات الأكسدة و *NAG* في مصل الدم بين المدخنين و غير المدخنين.

نستنتج مما سبق ان مرض حصى الجهاز البولي يؤثر على مستويات الدهون المؤكسدة و إنزيمات مضادات الأكسدة و *NAG*في مصل الدم . ليس هنالك تأثير لعمر المريض او جنس المريض و التدخين و عدد الحصى و حجم الحصى و تكرار المرض و التاريخ العائلي للمرض و الفترة الزمنية للمرض على مستويات تلك المتغيرات, ولكن الموقع التشريحي للحصى يؤثر بصورة معنوية على مستوى إنزيم مضاد الأكسدة *GPx*.

CHAPTER ONE

INTRODUCTION

AND

LITERATURE REVIEW

1.1. Anatomy and Physiology of Kidney, Ureter and Bladder

1.1.1. Kidneys

Kidney is a structurally complex organ that has evolved to subserve a number of important functions: excretory of the west product of metabolism, regulation of body water and salts, maintenance of appropriate acid-base balance, and secretion of a verity of hormones and autocoids (1). Two kidneys lie on the posterior wall of the abdomen, outside the peritoneal cavity. Each kidney of the adult human weighs about 150 grams and is about the size of a clenched fist. The medial side of each kidney contains an indented region called the hilum through which pass the renal artery and vein, lymphatics, nerve supply, and ureter, which carries the final urine from the kidney to urinary bladder, where it is stored until emptied. The kidney is surrounded by a tough, fibrous capsule that protects its delicate inner structures (2). On longitudinal section, the kidney is seen to be made up of an outer cortex, a central medulla, and the internal calices and pelvis. The cortex is homogeneous in appearance. The medulla consists of numerous pyramids formed by the converging collecting renal tubules, which drain into the minor calices at the tip of the papillae (3). The tips of the minor calices (8–12 in number) are indented by the projecting pyramids. These calices unite to form 2-3 major calices which join to form the renal pelvis (4). The pelvis may be entirely intrarenal or partly intrarenal and partly extrarenal. Inferomedially, it tapers to form the ureter (2) (Figure 1.1).

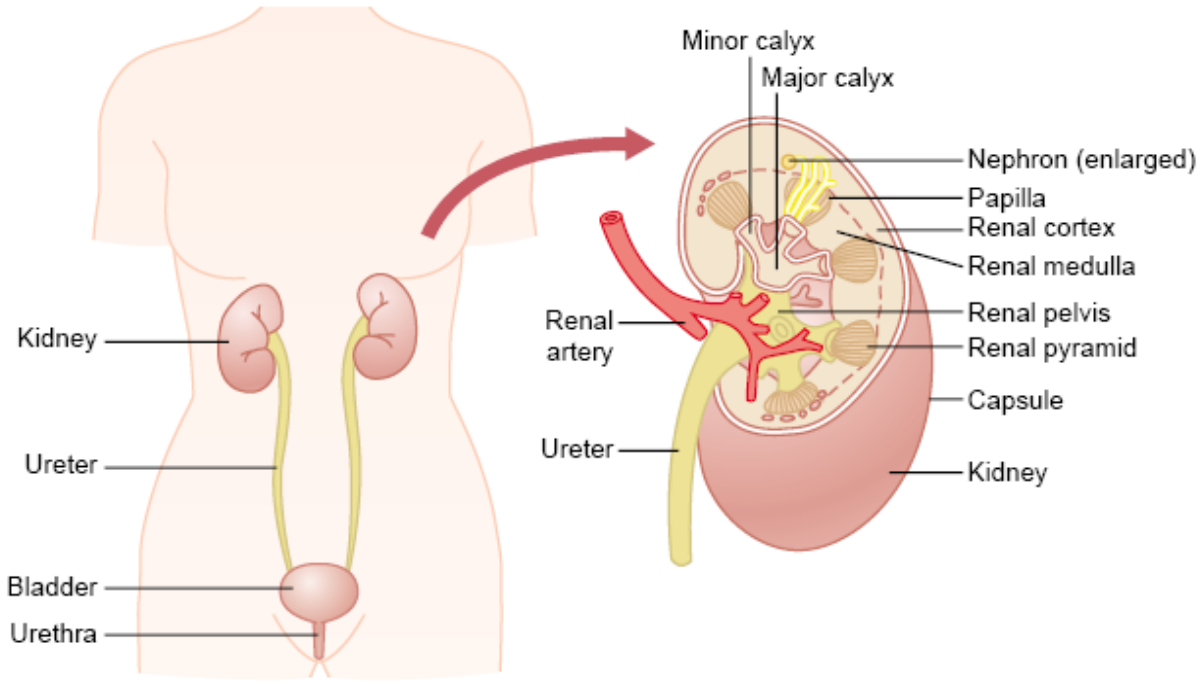


Figure 1.1. General organization of the kidneys and the urinary system (2).

Kidney in human contains about one million nephrons, which act as independent functional units. They have multiple physiological functions, which can be broadly categorized as the excretion of waste products, the homeostatic regulation of the extracellular fluid volume and composition, and endocrine (5). Each nephron contains a tuft of glomerular capillaries called the glomerulus, through which large amounts of fluid are filtered from the blood, and a long tubule in which the filtered fluid is converted into urine on its way to the pelvis of the kidney (2). The renal corpuscle is composed of the vascular glomerulus, which projects into Bowman's capsule, which, in turn, is continuous with the epithelium of the proximal convoluted tubule. The secretory portion of the renal tubule is made up of the proximal convoluted tubule, the loop of Henle, and the distal convoluted tubule (Figure 1.2). The excretory portion of the nephron is

the collecting tubule, which is continuous with the distal end of the ascending limb of the convoluted tubule. It empties its contents through the tip (papilla) of a pyramid into a minor calyx (3).

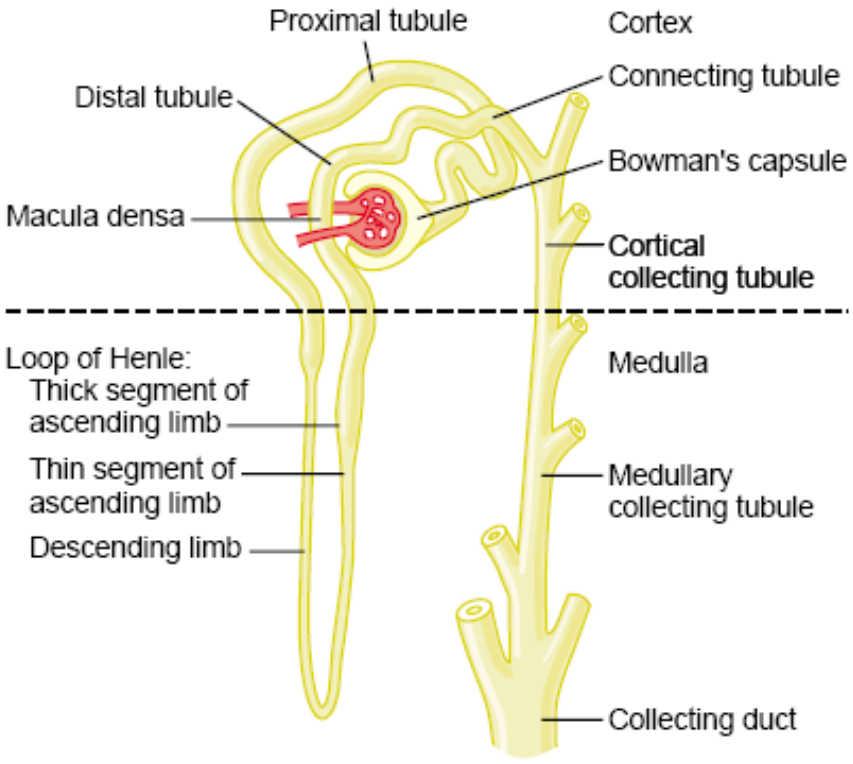


Figure 1.2. Basic tubular segments of the nephron (2).

1.1.2. Ureter

The adult ureter is about 30 cm long, varying in direct relation to the height of the individual. It follows a rather smooth S curve. Areas of relative narrowing are found at the ureteropelvic junction, where the ureter crosses over the iliac vessels, and where it courses through the bladder wall (3). Each ureter, as it enters the bladder, courses obliquely through the detrusor muscle and then passes another 1 to 2 centimetres beneath the bladder mucosa before emptying into the bladder (2).

1.1.3. Bladder

Bladder is a hollow muscular organ that serves as a reservoir for urine. The adult bladder normally has a capacity of 400–500 ml (3). The urinary bladder composed of two main parts: (a) the body, which is the major part of the bladder in which urine collects, and (b) the neck, which is a funnel-shaped extension of the body, passing inferiorly and anteriorly into the urogenital triangle and connecting with the urethra. Urine that is expelled from the bladder has essentially the same composition as fluid flowing out of the collecting ducts; there are no significant changes in the composition of urine as it flows through the renal calyces and ureters to the bladder (4). The walls of the calyces, pelvis, and ureter contain contractile elements that propel the urine toward the bladder, where urine is stored until it is emptied by micturition (2).

1.2. Urolithiasis

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 practic(6). The oldest urological object on record is a bladder calculus; in 1901, Elliott Smith discovered it in a prehistoric Egyptian tomb, in the pelvis of a mummy. The calculus is dated to 4800 BCE and has a uric acid nucleus with concentric laminations of calcium oxalate and ammonium magnesium phosphate (7).

1.2.1. Epidemiology

Urinary calculi are the third most common affliction of the urinary tract, exceeded only by urinary tract infections and pathologic conditions of the prostate (3). It is estimated that at least 10 % of the population in the industrialized part of the world is afflicted by urinary tract stone

disease. In some geographical areas the prevalence is even greater, for instance, in the Arabian countries (8). Environmental or occupational factors, as well as the presence of any bowel disease associated with diarrhea and/or malabsorption, can each predispose to stone disease. In this case, two factors have been hypothesized: climate induced perspiration resulting in a more concentrated urine, and sunlight-induced vitamin D conversion promoting calcium absorption from food (9). For both sexes, the peak age at first episode is between twenty and thirty years (10). About three males are afflicted for every female (11). However, there appears to be a changing pattern in as much as stone disease now is becoming more common in young females (8) and because stones in the urinary tract may be present but asymptomatic, prevalence estimates based on questionnaires or medical encounters are likely to be underestimates (12).

1.2.2. Urinary Stone Components

Stones are composed primarily of a crystalline component (3). The most common constituent of renal calculi is calcium, usually calcium oxalate which is present in 75% of all renal stones (13). Another 15-20% are composed of magnesium ammonium phosphate (struvite), and 10% are either uric acid or cystine stones (1) (Table 1.1). Xanthine and more rarely matrix and other substances may present (10). The urinary stone composes also from a noncrystaline protein like matrix. The matrix content varies from stone to stone, but most solid urinary calculi have a matrix content of about 3% by weight (11). Alternatively, matrix calculi, composed of an average of 65% of matrix by weight, may occur especially in association with urinary infection (14). The matrix is composed predominantly of protein, with small amounts of hexose and hexosamine (3)

Table 1.1. Urinary stone characteristics (4,13).

Chemical Composition	Percent of All Stones	Metabolic Causes	Radiographic Appearance
Calcium oxalate/ phosphate	75	Hypercalciuria (40-75%) Excessive absorption of dietary oxalate Primary hyperoxaluria.	Opaque, round
Magnesium-ammonium phosphate+(calcium phosphate carbonate)	20	Infection with urease positive organism (fall in $[H^+]$.	Opaque staghorn calculus
Uric acid	5	Myeloproliferative disorders High protein diet Uricosuric acid.	Radiolucent
Cystine	1-2	Hypercystinuria.	Opaque, staghorn±round calculi

1.2.3. Mechanism of Urinary Stone Formation

1.2.3.1. Supersaturation

The cause of stone formation is often obscure, particularly in the case of calcium containing stones. Probably involved is a confluence of predisposing conditions (1). Theories that explain urinary stone disease are incomplete. Urinary stones are polycrystalline aggregate composed of varying amounts of crystalloid and organic matrix (3). Saturation is the point at which the concentration of salts in the solution is just enough to prevent the crystals from dissolving. If more crystals are added, the crystals precipitate, unless the temperature or pH is changed or another chemical that allows the salts to dissolve is added (11). The most important cause of stone formation is increase urine concentration of the

stone's constituents, so that it exceeds their solubility in urine (supersaturation) (1). For crystals to occur, urine should be supersaturated with salts in consideration (11). In a large study of patients with complete metabolic work-ups and known stone composition, those with calcium oxalate stones have supersaturated urine with respect to calcium oxalate, and those with calcium phosphate stones tend to be supersaturated with respect to that crystalline phase. As a group, urine of patients with brushite stones have a higher pH and low citrate concentration, whereas calcium oxalate stone-formers have more acidic urine. The urine of those with uric acid stones are most acidic of all. Individuals with mixed calcium oxalate and calcium phosphate stones have urine that are supersaturated with respect to both crystallization phases (9). If urine is not sufficiently supersaturated, there will be no salt precipitation and accordingly, under such conditions, the fundamental prerequisite for stone formation is lacking (8). Supersaturation depends on urinary pH, ionic strength, solute concentration, and complexation. Urinary constituents may change dramatically during different physiologic states from a relatively acid urine in a first morning void to an alkaline tide noted after meals. Ionic strength is determined primarily by the relative concentration of monovalent ions. As ionic strength increases, the activity coefficient decreases. The activity coefficient reflects the availability of a particular ion (3). The point at which saturation is reached and crystallization begins is referred to thermodynamic solubility product (k_{sp}) (11). Concentrations above this point are metastable and are capable of initiating crystal growth and heterogeneous nucleation. As solutions become more concentrated, the activity product eventually reaches the formation product (K_{fp}). Supersaturation levels beyond this point are unstable, and spontaneous homogeneous nucleation may occur (3). The

concentration of most stone components in urine are in the metastable range between k_{sp} and k_{fp}(11).

