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Research Article

Total Antioxidant Capacity, Lipid Peroxidation, Thiol Group and Catalase Activity in Patients With Kidney Stone

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Abstract

Background: Urinary tract stones are one of the most common causes of kidney disease. There is evidence for the possible involvement of oxidative stress in the formation of kidney stones and renal cell injury.
Objectives: In this study, we aimed to determine the total antioxidant capacity (TAC), malondialdehyde (MDA), serum thiol group, ceruloplasmin (CP) levels and catalase (CAT) activity in the serum of the patients with kidney stones.
Materials and Methods: This study was conducted as a case-control study. A total of 31 patients (16 males and 15 females) with kidney stone(s) and a comparative normal control group including 21 (12 males and 9 females) healthy subjects were included.
Results: The present study revealed that TAC level was significantly higher in the control group ($P=0.004$), and the mean thiol group was remarkably decreased in the patient group ($P<0.001$). Moreover, CAT activity decreased in the patients but it was not significant ($P=0.23$). On the other hand, the findings showed that the serum CP level significantly increased in the patient group ($P<0.001$). MDA level also increased in the patient group, although this increment was not significant ($P=0.87$).
Conclusions: It can be concluded that the reduction of antioxidant indices in the patients with kidney stones can damage the renal tubular cells and strengthen the accumulation of crystals and the formation of kidney stones.
Keywords: Kidney stone, Oxidative stress, Total antioxidant capacity, Ceruloplasmin

Background

Urinary tract stone is the most common cause of kidney disease and poses a remarkable health care burden on adults. Recent studies have shown that the prevalence of kidney stones is increasing, and that 1 out of every 11 Americans have kidney stones. On the other hand, global statistics have shown that the number of people with kidney stones has almost doubled in the last 15 years (1). Several factors are effective in the development of kidney stones including sex, race, age, climate, nourishment, and genetics (2,3). Recent studies have also shown that nutrition, lifestyle factors, and metabolic factors are instances of the most important factors involved in developing kidney stones (3,4). In economically advanced countries, 70% of the total kidney stones contain calcium oxalate or phosphate (3). The disease is more common among the people aged 30-60 years and, more common to men than to women (3).

The formation of kidney stones is multifactorial pathogenesis, and there are several stages in the formation of stones in the renal tubes (5). Among various mechanisms for the formation of kidney stones, the damage of tubular cells and thus facilitation of the crystallization of sediment could be considered as the most possible mechanism (6,7). Numerous studies have determined that damage to tubular cells could be caused by the presence of oxalate and calcium oxalate crystals (3). However, it is known that damage to renal epithelial cells in the patients with kidney stones could be due to oxidative stress (8). Oxalate-induced membrane damage is promoted by lipid peroxidation (LPO) and oxidative stress, which is a degradative process due to the presence of reactive oxygen species (ROS) (8,9). LPO could be assayed by measurement of serum malondialdehyde (MDA) levels in the patients with renal stones. In addition, various studies have reported that the vicinity of kidney epithelial cells with different crystals leads to the production of ROS (8). On the other hand, ROS through

damage to and chemical changes on proteins, lipids, and carbohydrates, alter the kidney function. Association of calcium stone formation and the reduction of antioxidant levels have been suggested (10). To avert cell damage caused by ROS, aerobic cells are enriched with chemical and enzymatic antioxidant defense systems. One of the important antioxidant enzymes is catalase (CAT), which catalyzes the conversion of H_2O_2 to H_2O and supports the body against oxidative stress (11). The antioxidants such as glutathione (GSH) may play a key role against the renal tubular cell damage caused by oxidative stress, through the diminution of the ROS accumulation in the kidney. Ceruloplasmin (CP) is an acute phase response protein. It is an abundant, blue plasma protein that carries approximately 95% of total circulating copper in a healthy, human adult (12), however, pro-oxidant activity of CP is not known. Similar to Cu, CP is related to inflammation and oxidative stress and has both antioxidant and pro-oxidant effects (13).

During the reduction of antioxidant levels, ROS production leads to the reduction in the activity of suppressor molecules in crystalline formation; thereby, elevating crystalline formation in the kidney (14). Generally, evidence suggests that oxidative stress and ROS are possibly involved in damage to renal cells and in kidney stone formation.

Objectives

Few studies have been done in this area, mainly on laboratory animals or in vitro. The aim of this study was to evaluate oxidative stress in the patients with kidney stone. To this end, we measured total antioxidant capacity (TAC), LPO, serum thiol group of protein, and serum CP level in the patients with kidney stones.

