State of acute phase markers and oxidative stress in patients with kidney stones in the urinary tract

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Received 3 August 2011; accepted 21 August 2011

Abstract
Objective: This present study has aimed to assess the state of acute phase markers and oxidative stress in patients with kidney stones. Material and methods: A prospective study was carried out on 100 patients with kidney stones and 25 healthy controls. Albumin, β2microglobulin, gamma-glutamyl transpepsidase, lactate dehydrogenase (LDH), tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) were evaluated as acute phase markers and lipid peroxidation products, superoxide dismutase (SOD) and glutathione peroxidase (GPx) levels acted as oxidative stress markers. Results: An increase in renal cell damage markers as expressed by the β2microglobulin (p = 0.04), albumin (p = 0.004), LDH (p = 0.001) and Gamma glutamyl trans-pepsidasa (p = 0.01) was observed in the patient group. There was a direct correlation between levels of β2microglobulin and stone size (r = 0.3, p = 0.03). The association between stone size and cytokine activation was observed to be stronger in patients with staghorn calculi. In these patients, TNF-α (p = 0.011), IL-1 (p = 0.004) and IL-6 (p = 0.004) were significantly higher. Patients with stones in the urinary tract showed data of significantly higher oxidative stress, expressed as an increase in levels of lipid peroxidation products (p = 0.03) and a decrease in the antioxidant activity of SOD (p = 0.03) and GPx (p = 0.002).

Conclusions: Patients undergoing urolithiasis showed an elevation of acute phase markers, associated with oxidative stress as expressed by an increase in lipid peroxidation products and a decrease in the antioxidant enzyme activity.

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PALABRAS CLAVE
Litiasis renal; Daño glomerular; Estrés oxidativo

Estado de los marcadores de fase aguda y estrés oxidativo en los enfermos con litiasis de la vía urinaria

Resumen
Objetivo: El objetivo del presente estudio es evaluar el estado de los marcadores de fase aguda y de estrés oxidativo en enfermos con litiasis renal.
Material y métodos: Estudio prospectivo en enfermos con litiasis renal. Se incluyeron 100 enfermos y 25 controles sanos. Se evaluaron como marcadores de fase aguda: albúmina, β2microglobulina, gamma-glutamil transpepsidasa, lactato deshidrogenasa, factor de necrosis tumoral alfa, interleucina 1 e interleucina 6 y como marcadores de estrés oxidativo los niveles de liperoxídos, superóxido dismutasa y glutatión peroxidasa.
Resultados: En los enfermos estudiados se apreció un incremento de los marcadores de daño celular renal expresado por la β2microglobulina (p = 0.04), albúmina (p = 0.004), lactato deshidrogenasa (p = 0.001), así como gamma-glutamil transpepsidasa (p = 0.01). Existió una correlación directa entre los niveles de β2microglobulina con el tamaño de la litiasis (r = 0.3; p = 0.03). La asociación entre la extensión del cálculo y la activación de las citocinas se apreció de forma más intensa en los enfermos con cálculos coraliformes en los que las cifras de factor de necrosis tumoral alfa (p = 0.011), interleucina 1 (p = 0.004) e interleucina 6 (p = 0.004) fueron significativamente mayores. Los enfermos con litiasis de la vía urinaria presentaron cifras significativamente elevadas de radicales libres en plasma, expresados como liperoxídos (p = 0.03), que se acompañaron de un descenso en la actividad de las enzimas antioxidantes superóxido dismutasa (p = 0.03) y glutatión peroxidasa (p = 0.002).
Conclusión: Los pacientes con litiasis urinaria presentan una elevación de los marcadores de fase aguda, asociada a un incremento de especies reactivas del oxígeno y un descenso en la actividad de las enzimas antioxidantes.
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Introduction

Urolithiasis is a very common disease whose incidence has increased in recent decades. Estimated prevalence figures range between 4% and 20%, although they vary widely depending on factors such as geography, climate, or diet. In Spain, according to the Registry of the Urolithiasis Group of the Spanish Association of Urology, the prevalence is 4.6%.1

There are studies showing that crystal deposition causes tubular atrophy, interstitial fibrosis, and inflammatory infiltrate, as it has been observed in vitro, where monocytes exposed to crystals secrete tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), and interleukin 6 (IL-6) which could participate in the inflammatory phenomena occurred.2

On the other hand, it has been found that administration of oxalate loads to experimental animals raises urinary markers of cellular damage. The exact mechanism by which cell injury induced by oxalate occurs is unknown, but some experimental studies suggest that free radicals play an important role associated with acute phase proinflammatory cytokines (IL-1, IL-6, and TNF-α).3

Oxidative stress describes a state of damage caused by reactive oxygen species (ROS).4 These are highly reactive compounds formed during the normal metabolism of oxygen in redox processes, which occur in cells when a disruption in the existing balance between prooxidant and antioxidant substances is given in favor of the former.5 One of the consequences of this imbalance is manifested in the formation of lipid peroxides (LPO) in cell membranes, resulting in dysfunction of the same.