1.2.3.2. Nucleation, Crystal Growth and Aggregation

Nucleation initiates the stone process formation may be induced by a variety of substances, including proteinaceous matrix, crystals, foreign bodies, and other particulate tissues (3). The process by which nuclei form in pure solution is called homogenous nucleation. These nuclei form the earliest crystal structure that will not dissolve and have the form of lattice that is a characteristic of that crystal (11). For non-calcium stones, which is stones composed of uric acid, urate salts, cystine, and struvite, the supersaturation of each one of the salts under physiological conditions also might exceed its formation product. This situation most certainly makes a homogeneous nucleation possible (8). In urine crystal nuclei usually form on existing surface (heterogeneous nucleation) (11). Epithelial cells, cell debris, urinary casts, other crystals and red blood cells can all act as heterogeneous nuclei (14). It is reasonable to assume, however, that nucleation of calcium phosphate most commonly is the result of heterogeneous crystallization (8). The stones then grow on these performed nuclei. Aggregation is necessary concept to promote the genesis of urinary stone (11). Growth and aggregation of calcium oxalate crystals can proceed as long as the ion-activity product of calcium oxalate exceeds the solubility product (8). Magnesium and citrate inhibit crystal aggregation (11). Nephrocalcin, an acidic glycoprotein of renal origin, inhibits calcium oxalate nucleation, growth and aggregation (16). Tamm-Horsfall mucoprotein, the most abundant protein in urine, inhibit aggregation (17). Interference with crystal growth and aggregation is a possible therapeutic strategy for the prevention of recurrent stone disease(11).

1.2.3.3. Crystal Retention

Whether the initial crystallization takes place as free or fixed particles has been a matter of debate over the years and it has generally been assumed that the precipitation of calcium oxalate is too slow to give crystals of sufficient size to be trapped in the tubular system unless there is some kind of fixation of the crystalline material. Irrespective of whether the initial crystallization is the result of free or fixed particles, stone formation cannot occur unless crystal material is retained in the renal collecting system (8) (Figure 1.3).

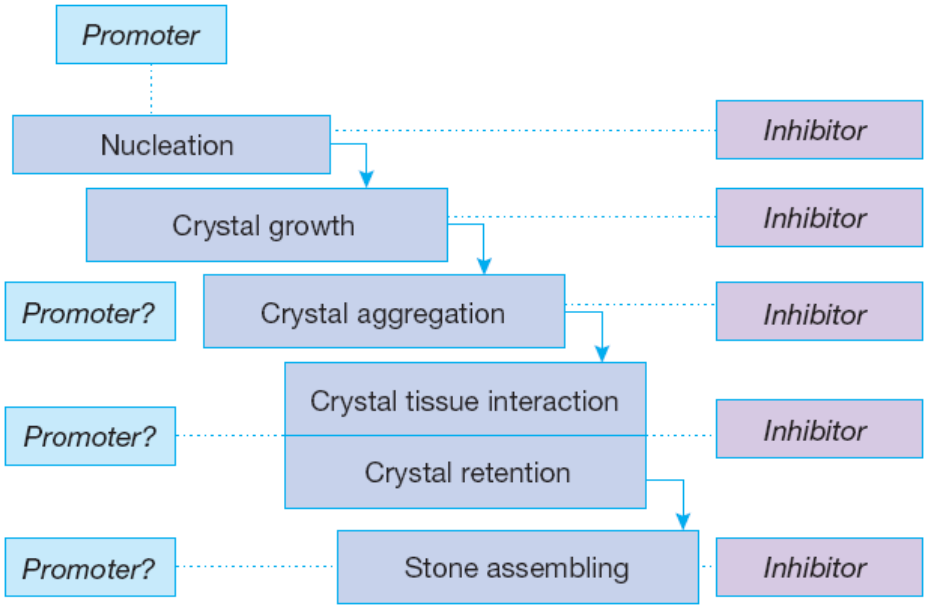


Figure 1.3. Major steps in calcium salt crystallization (8).

Many kidney stones have a layered structure, suggesting intermittent growth during the period of supersaturation (11). Crystal formation and retention are critical events for the formation of kidney stones. Oxalate and calcium oxalate crystals are injurious to renal epithelium, and membranes of injured cells promote crystal adherence and retention. Crystals are injurious to cells of both the proximal tubules as well as collecting ducts and the injury is mediated by reactive oxygen species (ROS) (17). Figure 1.4 summarizes a tentative series of events that possibly precedes stone formation and this model might help to understand the rationale for various therapeutic regimens. Such a background is important to clarify how the treatment might interfere with intranephronic crystallization (18).

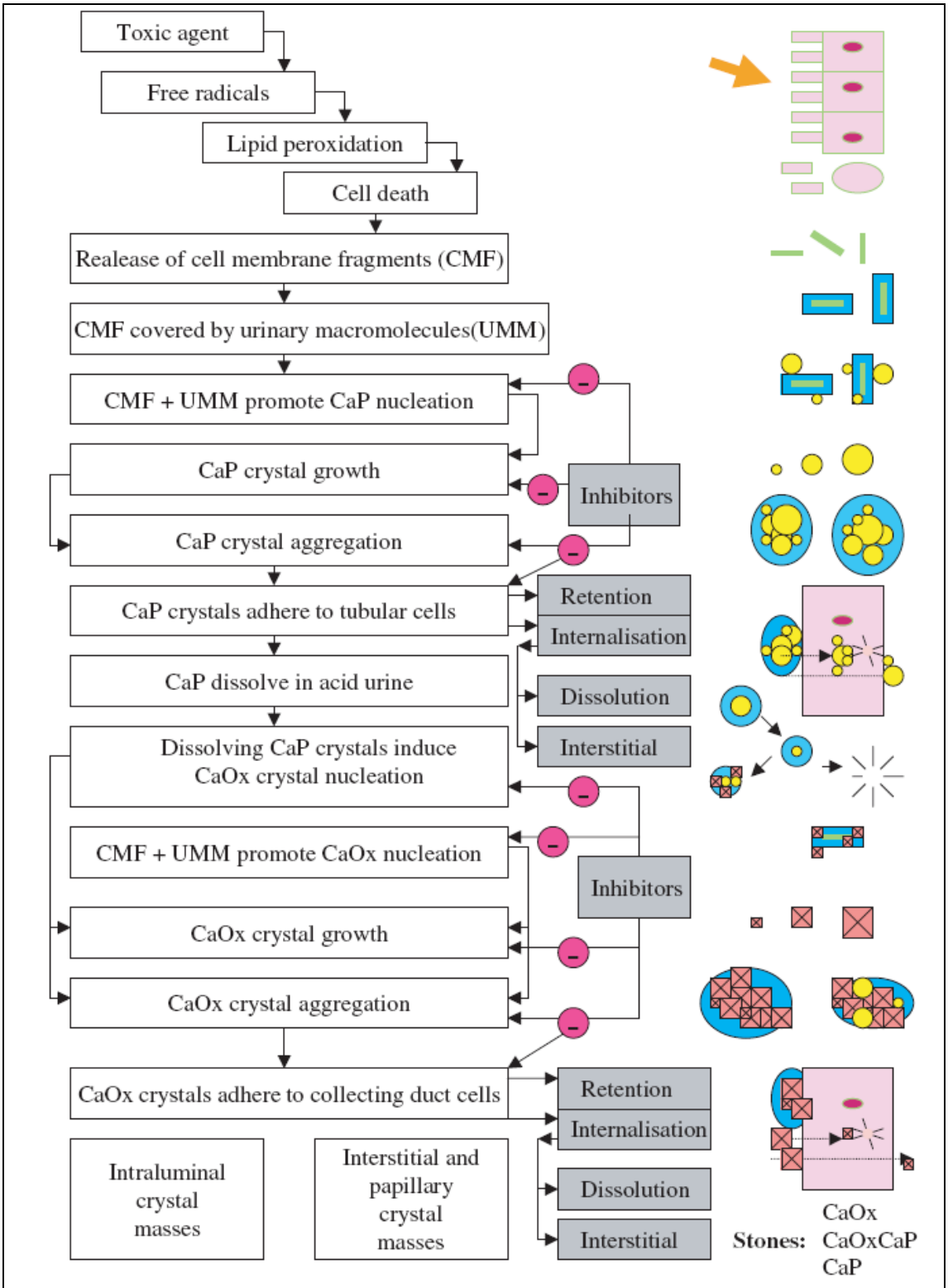


Figure 1.4. A tentative model for formation of CaOx/CaP stones in the nephron (18)

1.2.4. Types of urinary stones

1.2.4.1. Calcium calculi

Calcium nephrolithiasis is most commonly due to elevated urinary calcium, elevated urinary uric acid, elevated urinary oxalate, or a decreased level of urinary citrate (3). Calcium oxalate stones are either composed of pure calcium oxalate or of mixtures of calcium oxalate and calcium phosphate (8). Fifty percent of patients who develop calcium stones have hypercalciuria that is not associated with hypercalcaemia. Mostly in this group absorb calcium from the gut in excessive amount (absorptive hypercalciuria) and promptly excrete it in the urine, and some have a primary renal defect of calcium absorption (renal hypercalciuria) (1). Absorptive hypercalciuria is secondary to increased calcium absorption from the small bowel, predominantly from the jejunum. This results in an increased load of calcium filtered from the glomerulus. The result is suppression of parathyroid hormone, leading to decreased tubular reabsorption of calcium, culminating in hypercalciuria (>4 mg/kg). This physiologic cascade is in response to the primary defect, an increased absorption of calcium from the small bowel (3). In 5-10% of patients with calcium calculi there is hypercalcaemia (due to hyperthyroidism, vitamin D intoxication, or sarcoidosis). In 20% of hypercalciuria there is excessive excretion of uric acid in urine, which favours calcium stone formation; presumably the urates provide a nidus for calcium deposition. In about 15% there is hyperoxaluria or hypercitrateuria (1). It is important to emphasize, however, that despite considerable research efforts in order to increase the knowledge of calcium stone formation several fundamental details of the process are still poorly understood. Due to these shortcomings the therapeutic tools to prevent recurrent stone formation are less powerful than desirable (8).

1.2.4.2. Noncalcium calculi

a. Struvite

Struvite stones are composed of magnesium, ammonium, and phosphate (MAP). Although struvite calculi represent a small percentage of stones, they cause disproportionate morbidity. Untreated, struvite stones are often progressive and grow quickly and tend to provoke repeated episodes of pyelonephritis and sepsis (9). They frequently present as renal staghorn calculi and rarely present as ureteral stones except after surgical intervention (3). They almost always occur in patients with a persistently alkaline urine (normal urinary pH is 5.85) due to urinary tract infections (1). It is generally considered that this combination of salts, with a few exceptions, is the result of urinary urease activity (8). Infection with urease-producing microorganisms results in increased urinary concentrations of ammonium and carbonate ions, and gives an alkaline pH (18). *Proteus* is the most common urease producing microorganism. Other urease-producing microorganisms often present in stones are *Staphylococcus, Enterobacter, Providencia, Haemophilus, Mycoplasma, Pseudomonas, Corynebacterium, Klebsiella, Bacteroides,* and *Micrococci* (8).

b. Uric acid

Uric acid stones account for 5–10% of all those analyzed (9). They are radiolucent on a plain abdominal film, but radio-opaque on CT (18). They are usually found in men. Patients with gout, myeloproliferative diseases, or rapid weight loss, and those treated for malignant conditions with cytotoxic drugs have a high incidence of uric acid lithiasis (3). About half of the patients with uric acid stones have neither hyperuricaemia nor increase urine urate but an unexplained tendency to

excrete a persistently acid urine, favouring stone formation (1). Crystals of uric acid are precipitated when supersaturation with uric acid is high. The important factors are a high excretion of urate, a small urine volume and a low urinary pH. Uric acid can precipitate at normal urate concentrations if the pH is sufficiently low (18).

c. Cystine

Cystine lithiasis is secondary to an inborn error of metabolism (homozygous cystinuria) resulting in abnormal intestinal (small bowel) mucosal absorption and renal tubular absorption of dibasic amino acids, including cystine, ornithine, lysine, and arginine (3). Cystine stones are relatively hard, not easily amenable to fragmentation, and can quickly grow into large, potentially organ-threatening staghorn calculi. Therefore, medical therapy to prevent their formation is particularly important. About 25% of patient present in their first decade of life and another 30–40% as teenagers (9).

d. Xanthine

The formation of xanthine stones is seen in patients with a deficiency of xanthine oxidase (also called xanthine dehydrogenase). The conversions of xanthine to urate and that of hypoxanthine to xanthine are blocked and the urinary excretion of xanthine is increased as a result of guanine conversion to xanthine (8).

e. Indinavir

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 stones in up to 6% of patients who are prescribed this medication (3). It is excreted in urine

and have low solubility, tending to form precipitates. Indinavir calculi are the only urinary stones to be radiolucent on noncontrast computed tomography (CT) scans. They may be associated with calcium components and in these situations will be visible on noncontrast thin-cut helical computed tomography (CT) images (9).

f. Rare

Silicate stones are very rare and are usually associated with long-term use of antacids containing silica (3). Triamterene stones are radiolucent and have been identified with an increased frequency. They are associated with antihypertensive medications containing triamterene, such as Dyazide (9).

1.2.5. Risk Factors for Recurrent Stone Formation

Risk factors for stone formation are :

1. Onset of disease early in life, i.e., below 25 years of age.
2. Stones containing brushite (calcium hydrogen phosphate; $CaHPO_4.2H_2O$).
3. Strong family history of stone formation.
4. Diseases associated with stone formation: hyperparathyroidism, renal tubular acidosis (partial/complete), cystinuria, primary hyperoxaluria, jejunoileal bypass, Crohn's disease, intestinal resection, malabsorptive conditions, sarcoidosis and hyperthyroidism.
5. Medication associated with stone formation: calcium supplements, vitamin D supplements, acetazolamide, ascorbic acid in megadoses (>4 g/day), triamterene and indinavir (6).
6. The anatomy of the upper and lower tracts may also influence the likelihood of stone formation by predisposing to urinary tract infection or stasis (12).