Materials and Methods

This study was conducted as a case-control study. A total of 31 patients (16 males and 15 females) with kidney stone(s) as determined by clinical examination, history, paraclinical tests and renal ultrasonography who had referred to Shahid Beheshti Hospital of the Hamadan University of Medical Sciences, Hamadan, Iran, were included. The mean age in the subject group was 37.8 ± 4 years and maximum and minimum age of this group was 51 and 31 years, respectively. The comparative normal control group included 21 (12 males and 9 females) healthy subjects, age matched without renal stone symptoms, living in Hamadan, Iran. The mean age in the control group was 36.4 ± 3.4 years and the maximum and the minimum age of this group was 42 and 29 years, respectively. Exclusion criteria for both groups included smoking, chronic underlying diseases such as hypertension, heart disease, hyperlipidemia, diabetes, renal failure and body mass index (BMI) greater than or equal to 30. Written informed consent was obtained from all participating subjects. This study was approved by the Research Ethics Committee of the Hamadan University of Medical Sciences, Hamadan, Iran.

It should be noted that the height and the weight of the participants were measured, and BMI of patients was calculated using the following formula:

Normal: 24.9>BMI>18; skinny: 18>BMI; over weight: 29.9>BMI>25; obese: BMI >40 (15).

Blood Analysis

Blood samples were collected after 8 hours of fasting and then serum was separated. For this purpose, we used centrifugation at 2500 rpm. We measured TAC, CAT activity, LPO, and serum levels of CP.

TAC measurements were performed using the FRAP method. This determination is based on the ability of the serum in reducing the Fe^{3+} (ferric) ions to Fe^{2+} (ferrous) in the presence of TPTZ. The TPTZ-Fe^{2+} complex is a blue compound with a maximum absorption at the wavelength of 593 nm (16).

Catalase activity was determined based on the decomposition of hydrogen peroxide into H_2O and O_2. Absorption decrement was measured in the 240 nm (17).

To measure the LPO, we used thiobarbituric acid (TBA). In this method, MDA as the most important LPO product was measured. MDA reacted with TBA in acidic and high temperature conditions and the absorbance of the complex was measured at 535 nm (18).

Measurement of thiol group of serum (thiol group of protein and glutathione) was performed using the HU method. 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB or Ellman's reagent) was used and absorbance of yellow complex was determined at 412 nm (19).

We measured total CP with the colorimetric method using o-Dianisidine dihydrochloride. To determine the serum level of CP, we used oxidative activity, in which o-Dianisidine dihydrochloride acts as a substrate. CP catalyzed the oxidation of substrate and the purplish-red product was measured in a spectrophotometric assay at 540 nm (20).

Statistical Analysis

Statistical analysis was performed using SPSS software version 16.0. To determine the significance and compare the oxidative stress indices, the mean difference between 2 independent groups, independent *t* test was used and the significance level was considered to be <0.05.

Results

As shown in Figure 1, the results indicated that the mean thiol level remarkably decreased in the patient group

in comparison with the control group ($P<0.001$). The findings also showed that the MDA level increased in the patient group compared to the control group, but this increase was not statistically significant ($P=0.87$).

Table 1 shows the results of serum CP, TAC levels and CAT activity for the patient and healthy groups. The serum CP level significantly increased in the patient group ($P<0.001$). Moreover, the results of the present study showed that CAT activity decreased in the patient group compared to the control group, though this decrease was not statistically significant ($P=0.23$).

As shown in Table 1, comparison of the mean TAC between the groups revealed that the mean TAC was significantly higher in the control group compared to the patient group ($P=0.004$).

Discussion

An imbalance between pro-oxidants and anti-oxidants is defined as oxidative stress (21). Different cells of the body use different antioxidant systems, including enzymes, vitamins and various elements to cope with oxidative stress conditions. Under the conditions of oxidative stress, the amount of free radicals produced by the oxidation-reduction (redox) reactions greatly increases in the body. Free radicals damage the intracellular proteins and other macromolecules and oxidize them. In addition, ROS as a mediator leads to renal endothelial cells dysfunction with damage to extracellular matrix and other proteins, eventually resulting in glomerulosclerosis (hardening of the glomerulus in the kidney) and endothelial dysfunction (22, 23).

The present study showed that the CP level in the patient group was higher in comparison to the healthy control group. It was shown that CP increases as an acute phase protein in urine of the individuals with kidney stones. The results of this study were consistent with the study of Wright et al who reported that the level of serum CP increased in the patients with kidney stone (24). CP as an acute phase protein has an important mechanism in inflammation, but the role of CP in the generation of kidney stones is not well distinguished. Nonetheless, it has been seen in various in vitro studies that CP promotes crystallization of calcium oxalate (25). In agreement with the results of the present study, Howles et al reported that CP increased in the subjects with kidney stones (25). CP which is synthesized in the liver can have pro-oxidant as well as antioxidant activity in vitro (13). Recent studies reported high level of CP in oxidative stress condition, infection and in diseases such as diabetes mellitus, cancer and cardiovascular disease, though its pro-oxidant activity in oxidative stress is not well understood. Several studies demonstrated that CP increases in oxidative stress and exhibits potent oxidant activity in vitro (13,26-28).