1.2.6. Clinical Course of Urolithiasis

Nephrolithiasis is a common medical problem. It leads to considerable morbidity, causing renal colic, haematuria, and infection (19). Stones may be present without producing symptoms or significant renal damage. This is particularly true with large stones lodged in the renal pelvis. The smaller stones may pass into the ureter, producing a typical intense pain known as renal or ureteral colic, characterized by paroxysms of flank pain radiating towered the groin (1). The celiac ganglion serves both kidneys and stomach, therefore, nausea and vomiting are commonly associated with renal colic (11). Urinary stones may cause obstruction of urinary tract which usually is expressed in mid lifetime between 30 and 40 years of age (20). There are five typical locations where the urinary calculi become entrapped. Stones may become impacted in a renal calyx (the individual calyces become distended and painful and create haematuria) , at the uretropelvic junction, at the pelvic brim where the ureter arches over the iliac vessels, in the posterior pelvis where the ureter is crossed anteriorly by pelvic vessels , or at the uretrovesical junction (10). The majority of impacted ureteral stones are found in the pelvic portion of the ureter. To become impacted, calculi usually must have one diameter in excess of 2mm. If the smaller diameter is less than 4 mm, spontaneous stone passage is likely (21).

1.2.7. Diagnosis of Urinary Stones

1.2.7.1. Urinalysis

Urinalysis in most patient with urinary lithiasis reveals the presence of microscopic or gross haematuria. Moderate pyuria may occur even in patients with uninfected urinary lithiasis. On occasion, a patient who is in

active phase of urinary lithiasis has the urine crystals of the same type that are creating the calculus (11).

1.2.7.2. Diagnostic Imaging

The clinical diagnosis should be supported by an appropriate imaging procedure (6). In case of an acute stone colic, excretory urography (intravenous urography IVU) has been established as a gold standard diagnosis (22). Although IVU is sensitive to detect urinary stones and provides more anatomic information, but IVU can miss small or radiolucent nonobstructing stones (12). Unenhanced helical CT examinations have been used as a quick and contrast-free alternative (23). In randomized prospective studies, the specificity and sensitivity of this method for patients with acute flank pain was found to be similar to that obtained with urography (24). In selected cases, additional information regarding renal function may be obtained by combining CT with contrast infusion. One great advantage of CT is the demonstration of uric acid and xanthine stones, which are radiolucent on plain films. Another advantage is the ability of CT to detect alternative diagnoses (25). An alternative and commonly applied method for evaluating patients with acute flank pain is a plain film of kidneys, ureters and bladder (KUB) combined (KUB can fail to reveal small or radiolucent stones) with ultrasonography (US) (US is less sensitive in delineating stone size and number and cannot detect most ureteral stones)(12). There is a huge bulk of experience to show that these two methods are sufficient in a large proportion of patients for the diagnosis of a ureteral stone (6) (Figure 1.5).

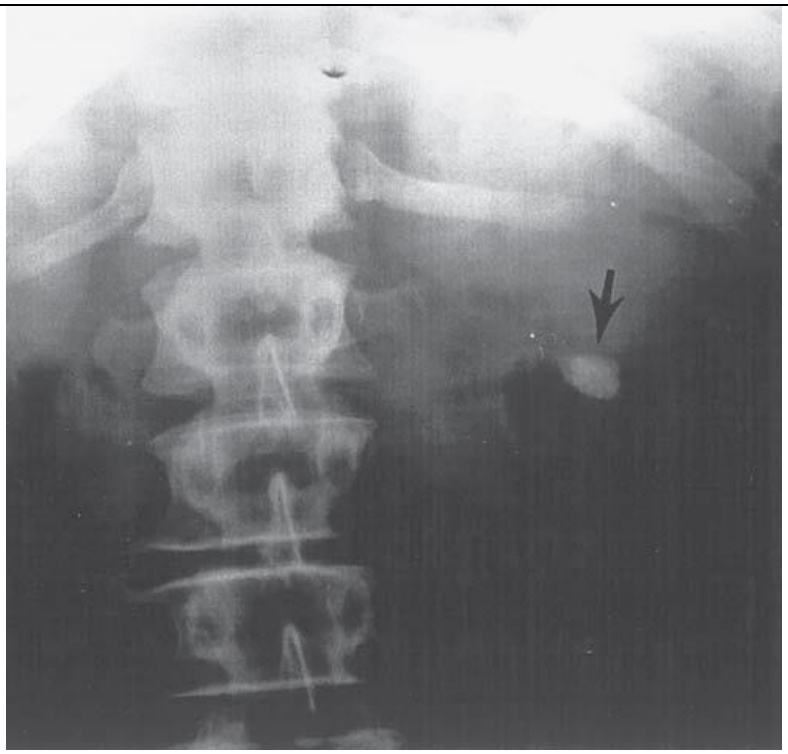


Figure 1.5. Plain-film KUB showing a round density (arrow) overlying the left renal shadow indicative of a left renal calculus (12).

1.2.7.3. Analysis of Stone Composition

Pathological morphology of urinary stones revealed that the stones are unilateral in about 80% of patients. Common sites of formation are renal pelvis and calyces and the bladder. Often, many stones are found in one kidney. They tend to be small (average diameter 2-3 mm) and may be smooth or jagged. Occasionally, progressive accretion of salts leads to the development of branching structures known as staghorn calculi, which create a cast of the renal pelvis and calyceal system. These massive stones are usually composed of magnesium ammonium phosphate (1).

The preferred analytical procedures are X-ray crystallography and infrared spectroscopy (26). Typically analysis is accomplished on a pulverized sample of the stone by comparing an infrared spectrum profile

to reference spectra of known stone components. Although most are composed of calcium oxalate, calcium phosphate, uric acid, or an admixture of these components, stone analysis can confirm the presence of struvite stones (indicating infection), more unusual stone components such as cystine, or even drugs (e.g., Indinavir) (9). All patients should have at least one stone analyzed and repeated analysis is indicated when any changes in urine composition (26). When stones have not been retrieved, conclusions on stone composition may be based on the following observations: qualitative cystine test, bacteriuria/urine culture , demonstration of crystals of struvite or cystine upon microscopic examination of the urinary sediment, serum urate, urine pH (low in patients with uric acid stones, high in patients with infection stones) and radiographical characteristics of the stone (6).

The stones which are not associated with infection are referred to as uric acid/urate stones. Urate stones include uric acid, ammonium urate and sodium urate. The typical constituents of infected stones are magnesium ammonium phosphate and carbonate apatite. Calcium stones, uric acid/urate stones and cystine stones associated with infection are referred to as stones with infection (27).

1.3. Lipid Peroxidation and Antioxidant Enzymes

1.3.1. Free Radical Injury

A free radical is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital (28). Several powerful oxidants are produced during the course of metabolism, in both blood cells and most other cells of the body. These include superoxide ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), peroxyl radicals ($ROO^{\bullet}$), and hydroxyl radicals ($OH^{\bullet}$), hydrpoperoxyl radical ($HOO^{\bullet}$), peroxy nitrate ($ONOO^{-\bullet}$)

and nitric oxide ($NO^{\bullet}$) (29). These radicals may be generated nonenzymatically or enzymatically as accidental by products or major products of reactions. Superoxide may be generated nonenzymatically from coenzyme Q (CoQ), or from metal-containing enzymes (e.g., cytochrome P450, xanthine oxidase, and NADPH oxidase). The highly toxic hydroxyl radical is formed nonenzymatically from superoxide in the presence of Fe^{3+} or Cu^{2+} by the Fenton reaction, and from hydrogen peroxide in the Haber–Weiss reaction (30). Free radicals can react with proteins, nucleic acids, lipids, and other molecules to alter their structure and produce tissue damage (29).

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 (31). Damaged epithelia might act as nidi for stone formation aggravating calcium oxalate precipitation during hyperoxaluria (32). Renal injury and inflammation appear to play a significant role in stone formation. Reactive oxygen species (ROS) are produced from many sources and involve a variety of signalling pathways (29). Several studies have been revealed that in urolithic rat kidney or oxalate exposed cultured cells, both superoxide anion and hydroxyl radicals were generated in excess, causing cellular injury (33**,**34).

1.3.2. Lipid Peroxidation

Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation (29). Lipid peroxidation is initiated by a hydroxyl or other radical that extracts a hydrogen atom from a polyunsaturated lipid, there by forming a lipid radical. The free radical chain reaction is propagated by reaction with O_2, forming the lipid peroxy radical and lipid peroxide. Rearrangements of the single electron result in degradation of the lipid (30).

Lipid peroxidation process can be depicted in the following steps of reactions (29):

1. Initiation:

$$ROOH + Metal^{(n)+} \longrightarrow ROO^{\bullet} + Metal^{(n-1)+} + H^{+}$$

$$X^{\bullet} + RH \longrightarrow R^{\bullet} + XH$$

2. Propagation:

$$R^{\bullet} + O_2 \longrightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}, \text{ etc}$$

3. Termination:

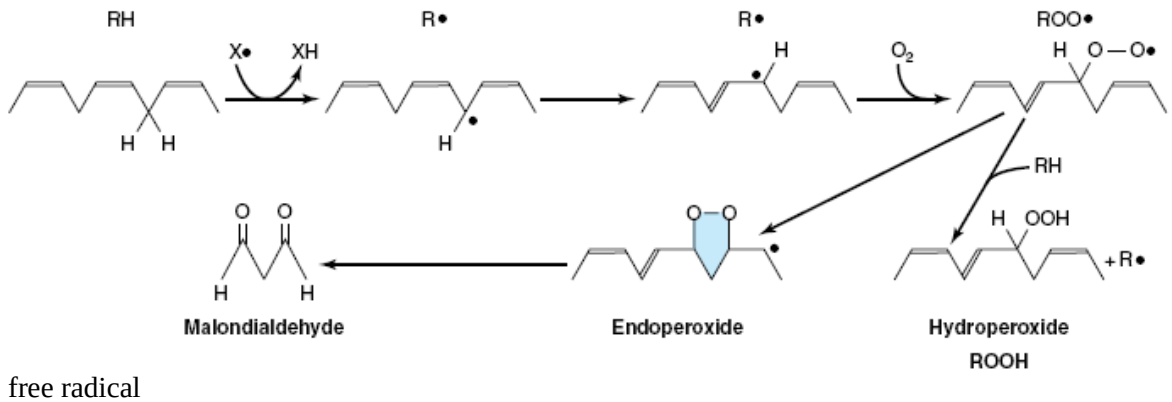
$$ROO^{\bullet} + ROO^{\bullet} \longrightarrow ROOR + O_2$$

$$ROO^{\bullet} + R^{\bullet} \longrightarrow ROOR$$

$$R^{\bullet} + R^{\bullet} \longrightarrow RR$$

The process of peroxidation of polyunsaturated fatty acids in plasma membrane leads to loss of membrane function. Lipid peroxidation and consequence degradation products such as malondialdehyde ($CHO\text{-}CH_2\text{-}CHO$) are seen in biological fluids (28). Malondialdehyde is only formed by fatty acids with three or more double bonds and is used as a measure of lipid peroxidation together with ethane from the terminal two carbons of ω3 fatty acids and pentane from the terminal five carbons of ω6 fatty acids (29) (Figure 1.6).

Lipid peroxidation remains one of the most widely used indicators of oxidant/free radical formation *in vitro* and *in vivo* (33). Malondialdehyde appears in the blood and urine and is used as an indicator of free radical damage (30).



X•: free radical

Figure 1.6. Lipid peroxidation reactions (29).

1.3.3. Lipid Peroxidation and Urolithiasis

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 (35). 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 (8). 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 (36). the brush border membrane might be injured by free radicals formed as the result of toxic effects on the cell (8). This might lead to lipid peroxidation and cell death (37). 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 (8). Studies revealed that the lipid peroxidation products, thiobarbituric acid-reactive substances, hydroperoxides, and diene conjugates were excessively released in tissues of urolithic rats and in plasma of rats as well as stone patients (33,38).

1.3.4. Antioxidant Defence

Reactive oxygen species (ROS) damage to lipids and proteins is addressed largely by degradation and re-synthesis. Oxidized proteins, for example, are preferred targets for proteasomal degradation and damaged DNA is repaired by a number excision-repair mechanisms. The process is not perfect. Some proteins, such as collagens NAD crystallins, turn over slowly, so that damage accumulates and function may be impaired (39).

Our defences against oxygen toxicity fall into the categories of antioxidant defence enzymes, dietary and endogenous antioxidants, cellular compartmentation, metal sequestration, and repair of damaged cellular components (30). Our first line of defence against oxidative damage is sequestration or chelation of redox-active metal ions. These chelatores 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, ROS 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 (39). These include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Catalase and peroxidases are preventive antioxidants (reduce the rate of chain initiation) that react with ROOH and chelators of metal ions such as ethylenediaminetetra acetate (EDTA) and diethylene-triaminepentaacetate (DTPA). Superoxide dismutase is the principle chain braking antioxidants which interfere with chain propagation (29).

Three antioxidant vitamins, A, C and E, provide the third line of defence against oxidative damage. Vitamin C (ascorbate) in the aqueous phase and vitamin E (α- and γ-tocopherol) in the lipid phase act as chain-breaking antioxidants. These vitamins are reducing agents; they donate a

hydrogen atom ($H^{\bullet}$) to radical intermediates formed by reaction of ROS with biomolecules. Vitamin C and E works together to inhibit lipid peroxidation reaction in plasma lipoproteins and membranes. Vitamin A is a lipophilic antioxidant that is a potent singlet oxygen scavenger (39).

A number of compounds synthesized endogenously for other functions, or as urinary excretion products, also function nonenzymatically as free radical antioxidants, such as uric acid, and melatonin (30).

1.3.4.1. Superoxide dismutase (EC 1.15.1.1)

In *vivo*, the principal chain breaking antioxidant is superoxide dismutase (SOD), which acts in the aqueous phase to trap superoxide free radicals ($O_2^{\bullet -}$), as shown in the following reaction: (29)

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Superoxide dismutase exists as three isoenzyme forms, a Cu^+-Zn^{2+} form present in the cytosol, a Mn^{2+} form present in mitochondria, and a Cu^+-Zn^{2+} form found extracellular. The activity of Cu^+-Zn^{2+} SOD is increased by chemicals or conditions (such as hyperbaric oxygen) that increase the production of superoxide (30). Calcium stones usually contain an organic matrix constituting 1-3 percent of stone weight. One of these compounds which is extracted from stone matrix is the superoxide dismutase (8).

1.3.4.2. Glutathione Peroxidase (EC 1.11.1.9)

Glutathione is a tripeptide comprised of glutamate, cysteine, and glycine (γ-glutamylcysteinylglycine), where the amino group of cysteine joined in peptide linkage to the γ-carboxyl group of glutamate (40).

Glutathione is one of the body's principal means of protecting against oxidative damage (30). Glutathione present at high levels in animal cells, serve as a sulfhydryl buffer. It cycles between a reduced thiol form (GSH) and an oxidized form (GSSG) in which two tripeptides are linked by a disulfide bond (41). Reduced glutathione removes H_2O_2 in a reaction catalyzed by glutathione peroxidase , which is an enzyme containing the selenium analogue of cysteine (selenocysteine) at the active site (29). Glutathione peroxidase thus protects against oxidative damage by reducing the reactive oxygen species. The enzyme is interesting in that it contains the rare amino acid, four selenocysteine (42). (figure 1.7).

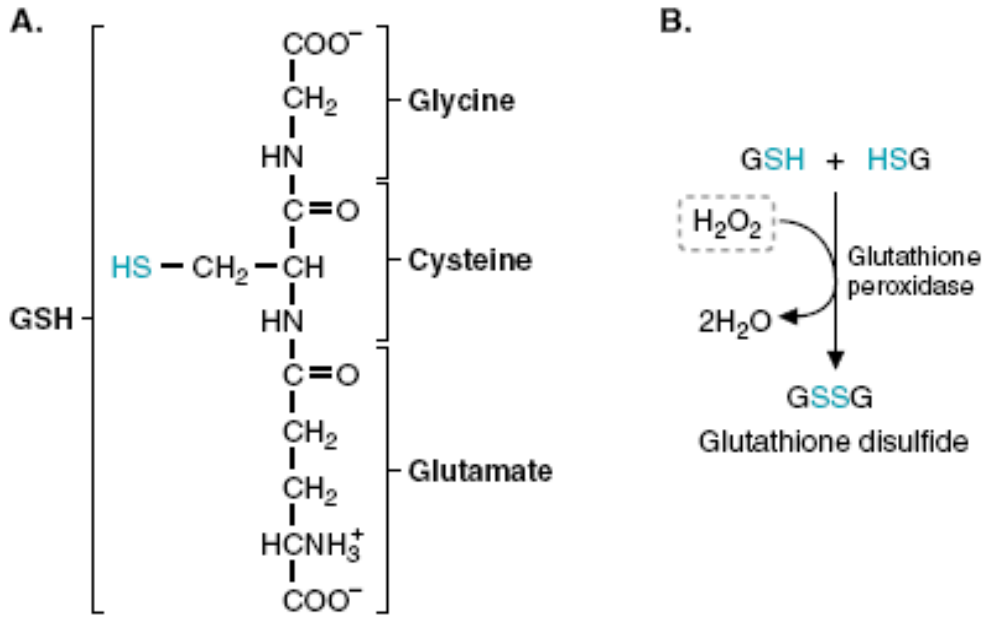


Figure 1.7. Glutathione peroxidase. A: The structure of glutathione. B: Glutathione peroxidase transfer electrons from glutathione (GSH) to hydrogen peroxide (30).

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 (43). Within cells, glutathione peroxidases are found principally in the cytosol and mitochondria, and are the major means for removing H_2O_2 produced outside of peroxisomes. They contribute to our dietary requirement for selenium and account for the protective effect of selenium in the prevention of free radical injury (30). Humans have five selenoprotein glutathione peroxidases, including GPx1, gastrointestinal GPx2, plasma GPx3 and its close homolog GPx6, and phospholipid hydroperoxide glutathione peroxidase, known as PHGPX or GPx4 (40).

GPx has been measured in erythrocytes of human diseased with urolithiasis (44). Other studies have measured GPx in tissue (31) and mitochondria (45) of urolithic rat kidney. While serum GPx have been studied by several studies in human with different types of diseases other than urolithiasis (46-49).

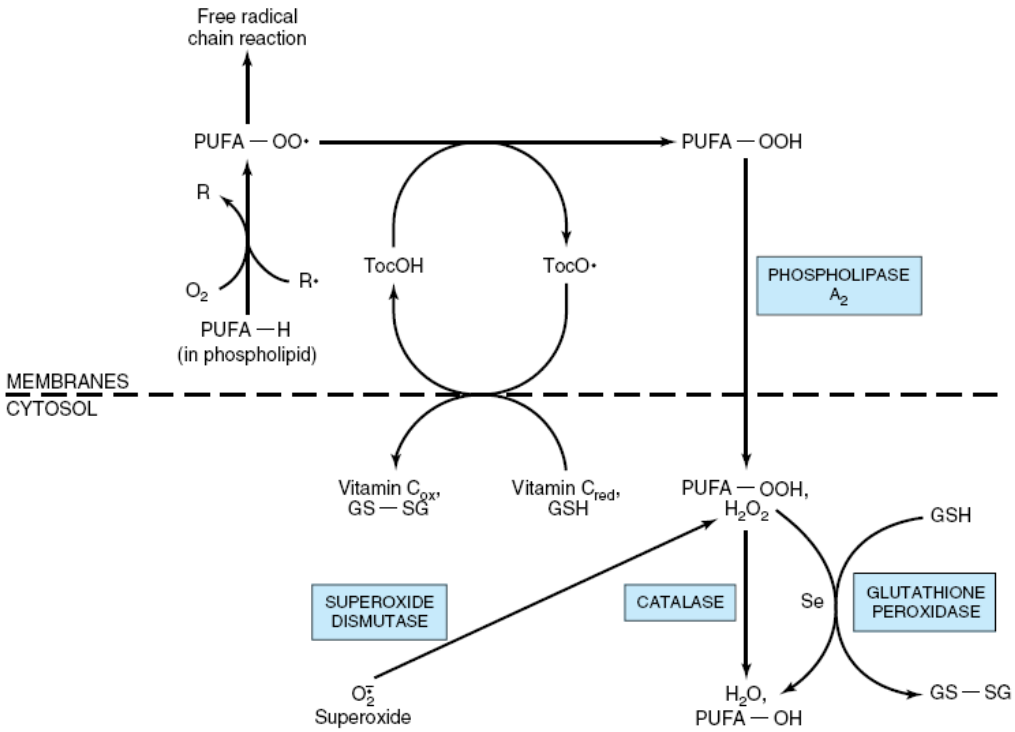
1.3.4.3. Catalase (EC 1.11.1.6)

Catalase (CAT) is a haemoprotein containing four haem groups (41). Catalase is used to remove the H_2O_2 when it is generated in large quantity(28), as shown in the reaction below (30).

$$\mathbf{2H_2O_2 \xrightarrow{Catalase} O_2 + 2H_2O}$$

Catalase is found principally in peroxisomes, and to a lesser extent in the cytosol and microsomal fraction of the cell. The highest activities are found in tissues with a high peroxisomal content (kidney and liver). In cells of the immune system, catalase serves to protect the cell against its own respiratory burst (30). Interaction and synergism between antioxidant systems is shown in figure 1.8.

Experimental rats model of calcium oxalate stone have been used by researchers to measure the level of catalase in kidney homogenates (31,37). In human, erythrocyte catalase level has been studied in patients with urolithiasis (36, 50). Several studies have used serum to measure the level of catalase in different human diseases such as acute pancreatitis (51), ankylosing spondylitis (52) and liver diseases (53).



(R•; free radical, PUFA-OO•; peroxyl free radical of polyunsaturated fatty acid in membrane phospholipid, PUFA-OOH; hydroperoxy polyunsaturated fatty acid, PUFA-OH; hydroxy polyunsaturated fatty acid, TocOH; vitamin E (α-tocopherol), TocO•; free radical of α-tocopherol; Se; selenium, GSH; reduced glutathione; GS-SG; oxidized glutathione, PUFA-H; polyunsaturated fatty acid)

Figure 1.8. Interaction and synergism between antioxidant systems operating in the lipid phase (membranes) of the cell and the aqueous phase (cytosol) (29).

1.4. *N*-acetyl-*ß*-D-glucosaminidase (EC 3.2.1.52)

The enzyme *N*-acetyl-*ß*-D-glucosaminidas*e* (NAG) catalyses hydrolytic release of terminal ß (1-4)-linked *N*-acetyl-D-hexosamine (-glucosamine or -galactosamine) residues from the non-reducing end of a variety of substrates including glycoproteins, glycolipids and glycosaminoglycans (54). *N*-acetyl-*ß*-D-glucosaminidase is widely distributed in animal tissue and serum, including that of humans. It is particularly abundant in organs in which high rates of mucoid turnover might be expected, for example, epididymis, kidney, liver, testis, spleen, and salivary glands (55). In humans the enzyme's function as a lysosomal acid hydrolase is of clinical significance in Tay–Sachs and Sandhoff diseases, where deficiency in one or more of the A or B isoenzymes leads to imperfect degradation and altered lysosomal storage of glycoconjugates, with resulting pathology (54). On the other hand, elevated levels of serum NAG have been implicated in a variety of diseases, such as renal disorders, diabetes mellitus, liver disease (56) and cancer (57). The lysosome in the renal proximal tubular cells contains high amount of NAG, which is secreted in urine when kidney is damaged (58), so that increased activity of this enzyme in urine suggests injury to tubular cells (59). It is released into serum from cells by extracellular secretion of this lysosomal enzyme (exocytosis) or from the breakdown of cells (58). Serum NAG activity is reported to be influenced by oxidative stress (59, 60).

Aim of the study

The present study has aimed to assess the clinical significance of lipid peroxidation, antioxidant enzymes, and N-acetyl-β-D-glucosaminidase in patients with urolithiasis and control group. The aim is achieved by the following :

1. Serum MDA measuring as an indicator of lipid peroxidation.
2. Serum glutathione peroxidase and catalase measuring as antioxidant enzymes and their correlation to MDA.
3. Serum *N*-acetyl-*ß*-D-glucosaminidase measuring as a lysosomal enzyme and if it is influenced by oxidative stress.
4. Effect of age, gender and smoking on theses parameters in urolithiasis patients.
5. Assessment of the mentioned biochemical parameters clinically, according to number, size and anatomical site of stones, and duration, recurrence and family history of urolithiasis disease.

CHAPTER TWO

MATERIALS

AND

METHODS

2.1. Materials

2.1.1. Patient and control subjects

The project has covered the period from November 2007 till October 2008. A total of sixty urolithiasis patients in the age group ranging from 21-74 years old, who have been admitted to Al-Hilla Teaching Hospital, Urology Department , and proved to have urinary stone as diagnosed by radiological investigations. The study group comprises of 30 males and 30 females.

The patients have been having the following investigations and criteria:

1. Demonstrated Radio-opaque stone(s) with detection of the stone size, site and position by using KUB or IVU and/or Ultrasound examination
2. The patients have not been in acute state of the disease.
3. The patients have been free of medical diseases including diabetes mellitus, hypertension, and rheumatologic disease.
4. Pregnant patients have been excluded from the study group.

All patients underwent full history and physical examination including: age, gender, smoking, family history of urolithiasis, past history of recurrent stone and any current medical diseases. The patients underwent general urine examination (GUE), random blood sugar (RBS), with ultrasonography (US), plain abdominal X ray film of kidney, ureter and bladder (KUB), and intravenous urography (IVU). The work of this study have not included the results of GUE and RBS in the study work, which have been done on the coast of the hospital as part of their investigations, but they have been checked to exclude urinary tract infection and diabetes mellitus.

Twenty eight apparently healthy subjects, age and gender matched from medical and college staff, have been included as a control group. They have been free from history of smoking, alcoholism, and coexistence of any medical disease which can also lead to similar changes such as diabetes mellitus and hypertension. Pregnant females have been excluded from the control group too.

All tests have been performed on serum in Biochemistry Department in College of Medicine of Babylon University. Blood samples have been collected from patient and control subjects. Clean and sterile vials without any anticoagulant have been used to collect 10 ml of blood sample in each tube. The blood has been allowed to clot and then centrifuged (450 Xg for 10 minutes) to be used serum samples immediately for detection of MDA and GPx, and the rest of serum have been stored at deep freezing (-20°C) for 10 days till using for detection of catalase and NAG.

2.1.2. Statistics

Student's *t*-test has been used to determine the significant difference between two groups at p=0.05 level. When multiple means have been compared, significance (p=0.05) has been determined by analysis of variance (ANOVA), followed by Fisher's protected least significant difference test (LSD). While the correlation between two variables have been estimated by Pearsons's correlation coefficients at 0.05 level (61).