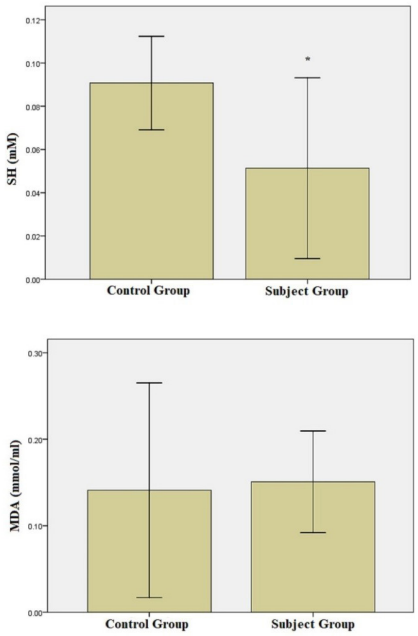

Figure 1. Comparison of Thiol Group (SH) and Malondialdehyde Levels Between Control Group (n = 21) and Patient Group With Renal Stone (n = 31); * $P<0.001$

Table 1. Comparison of Ceruloplasmin, Total Antioxidant Capacity and Catalase Levels Between Healthy Subjects (n = 21) and Patients With Renal Stone (n = 31)

	Groups	
	Control	Subject
CP (U/L)	105.9 ± 48	181.8 ± 83[a]
CAT (U/mL)	3.92 ± 0.58	2.09 ± 1.8
TAC (µmol/mL)	0.56 ± 0.29	0.39 ± 0.14[a]

Abbeviations: TAC, total antioxidant capacity; CP, ceruloplasmin; CAT, catalase.
Results are presented as mean ± SD; [a] $P<0.05$ compared with control group.

In this study, oxidative stress caused the reduction of protein thiol group (protein-SH) level in the patients with kidney stones. In addition, GSH is a free radical scavenger which is directly reduced duo to oxidative stress and decline in its level can exacerbate the damage caused by ROS and oxidative stress conditions. On the other hand, decrease in GSH concentration is a remarkable alteration in the antioxidant defense system (29). Tungsanga et al reported that oxidative stress and tubular damage was directly linked to the formation of kidney stones and the GSH level remarkably decreased in the kidney stone patients compared to the controls (8).

LPO is one of the important events caused by oxidative stress. Determination of serum MDA level was used to assess the LPO. A direct association between LPO and renal stone formation was shown. Carrasco et al reported

that LPO was significantly higher in the subjects with kidney stones compared to the subjects without kidney stones (30). In a study conducted by Kato et al, results showed that MDA levels were higher in the subjects with kidney stones (31). The results of the present study showed MDA levels were higher in the subjects with kidney stones compared to the healthy subjects, though this increase was not significant.

The findings of the current study showed that the level of TAC and CAT activity in the subjects with kidney stones was lower than those in the healthy subjects; however, decline in CAT activity was not statistically remarkable. A decline in the antioxidant defense may initiate the oxidative stress in the body. Decrease of antioxidant defense in the individual with kidney stone supports the hypothesis that oxidative stress may play an important role in the pathophysiology of renal stone formation. The study conducted by Gomathi et al revealed that potassium oxalate accumulation in the kidney is directly related to the reduction of CAT activity (32). Ceban et al reported decrease of antioxidant capacity in the patients with kidney stone which is consistent with the results of the present study (33). TAC indicates the antioxidant state of the body, which reduces in oxidative stress condition. Loss of balance between the pro-oxidants and antioxidants results in TAC reduction (34). Under oxidative stress conditions, the produced free radicals lead to tubular cells damages and accelerate the formation of renal stones. On the other hand, resistance of renal cells to the accumulation of crystals could be reduced by oxidative stress, resulting in the reduction of the levels of antioxidant agents and activities of antioxidant enzymes. Finally, the oxidative stress status exacerbates and the crystals deposition elevates, eventually leading to kidney stones formation (10).

Conclusions

It can be concluded that the reduction of antioxidant indices and the increase of pro-oxidant indices in the patients with kidney stones indicate an imbalance between the pro-oxidant and antioxidant levels. Oxidative stress conditions may damage renal tubular cells and strengthen the accumulation of crystals and the formation of kidney stones.

Authors' Contributions

Designed the study and contributed to the critical revision: FR, HT, SHM, AR; wrote the article: HG; Contributed to sample collection and performing experiments: FR, THS.

Conflict of Interest Disclosures

None.

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