2.1.3. Chemicals

Table 2.1. Chemical compounds

No.	Chemicals	Production
1	Disodium hydrogen orthophosphate	BDH
2	DTNB	Fluka
3	Distilled water	Labroratory Distillator
4	Glutathione standered (GSH) 99%	SIGMA-ALDRICH CHEMIE
5	Hydrogen peroxide 30%	Fluka
6	Potassium dihydrogen orthophosphate	CHEM GILLMAN
7	Sodium azide	Fluka
8	Tertiary butyl hydroperoxide	Fluka
9	Thiobarbutric acid 98% (4,6-dihydroxy-2-mercaptopyrimidine)	Fluka
10	Trichloroacetic acid 99%	Hopkin Williams
11	*N*-acetyl-ß-D-glucoseaminidase kit	PPR Diagnostics Ltd,London.

2.1.4. Instruments and Apparatuses

Table 2.2. Instruments and apparatuses.

No.	Instruments /Apparatuses	Company
1	Balancer	Sartorius AG GÖTTINGEN BL2105 / Germany
2	Centrifuge	EBA20-Hettich/ Germany
3	Distillator	Bibby Science Product Limited/ England.
4	Incubator	Fisher Scientific, model 5370, CAT.11-690-538D / USA
5	Magnetic stirrer with hot plate	Classico / India
6	Micropipettes-automatic	Slamed / Germany
7	pH-meter	HANNA HI-9321 / Portugal
8	Spectrophotometer (Digital ultraviolet and visible)	Spectronic 21,MILTON ROY COMPANY,Bouch and Lamp/ USA
9	Vortex (electronic)	VIBROFIX, VF-1 JANKE and KUNKEL IKA-Labortechnik/ Germany
10	Water bath	Schutzart/Germany

2.2. Methods

2.2.1. Determination of Serum Malondialdehyde (62).

Principle

The principle of serum MDA determination is based on the spectrophotometric measurement of the color which is produced during the reaction of thiobarbutric acid (TBA) and MDA.

Reagents

1- 17.5% Trichloroacetic acid (TCA).
2- 0.6% Thiobarbutric acid (TBA).
3- 70% Trichloroacetic acid (TCA).

Procedure

1- Two set of tubes have been prepared as follow:

Reagent	Sample	Blank
Serum	0.15 ml	-
TCA 17.5%	1 ml	1 ml
TBA 0.6%	1 ml	1 ml
All tubes have been mixed well by vortex, incubated in boiling water bath for 15 minutes then allowed to cool.		
TCA 70%	1 ml	1 ml

2- The mixture has been left to stand at room temperature for 20 minutes and centrifuged at 450Xg for 15 minutes. The supernatant has been taken out to read the absorbance of sample at 532 nm.

Calculation

$$\text{The conc.of MDA } (\mu\text{mol/L}) = \frac{\text{A of sample at 532nm}}{\text{L} \times \varepsilon} \times \text{D}$$

A= Absorbance of sample at 532 nm.

L= Light path (1 cm).

ε= Extinction coefficient ($1.56 \times 10^5 M^{-1}cm^{-1}$).

D= Dilution factor (6.7).

2.2.2. Assay the activity of serum glutathione peroxidase (63).

Principle

Selenium-dependent glutathione peroxidase is measured with tertiary-butyl hydroperoxide (*t*-BuOOH) (This substrate is preferable to H_2O_2 which gives too high blank values compared to the assay values). Glutathione peroxidase catalyzes the reduction of *t*-BuOOH as a substrate by using glutathione (GSH) as the reducing substrate :

$$t\text{-BuOOH} + 2GSH \xrightarrow{\text{Glutathione peroxidase}} t\text{-BuOH} + H_2O + GSSG$$

Reagents

1- Phosphate buffer solution (pH 7.0) composed of 11 g of Na_2HPO_4 and 2.7 g of KH_2PO_4 in 1 L.
2- Sodium azide (NaN_3) (10 mM).
3- Glutathione peroxidase (GSH) (2mM).
4- Tertiary-butyl hydroperoxide (*t*-BuOOH) (2mM).
5- TCA 30%.
6- Na_2HPO_4 (0.4 M).
7- 5,5'-Dithio-Bis (2-Nitrobenzoic Acid) (DTNB) : 0.02% of DTNB in 1% sodium nitrate ($NaNO_3$).

Procedure

Three sets of tubes have been prepared as follow:

Reagent	Stander (std)	Test	Blank
Phosphate buffer	1.5 ml	1.3 ml	1.7 ml
NaN_3	0.1 m	0.1 ml	0.1 ml
Serum	-	0.2 ml	-
GSH	0.2 ml	0.2 ml	0.2 ml
t-BuOOH	0.2 ml	0.2 ml	0.2 ml
Incubation at 37 C° for 5 minutes.			
TCA	0.4 ml	0.4 ml	0.4 ml
Mixing and centrifugation at 3000 Xg for 5 minutes.			

After that, 0.5 ml of supernatant has been mixed with 1 ml of Na_2HPO_4 (0.4 M) and 1 ml of DTNB reagent. Then, absorbance has been read at 412 nm.

Calculation

$$C_{test}\ (\mu M/L) = \frac{A_{test}}{A_{std}} \times C_{std}$$

$$Se - GPx\ (IU/L) = (C_{std} - C_{test}) \times \frac{D.F}{Time}$$

C_{std} =concentration of the stander which is equal to 200 $\mu M/L$.

D.F= dilution factor which is equal to 10.

Time = 5 minutes.

2.2.3. Assay the activity of serum catalase (64).

Principle

Catalse activity is determined by the decrease in absorbance due to hydrogen peroxide (H_2O_2) consumption.

Reagents

1- Phosphate buffer solution (50 mM, pH 7.0). The solution has been prepared as follow:

   A- Potassium dihydrogen orthophosphate (KH_2PO_4) (50 mM). This solution has been prepared by dissolving 6.81 gm of KH_2PO_4 in small amount of distilled water, and then the toltal volume has been completed to 1 L.

   B- Disodium hydrogen orthophosphate (Na_2HPO_4) (38.772 mM). This solution has been prepared by dissolving 6.9 gm of Na_2HPO_4 in small amount of distilled water, and then the toltal volume has been completed to 1 L.

   The phosphate buffer has been prepared by mixing 390 ml of solution A and 610 ml of solution B. The pH is adjusted to 7.0.

2- Hydrogen peroxide (H_2O_2) (30mM).

   Fresh solution has been prepared by diluting 0.34 ml of 30% H_2O_2 with phosphate buffer to 100 ml.

Procedure

1- 50 µl of serum has been diluted with 5 ml of phosphate buffer solution (50 mM), pH 7.0 immediately prior to assay.

2- Two set of tubes have been prepared as follow:

Reagents	Sample	Blank
Phosphate buffer solution	-	1 ml
Diluted serum	2 ml	2 ml
H_2O_2	1 ml	-

The reaction is started by adding hydrogen peroxide (freshly prepared), then all tubes have been mixed immediately and initial absorbance after 15 seconds (t_1) and the final absorbance after 30 seconds (t_2) have been read at 240 nm.

Calculation

Enzyme activity in units (U), which is expressed as the rate constant of first reaction (K), has been used according to the following equation:

$$K\ (K/ml) = \frac{V_t}{V_s} \times \frac{2.3}{\Delta_t} \times \log\frac{E_1}{E_2} \times 60$$

K= Rate constant

Δ_t= ($t_2 - t_1$) and it is equal to 15 seconds.

E_1= the initial absorbance at 15 seconds.

E_2= the final absorbance at 30 seconds.

V_t=the total assay volume.

V_s= the sample volume in assay mixture.

2.2.4 Assay the activity of *N*-Acetyl-*ß*-*D*-glucosaminidase.

Colorimetric assay for quantization of NAG enzyme in serum is assayed by using the commercially kit.

Principle

The 2-Methoxy-4-(2'-nitrovinyl)-phenyl-2-acetoamido-2-deoxy-ß-D- glucopyrinoside (MNP-GlcNAc) is hydrolysed by *N*-Acetyl-ß-D-glucosaminidase with the release of 2-Methoxy-4-(2'-nitrovinyl)-phenol, which on the addition of alkaline produces a color which can be measured at 505 nm.

Reagents

1- The substrate which is a yellow solution containing MNP-GlcNAc in 0.005M HCL.
2- The Incubation buffer which is a mixture of citric acid monohydrate and dipotassium hydrogen phosphate.
3- Colour developing buffer which is a solution of potassium carbonate and potassium hydrogen carbonate pH 9.5.
4- Calibrant which is a freeze dried partially purified sample of bovine kidney NAG of defined activity.

Procedure

Four sets of tubes have been prepared as follow:

Reagent	Reagent blank	Sample blank	Calibrant	Sample
Distilled water	50 µl	750 µl	-	-
NAG calibrant	-	-	50 µl	-
Sample	-	50 µl	-	50 µl

Reaction solution	750 µl	-	750 µl	750 µl
Incubation for 30 minutes at 37 C° and then add:				
Color developing buffer	250 µl	250 µl	250 µl	250 µl
The four tubes have been mixed and read at 505 nm after 10 minutes.				

Calculation

$$\text{Activity } (\mu\text{mol/h/L}) = \frac{S \times (OD_{SA} - OD_{RB} - OD_{SB})}{OD_{ST} - OD_{RB}}$$

S=activity of NAG calibrant.

OD_{SA} =sample absorbance.

OD_{SB}=sample blank absorbance.

OD_{ST}=calibrant absorbance.

OD_{RB}=reagent blank absorbance.

CHAPTER THREE

RESULTS

AND

DISCUSSION

3.1. Activities of GPx, CAT and NAG and their correlation MDA concentration in sera of urolithiasis patients.

The current study has revealed significant changes in concentration of MDA and activities of GPx, CAT and NAG in sera of urolithiasis patients in comparison to their levels in sera of controls. Concentration of MDA and activity of NAG have been increased significantly, while sera activities of GPx and CAT have shown a significant decrease in their levels (table 3.1).

Table 3.1. Concentration of MDA and activities of GPx, CAT and NAG in sera of urolithiasis patients and controls.

Serum Biochemical Test	Patients Mean±SD	Controls Mean±SD	P value
MDA (µmol/L)	7.01±2.51	3.25±1.08	0.0000[a]
GPx (U/L)	61.79∓42.64	188.8∓42.13	0.0000[b]
Catalase (K/ml)	0.13∓0.07	0.28∓0.08	0.0000[c]
NAG (µmol/h/L)	1152.72±390.24	356.36±146.78	0.0000[d]

[a] The mean difference is significant at 0.05 level, P value $=4\times 10^{-11}$.

[b] The mean difference is significant at 0.05 level, P value $=3\times 10^{-22}$.

[c] The mean difference is significant at 0.05 level, P value $=5\times 10^{-13}$.

[d] The mean difference is significant at 0.05 level, P value $=6\times 10^{-17}$.

The study has revealed no correlation of MDA concentartion to activities of GPx, CAT and NAG in sera of urolithiasis patients, also it have revealed no correlation of NAG activity to activities of GPx and CAT in sera of urolithiasis patients (table 3.2).

Table 3.2. The correlation of NAG activity to MDA concentration and activities of GPx and CAT, and the correlation of MDA concentration to activities of GPx and CAT in sera of urolithiasis patients.

The correlated parameters	NAG (μmol/h/L)		MDA (μmol/L)	
	Correlation coefficient	P value*	Correlation coefficient	P value*
MDA (μmol/L)	-0.02	0.89	----	-----
GPx (U/L)	-0.02	0.88	0.19	0.14
CAT (K/ml)	-0.12	0.37	0.03	0.78
NAG (μmol/h/L)	------	----	-0.02	0.89
* The mean difference is examined at 0.05 level.				

The result of a significant increase in MDA concentration has cohered with previous studies (50,65). Other workers have detected a significant increase in MDA concentartion in plasma, erythrocytes (65) and urine (65,66) of urolithiasis patients. However, this elevation has been supported by experimental rat studies which have been reported an elevation in lipid peroxidation in induced calcium oxalate nephrolithiasis rats administered sodium oxalate (67). 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 (68). 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 (44).

The activity of GPx in urolithiasis is controversy. It has been revealed that erythrocyte GPx activity has shown a significant reduction in urolithiasis by some workers (66), while others have revealed no significant changes (30). Glutathione peroxidase is one of the most important lines of defense 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 (69) so; the reduction in GPx activity can be explained due to the consumption of antioxidant enzyme by increasing the lipid peroxidation.

The current study has revealed a significant decrease in CAT activity. One study has been done on human in India, which has revealed a significant increase in CAT activity in haemolysate of urolithiasis patients (38), while the studies which have been done on experimental rats shown a significant increase in CAT activity in kidney homogenates (31), also the renal epithelial cells (LLC-PK1) have shown a decrease in its cellular activity of CAT after exposure to oxalate (36). 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 (67).

The correlation of MDA concentration to antioxidant enzymes activities is indistinct, because the previous studies have revealed a negative correlation (70), positive correlation (71), or no significant correlation (72), although these studies have been done in diseases other than urolithiasis, but it indicated the correlation of MDA concentration to antioxidant enzymes is variable. According to the current study, there

have been insignificant positive correlation of MDA concentration to GPx and CAT activities in sera of urolithiasis patients, but this may suggested a cause and effect relationship, i.e. if oxidative stress developed, then increase in level of antioxidant try to nullify the effect (73).

N-Acetyl-ß-D-glucosaminidase (lysosomal enzyme) is released into serum from cells by exocytosis or from the breakdown of cells (74). Previous studies have been revealed that NAG activity influenced by oxidative stress (59,60), but in this study no correlation have been found between NAG activity and MDA concentration and activities of GPx or CAT in sera of urolithiasis patients. However, another mechanism can explain the high activity of 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 (75). In rat aortic muscle cells the lysophosphatidylcholine (Lyso-PC) increases the expression of monocyte chemoattractant protein-1 (MCP-1). The MCP-1 is induced in renal cells following exposure to oxalate ions or to calcium oxalate crystals (76) and its expression has been associated with inflammatory responses in a variety of kidney diseases (77) including perhaps, the inflammation produced by crystal deposition in stone disease (76, 78).

3.2. Distribution of urolithiasis patients according to age and the influence of age and gender on the biochemical parameters

The mean age of urolithiasis patients has been 44.07 years (44.86 years of control group).

Table 3.3. The sampling characteristics of study groups.

Gender	Patients (n=60)	Controls (n=28)
Male (N0.)	30	16
Female (N0.)	30	12
Age (mean∓SD)	44.07 ∓ 13.8	44.86 ∓ 8.4
P value*	0.8	
*The difference in mean of age between patients and controls at level 0.05.		

Analysis of age groups has shown a high occurrence of urolithiasis in the age group 31-40 (30%), and the occurrence had started to decrease with progress of age (figure 3.1). There had been no difference in concentration of MDA and activities of GPx, CAT and NAG among age groups of urolithiasis patients (table 3.4).

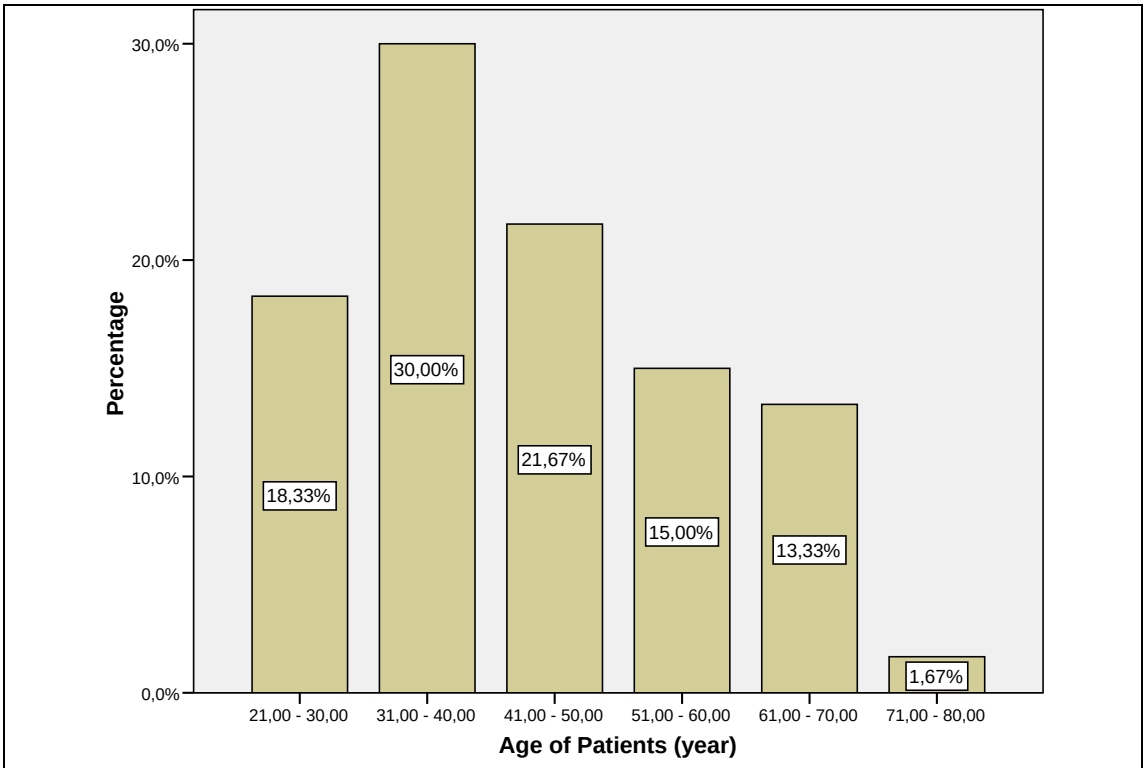


Figure 3.1. Distribution of urolithiasis patients according to the age and the percentage of each age group.

Table 3.4. Sera MDA concentration and activities of GPx, CAT and NAG among the age groups distribution of urolithiasis patients.

Age group (year)	MDA* (µmol/L)	CAT* (K/ml)	GPx* (U/L)	NAG* (µmol/h/L)
21-30	7.98±2.99	0.11±0.05	60.4±53.6	1166.6±369.9
31-40	6.86±2.46	0.13±0.05	59.8±42.9	1142.1±363.1
41-50	6.83±2.39	0.13±0.06	58.0±31.3	1100.7±503.3
51-60	6.79±2.51	0.10±0.04	71.6±54.4	1262.0±386.5
61-70	7.45±2.46	0.18±0.16	52.4±18.6	1122.7±375.2
71-80**	5.71	0.21	148.8	1123.5
P value	0.70	0.40	0.31	0.96

*The values are expressed as mean±SD and p value at 0.05 level of significance.
**This group has fewer than 2 cases.

The concentration of MDA and activities of GPx, CAT and NAG have shown no difference between male and female patients, but the difference in male or female have been significantly differ from their levels in controls (the results are similar to these in general) (table 3.5).

Table 3.5. Sera MDA concentration and activities of GPx, CAT and NAG in male and female urolithiasis patient and controls.

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}	
GPx (U/L)	Patient	64.55∓42.72	59.03∓43,11	0.62
	Control	200.93∓45.26	172.66∓32.63	0.08
	P value**	0.0000^{e}	0.0000^{f}	
NAG (µmol/h/L)	Patient	1130.2∓425.3	1175.23∓357.65	0.65
	Control	342.1∓170.2	375.26∓112.71	0.56
	P value**	0.0000^{g}	0.0000^{h}	

*P value of mean difference in serum biochemical variables 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] $p=4\times10^{-6}$ [b] $p=3\times10^{-6}$ [c] $p=1\times10^{-12}$ [d] $p=1\times10^{-5}$

[e] $p=4\times10^{-13}$	[f] $p=4\times10^{-10}$	[g] $p=8\times10^{-9}$	[h] $p=3\times10^{-9}$

The environmental, geographical, occupational and nutritional factors are known to affect epidemiology of urolithiasis (11). 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 (79). 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 (80). Oxalate the major stone forming constituent has been reported to induce free radical generation, which results in peroxidative injury to renal epithelial cells (66).

In this study, the changes in sera concentration of MDA and activities of GPx , CAT and NAG have not been influenced by the age of patients with urolithiasis. Absence of the correlation between antioxidant capacity and age according to this study is coherent with a previous study (81), but not with other which has reported a correlation of age to CAT activity but not to GPx activity (38). The concentration of MDA have been reported to be influenced by age in several studies (82,83), but others have been reported no correlation between MDA concentration and age (85,86). Also serum NAG activity has been reported to be age dependent (86), but not by other (57).

The difference in MDA concentration between male and female have been studied by other studies and shown no difference in its mean in the plasma between male and female (87). While GPx and CAT activities have been reported by some workers to have higher activity in women than in men (88-90) (these difference can be due to chance or to gender-related differences in life-style such as intake of dietary supplements (68)), but other studies have reported no difference between male and

female (89,91). These differences can be explained by environmental and nutritional factors. Serum NAG activity has known to be of no difference in its level between male and female and this has been coherent with several studies (56, 86).

3.3. The correlation of stone number, stone size, and duration of urolithiasis disease to MDA concentration and activities of GPx, CAT and NAG.

The current study has revealed a higher percentage of urolithiasis have had one stone (figure 3.2).

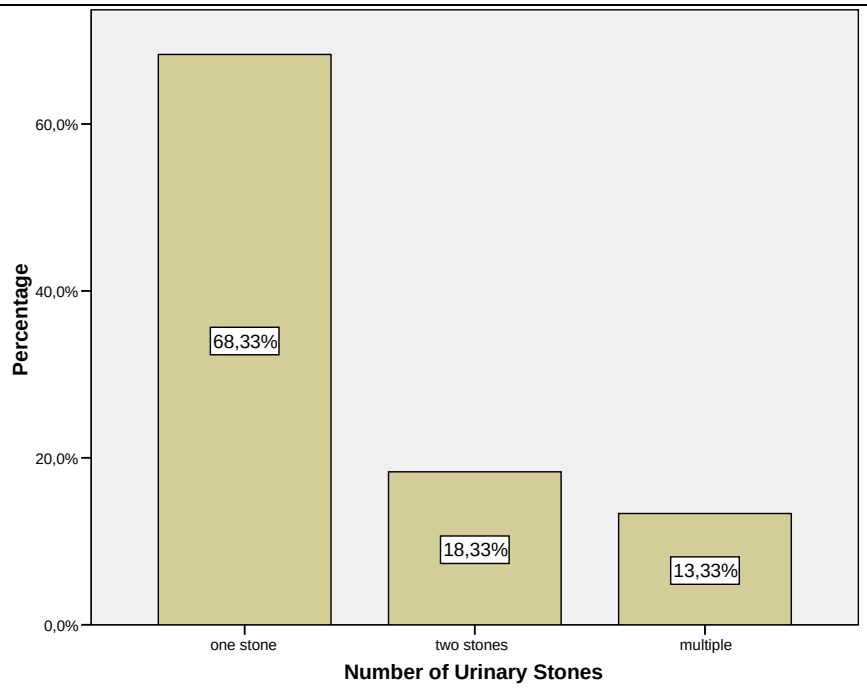


Figure 3.2. Distribution of urolithiasis patients according to the number of stones.

However, there have been no differences in MDA concentration and activities of GPx, CAT and NAG in sera of patients with one stone, two stones and multiple stones (table 3.6).

Table 3.6. Sera MDA concentration and activities of GPx, CAT and NAG among urolithiasis patients with one stone, two stones and multiple stones.

Serum biochemical test	One stone	Two stones	Multiple stones	P value**
MDA(µmol/L)*	7.39±2.6	6.20±1.9	6.15±2.0	0.22
GPx (U/L)*	66.44±47.9	58.50±28.1	42.48±22.5	0.34
CAT (K/ml)	0.13±0.08	0.12±0.05	0.11±0.05	0.75
NAG (µmol/h/L)*	1219.3∓384.6	1051.7∓325.5	949.9∓444.7	0.12
*The values express mean ± SD. ** The mean difference is significant at 0.05 level.				

The urolithiasis patients have been grouped into three groups according to the size of the stones (≤ 4, 4-7 and >7 mm). The highest stone size group (71.11%) has been >7 mm stone (figure 3.3).

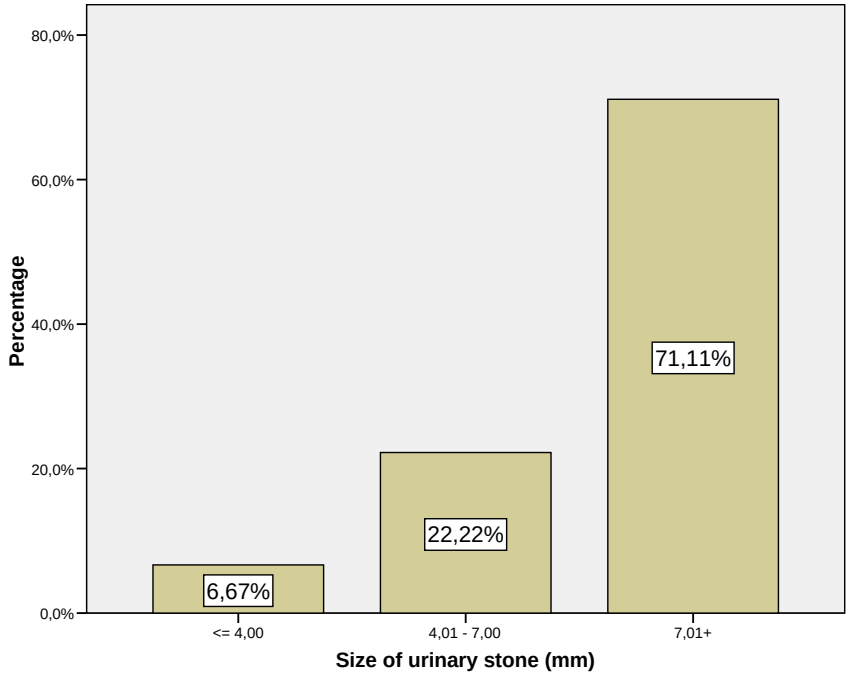


Figure 3.3. Distribution of urolithiasis patients according to the size of the stones.

The mean size of urinary stone in patients with one stone has been 11.65 mm and it revealed no significant correlation with the mean sera MDA concentration and activities of GPx, CAT and NAG of those patients (r=0.1, -0.05, -0.01 and 0.03, and p>0.05 for all). In addition, there have been no difference in mean MDA concentration and activities of GPx, CAT and NAG among patients with one stone, two stones and multiple stones, so the mean size of stones in patient with more than one stone have been taken and the results revealed that sera MDA concentration and activities of GPx CAT and NAG have shown no significant correlation with the size of urinary stone (table 3.7).

The duration of a disease symptoms represents the time when the patient aware the symptoms of urolithiasis till presentation. The mean duration of urolithiasis has been 7 months. There has been no correlation between the duration of urolithiasis symptoms and sera MDA concentration and activities of GPx, CAT and NAG (table 3.7).

Table 3.7. The duration of urolithiasis symptoms and the size of urinary stone and their correlation to sera MDA concentration and activities of GPx, CAT and NAG

Variables	Mean±SD	Correlation			
		MDA (µmol/L)	GPx (U/L)	CAT (K/ml)	NAG (µmol/h/L)
Duration of the disease symptoms (months)*	7 ± 8.2	r= 0.009 p= 0.95	r= -0.093 p= 0.48	r=-0.09 p=0.46	r=0.07 p= 0.59
Size of stone (mm)**	11.15±6.14	r= 0.01 p= 0.9	r= 0.005 p= 0.9	r= 0.08 p=0.6	r= -0.01 p= 0.9

*The minimum recorded duration of urolithiasis has been 2 days and maximally 36 months.
**The minimum recorded size of stone has been 4mm and maximally 30 mm.
r; correlation coefficient.
p; the level of significance is 0.05.

The radio-opaque stones (apart of staghorn calculus which are excluded) are usually multiple (13). The current study has recorded 68.3% of urolithiasis patients have had one stone. This might be overestimated, because the KUB, and IVU can miss small stones, and US can miss ureteral stones (The most sensitive imaging modality for the diagnosis of renal, ureteral, and bladder calculi is CT, which can detect stones as small as 1 mm in diameter) (12). Classification of urinary stones according to the size is useful for decision of treatment, since 80% of stones ≤4mm can expected to pass spontaneously, ≤7mm the chance of spontaneous passage is very low, and >7mm is indication for active stone removal (6). So, the highest percentage regarding the stone size of >7mm can be explained by the difficulty to pass spontaneously and the low sensitivity of KUB and IVU to detect small size stones. No correlation has been detected between the size of the stone and duration of the disease and MDA concentration and activities of antioxidant enzymes and NAG.

3.4. Recurrence and family history of urolithiasis and their correlation to sera concentration of MDA and activities of GPx, CAT and NAG.

The current study has revealed that higher percentage of urolithiasis patients have had recurrent stones (Figure 3.4). The concentration of MDA and activities of GPx, CAT and NAG have shown no significant difference in sera of patients with first episode and recurrent urolithiasis.

No family history of urolithiasis has presented in higher percentage among patients with urolithiasis (Figure 3.4). Also, sera concentration of MDA and activities of GPx, CAT and NAG have shown no significant difference concerning negative and positive family history of urolithiasis (table 3.8).

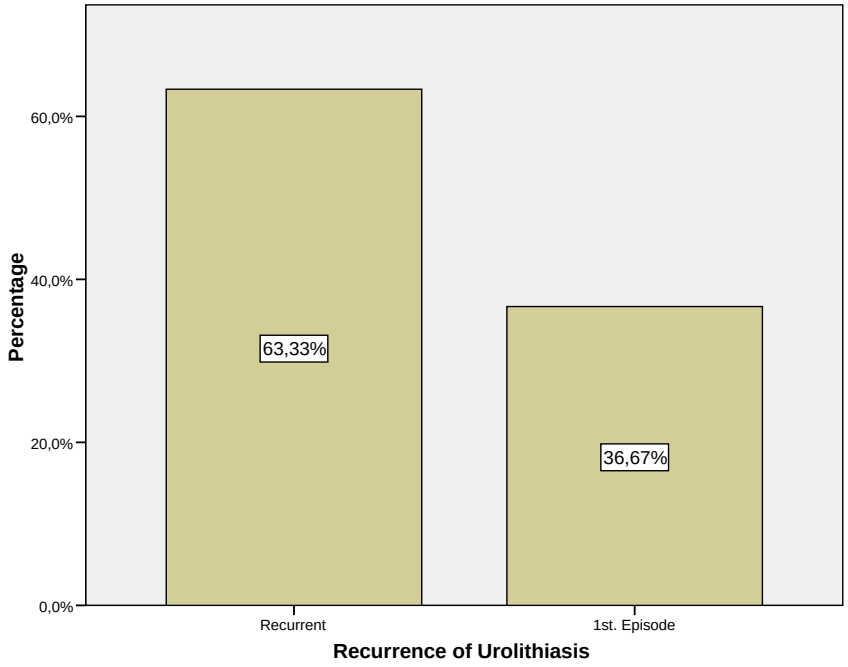


Figure 3.4. Distribution of urolithiasis patients according to the recurrence or first episode of the disease.

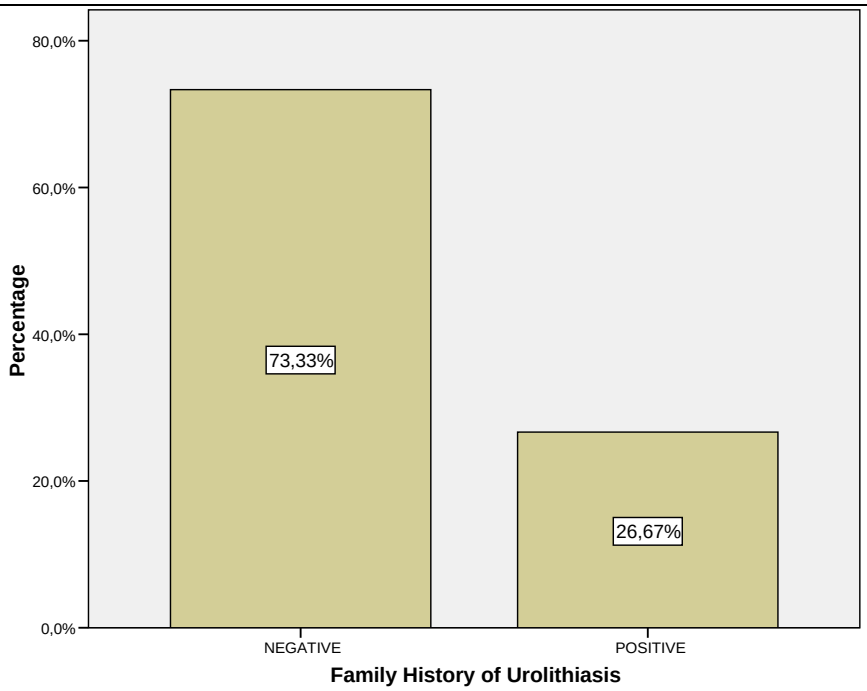


Figure 3.5. The percentage of family history of urolithiasis disease in patients with urolithiasis.

Table 3.8. Sera MDA concentration and activities of GPx, CAT and NAG of urolithiasis patients according to recurrence and family history of urolithiasis.

Serum biochemical variable		Recurrence of urolithiasis		Family history of urolithiasis	
		1st.episode	Recurrent	Negative	positive
MDA (µmol/L)	Mean±SD	7.63±2.83	6.64±2.33	6.7∓2.4	7.67±2.7
	P value*	0.14		0.22	
GPx (U/L)	Mean±SD	66.4±49.6	59.09±38.4	59.3±39.3	68.5±51.4
	P value*	0.52		0,46	
CAT (K/ml)	Mean±SD	0.12±0.05	0.13±0.09	0.12±0.08	0.14±0.04
	P value*	0.94		0.49	
NAG (µmol/h/L)	Mean±SD	1097.5∓ 430.9	1184.6∓ 366.9	1204.4∓ 357.4	1010.5∓ 450.9
	P value*	0.4		0.08	
*The difference in mean at p=0.05					

Recurrent stone formation in the urinary tract is a common and important problem that must be considered in daily urological practice. With a prevalence of >10% and an expected recurrence rate of 50%, stone disease has an important effect on the healthcare system (18).

It has been reported no significant difference between MDA concentration of recurrent stone formers and first episode stone formers (65), which has cohered to the result of this study.

According to this study, a positive family history of urolithiasis has been found in 26.67% of patients. Although, this percentage has been higher than the percentage (11.5%), which has been recorded by a previous study done in Baghdad in 2005, but this suggests that either a genetic or an environmental factor is important in stone formers (79). Strong family history of stone formation is regarded as a risk factor of recurrent stone formation (12).

3.5. Anatomical distribution of urinary stone and its correlation to sera concentration of MDA and activities of GPx, CAT and NAG.

The patients have been grouped into five groups according to anatomical site of the stone in consideration on unilateral or bilateral stones. Renal stones (unilateral or bilateral) have been found in 70.17% of patients and ureteric stones in 19.30%. Details of the anatomical distribution of the urinary stones are given in figure 3.6.

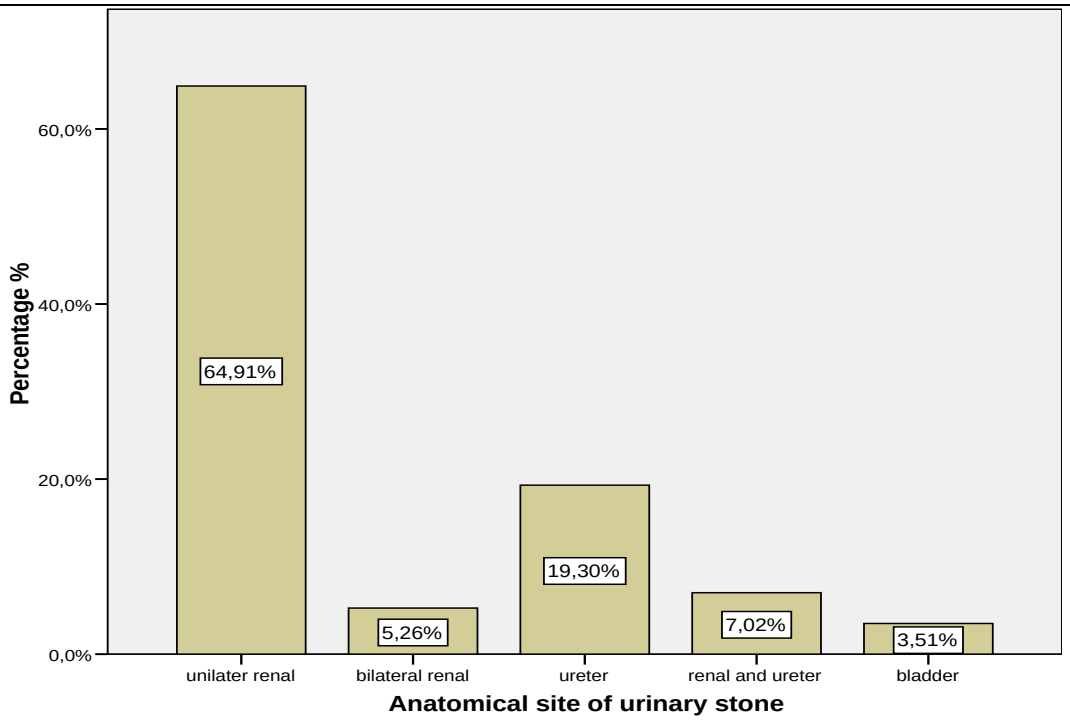


Figure 3.6. Distribution of urolithiasis patients according to the anatomical site of stone and percentage of each group.

Another anatomical distribution of urinary stone has been identified regardless of the unilateralist or bilateralism to restrict the exact correlated anatomical site to sera concentration of MDA and activities of GPx, CAT and NAG, so the patients have been grouped into four anatomical positions. These have been included the stone in renal calyx, renal pelvis, ureter, and urinary bladder .This anatomical distribution has revealed no significant difference in concentration of MDA and activities

of CAT and NAG among urinary stone in renal calyx, renal pelvis, ureter, renal calyx and ureter and urinary bladder (figure 3.7-3.9).

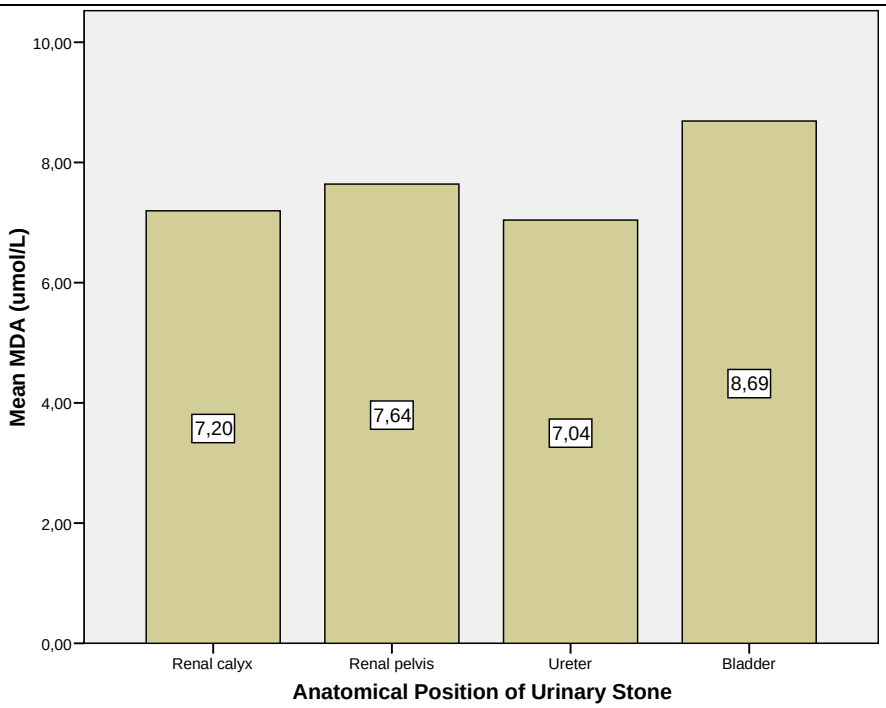


Figure 3.7. Mean MDA concentration in sera among the anatomical positions of urinary stone (renal calyx, renal pelvis, ureter, and urinary bladder).

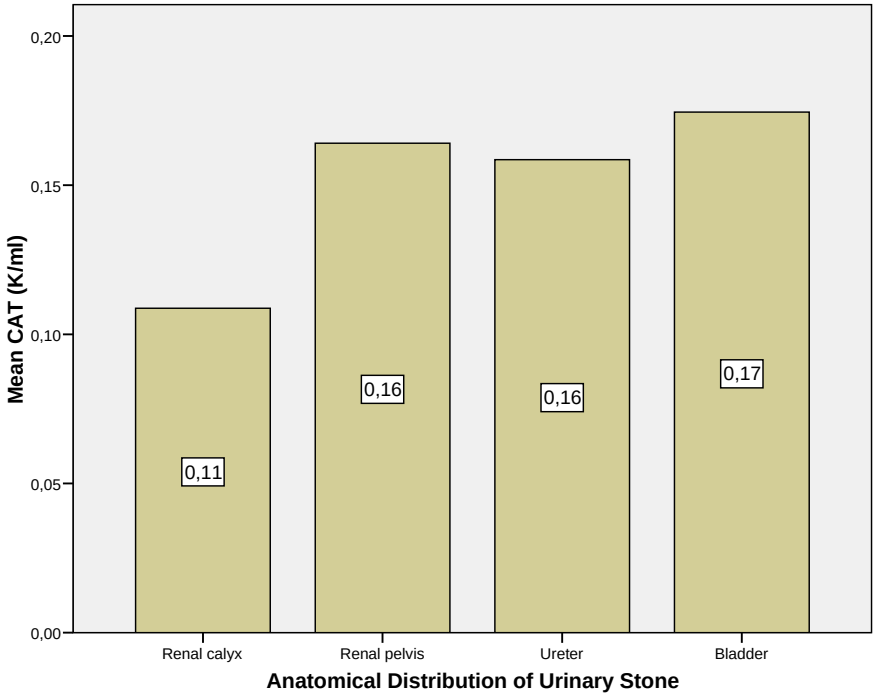


Figure 3.8. Mean CAT activity in sera among the anatomical positions of urinary stone (renal calyx, renal pelvis, ureter, and urinary bladder).

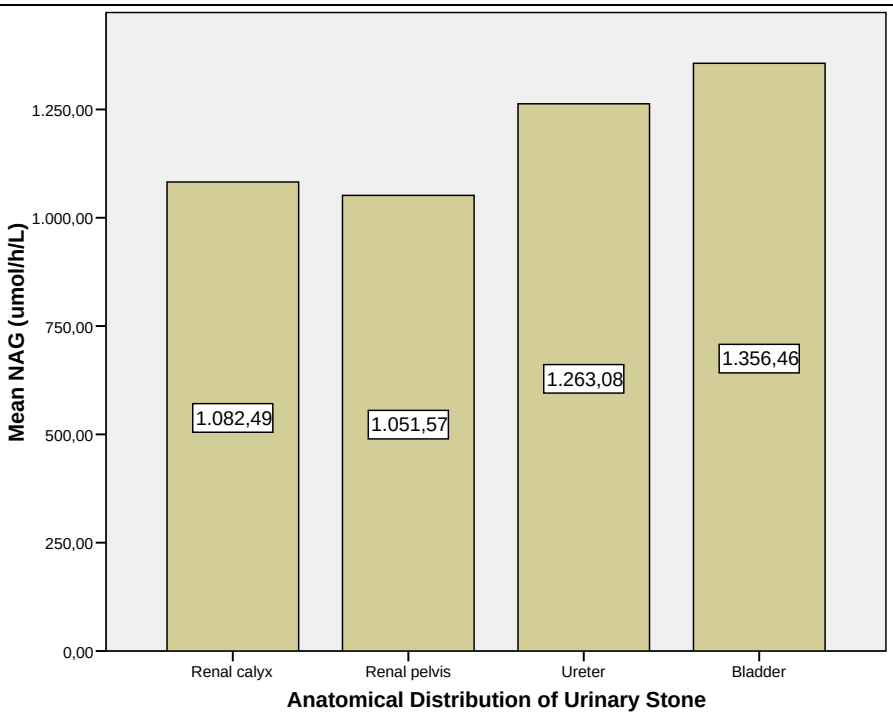


Figure 3.9. Mean NAG activity in sera among the anatomical positions of urinary stone (renal calyx, renal pelvis, ureter, and urinary bladder).

The activity of GPx has shown differences among the four anatomical positions of urinary stone. There has been a significant difference in GPx activity in seraof uretreic stone patients in comparison to its level in sera of renal calyceal and renal pelvic stone patients. Also, the difference in mean of GPx has presented significantly in comparison of vesicle stone to renal pelvic and renal calyceal stones (figure 3.10). The multiple comparison of MDA concentration and activities of GPx, CAT, and NAG according to anatomical stone position are shown in table 3.9.

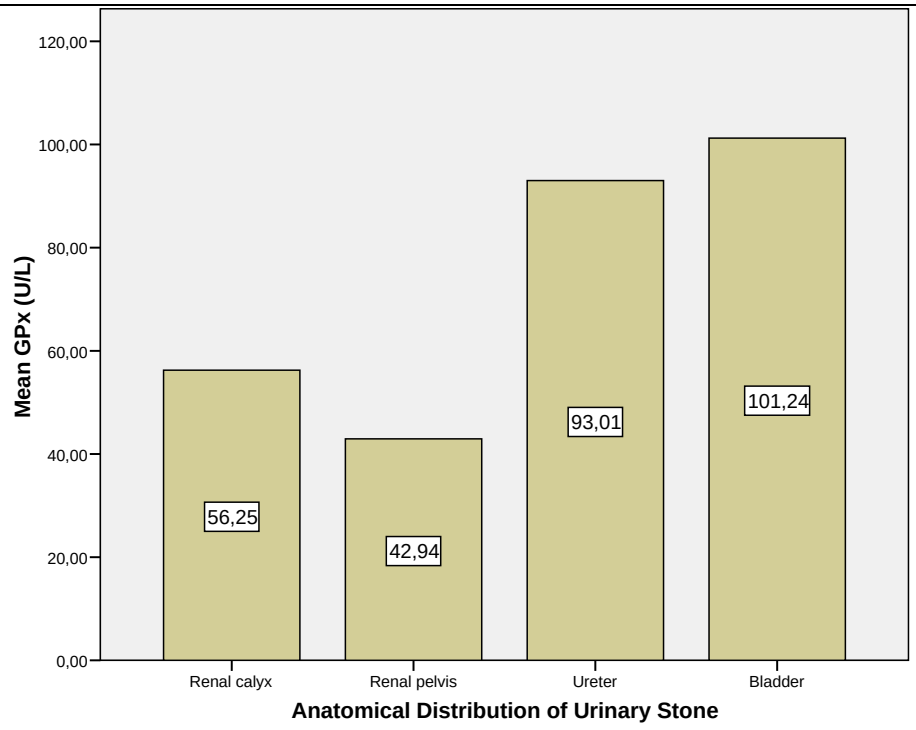


Figure 3.10. Mean GPx activity in sera among the anatomical positions of urinary stone (renal calyx, renal pelvis, ureter, and urinary bladder).

Table 3.9. Concentration of MDA and activities of GPx, CAT and NAG in sera of urolithiasis patients according to anatomical position of stone(s).

Dependent Variable	(I) anat.position	(J) anat.position	Mean Difference (I-J)	Sig.
MDA	Renal calyx	Renal pelvis	-,44533	,658
		Ureter	,15398	,869
		Bladder	-1,49311	,289
	Renal pelvis	Renal calyx	,44533	,658
		Ureter	,59931	,611
		Bladder	-1,04778	,507
	Ureter	Renal calyx	-,15398	,869
		Renal pelvis	-,59931	,611
		Bladder	-1,64709	,285
	Bladder	Renal calyx	1,49311	,289
		Renal pelvis	1,04778	,507
		Ureter	1,64709	,285
CAT	Renal calyx	Renal pelvis	-,05533	,076
		Ureter	-,04979	,085
		Bladder	-,06576	,128
	Renal pelvis	Renal calyx	,05533	,076
		Ureter	,00554	,877
		Bladder	-,01043	,828
	Ureter	Renal calyx	,04979	,085
		Renal pelvis	-,00554	,877
		Bladder	-,01597	,732
	Bladder	Renal calyx	,06576	,128
		Renal pelvis	,01043	,828
		Ureter	,01597	,732
GPx	Renal calyx	Renal pelvis	13,31259	,383
		Ureter	-36,75582*	,012
		Bladder	-44,98664*	,038
	Renal pelvis	Renal calyx	-13,31259	,383
		Ureter	-50,06840*	,007
		Bladder	-58,29922*	,018
	Ureter	Renal calyx	36,75582*	,012
		Renal pelvis	50,06840*	,007
		Bladder	-8,23082	,723
	Bladder	Renal calyx	44,98664*	,038
		Renal pelvis	58,29922*	,018
		Ureter	8,23082	,723
NAG	Renal calyx	Renal pelvis	30,91137	,839
		Ureter	-180,59804	,204
		Bladder	-273,96986	,199
	Renal pelvis	Renal calyx	-30,91137	,839
		Ureter	-211,50940	,238
		Bladder	-304,88122	,204
	Ureter	Renal calyx	180,59804	,204
		Renal pelvis	211,50940	,238
		Bladder	-93,37182	,686
	Bladder	Renal calyx	273,96986	,199
		Renal pelvis	304,88122	,204
		Ureter	93,37182	,686

*The difference is significant at level 0.05

The sequence of the anatomical sites percentage of urinary stone distribution in this study has cohered to a previous study (79). The concentration of MDA and activities of CAT and NAG have shown no difference among the anatomical site of urinary stone, while GPx activity has done. The activity of GPx has shown significant difference among anatomical position of urinary stones. The lowest GPx activity has been in renal pelvic stone and the highest in vesicles and ureteric stones, which it has indicated that the renal pelvic stone has the more consumption of GPx. Although, no difference in MDA concentration among the anatomical sites of the stone, but the oxidative pool in those patients may contain free radicals other than MDA that have a correlation to these anatomical site. Another possible mechanism that the selenium dependent glutathione peroxidase has five members, one of them is directly synthesized and secreted from proximal tubule cells of kidney (92).

3.6. Effect of smoking on MDA concentration and activities of GPx, CAT and NAG in sera of urolithiasis patients.

The higher percentage of patients with urolithiasis has been non smokers as shown in figure 3.11.

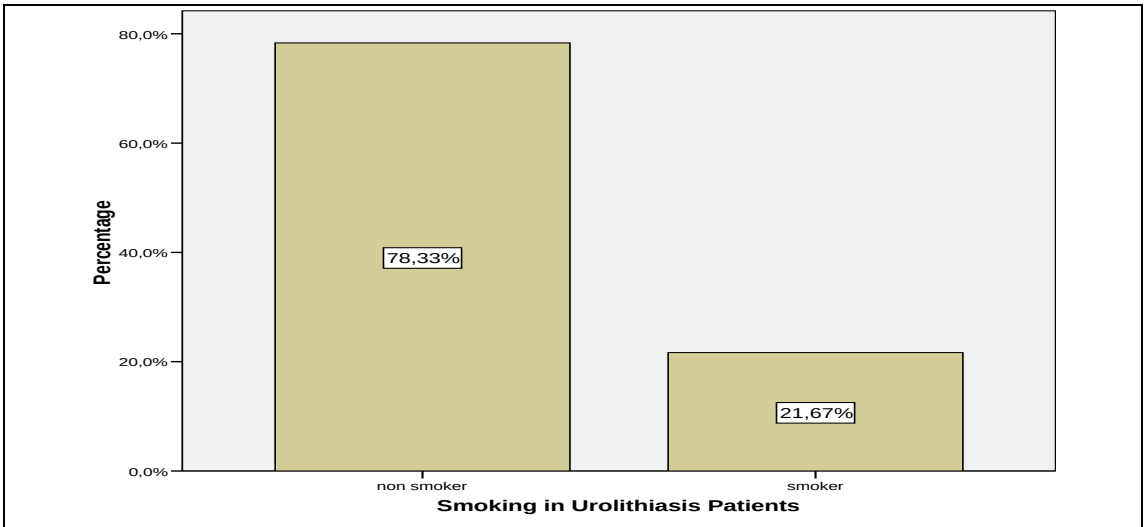


Figure 3.11. Percentage of smoking among urolithiasis patients.

There has been no difference in MDA concentration and activities of GPx, CAT and NAG between smokers and non smokers patients with urolithiasis (table 3.10).

Table 3.10. Concentration of MDA and activities of GPx, CAT and NAG in sera of smoker and non smoker patients with urolithiasis.

Biochemical variable	Smokers	Non smokers	P value**
MDA (µmol/L)*	7.3±2.3	6.9±2.6	0.6
GPx (U/L)*	68.07±47.2	60.05±41.7	0.5
CAT (K/ml)*	0.13±0.05	0.13±0.08	0.9
NAG (µmol/h/L)*	998.2±420.9	1195.5±374.8	0.1
*The values are expressed as mean ± SD. **The difference in mean at p=0.05.			

The current study has revealed that no effect of smoking on neither MDA concentration nor activities of GPx, CAT and NAG in sera of 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 MDA concentration and activities of GPx, CAT and NAG are due to urolithiasis as a cause or as a result.

Conclusions and Recommendations

Conclusions

- Patients with urolithiasis have associated with increase free radicals generation which have been observed by increasing MDA concentration and decrease in the activities of GPx and CAT in sera of patients with urolithiasis.
- *N*-acetyl-*ß*-D-glucosaminidase activity has increased in sera of urolithiasis patients, but it has been not influenced by oxidative stress.
- Age of urolithiasis patients and their gender, recurrence of urolithiasis, family history of urolithiasis, number of urinary stones, and size of stones have no effect on oxidative stress and NAG activity in sera of urolithiasis patients, but the anatomical site of urinary stone has affected glutathione peroxidase. However, it can be concluded that oxidative stress may affected the mechanism of stone formation rather than the disease itself.
- Smoking have no effect on the oxidative stress and NAG activity in sera of urolithiasis, in addition to the exclusion of diseases that known to be affect oxidative stress, it is concluded that the changes in MDA concentration and activities of GPx, CAT and NAG have been due to urolithiasis.

Recommendations

- Further studies dealing with the activity of superoxide dismutase isoenzymes and antioxidant vitamins in urolithiasis patients.
- Further studies dealing with serum NAG activity in urolithiasis patients to establish its site of release, its beneficial study to detect early complication, and comparison the results with 24-hour urinary NAG which is an indicator of proximal renal tubular injury.

- The role of antioxidants in urolithiasis patients particularly vitamin E as a preventive measures of urinary stone can be studied.
- Studies dealing with the isoenzymes of GPx activities in urolithiasis patients and detecting their different sites of release, to know which one is related to the disease.
- Further studies dealing with the role of lipid peroxidation and antioxidant enzymes in different types of urinary stones according to stone analysis and if they have the same role in radio-opaque stone.

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