While oxidative stress is a well-known mechanism of action in the genesis of cell injury in different pathologies,6 in the context of urolithiasis, the works supporting this hypothesis are mostly experimental, with no clinical studies to date that evaluate together the state of acute phase markers and oxidative stress in patients with urolithiasis.

Material and methods

We performed a prospective study of patients diagnosed with urinary tract lithiasis at the Hospital Universitario Reina Sofia in Córdoba, in the period between April 2009 and December 2010, in which a total of 125 subjects was included, and distributed as follows: 100 patients diagnosed with renal lithiasis (51 men and 49 women) considered ‘study group’ and 25 healthy subjects (11 men and 14 women) used as controls. We excluded patients with acute or chronic pathologies that could alter the markers studied (previous renal insufficiency, hypertension, diabetes, heart disease).

As acute phase markers, the following were evaluated: levels of albumin, β2microglobuline, lactate dehydrogenase (LDH), and gamma-glutamil transpepsidasa (GGT) in urine, and TNFα, IL-1, and IL-6 in serum. As markers of oxidative stress, we measured serum levels of LPO and the activity of the superoxide dismutase (SOD) enzymes and glutathione peroxidase (GPx).

All determinations were performed in the laboratories of the Department of Biochemistry and the laboratory of the Experimental Department of the Hospital Reina Sofia in Córdoba.
The blood samples were extracted after 8 h of fasting. The amount of venous blood sample was from 5 to 10 ml, it was extracted by means of Vacutainer® in tubes with EDTA as anticoagulant and the serum was separated immediately by centrifugation. After that, aliquots were made and the samples were frozen within the first 4 h at −70 °C until their analysis.

A urinalysis was performed in each analysis. Each urine sample was centrifuged at 2000 rpm for 5 min and stored at −20 °C until the performance of the appropriate analytical techniques. These samples corresponded to the entire first morning urine, measured in milliliter, representing a period of urinary excretion of 6–8 h.

The laboratory techniques used for the determination of the parameters were as follows: (a) microalbuminuria (MA) was quantified by the autoanalyzer Beckman CX7 following the turbimetry methods; (b) β2microglobulin was evaluated by the immunosassay method Synelisa B-2-H color; (c) LDH was measured with the biochemical analyzer Olympus AU560; (d) GGT by spectrophotometry (Genzyme®; Kent, UK); (e) lipid peroxidation products, expressed as malondialdehyde acid (MDA) were determined following a modification of the protocol described by Lepage7 by high pressure liquid chromatography (HPLC) using the commercial kit supplied by Bioxytech S.A. (Oxis International Inc., Portland, OR, USA): LPO-586; (f) GPx activity was evaluated by spectrophotometry, depending on the decrease in absorbance at 340 nm due to NADPH oxidation,8 with a commercial kit supplied by BioVision incorporated (Mountain View, CA, USA): Glutathione Assay kit (K264-100); and (g) the SOD by means of SOD Assay Kit-WST (SIGMA), method based on tetrazolium.

Serum concentrations of proinflammatory cytokines were measured using commercial kits from Bio Plex multi assay technology (Bio Rad laboratories, USA). The tests were carried out according to the manufacturer’s instructions.

For the statistical analysis of the data obtained, we used the SPSS (SPSS Inc.) programme version 18.0.0 for Windows (now called PASW Statistics 18). After confirming the adjustment of the sample to a normal distribution, according to the Kolmogorov–Smirnov test, the analyses of the samples were carried out. For comparison of independent means, Student’s t-test was used. For the comparison of qualitative variables, Chi square test with Yates corrections was performed, and for less than 5 observations, Fischer’s test was carried out. The correlations between variables were assessed using Pearson’s test.

Those values whose security level was above 95% (p < 0.05) were considered statistically significant.

The protocol was reviewed and approved by the Ethics and Research Committee of the Hospital Reina Sofía in Córdoba, and consent was obtained from all the participants after verbal and written explanation of the methods used in the study.

Results

The average age of the study group was 48.27 ± 12.9, of which 51 were men and 49 women. In the control group, the mean age was 42.65 ± 12, of which 11 were men and 14 women.

The correlation between the size of the calculus and β2microglobulin levels (r = 0.3; p = 0.03).

General characteristics of lithiasis

The location of the lithiasis within the kidney was 50% in the lower calyx, 32% in the upper, and the remaining 18% in the middle calyx. In 82% of the cases, renal involvement was unilateral, and in 10% they were coralliform calculi. The lithiasis was unique in 75% of the patients and multiple in the remaining 25%.

State of the markers of cell injury and acute phase

In the patients studied, we observed an increase in the markers of renal cell damage in urine expressed by the increase of β2microglobulin (p = 0.04 vs. control), along with high levels of albumin (p = 0.004), GGT (p = 0.01), and LDH (p = 0.011) (Table 1).

We observed a direct correlation between the levels of β2microglobulin and the stone size (r = 0.3; p = 0.03) (Fig. 1). In fact, β2microglobulin levels were significantly higher in patients with coralliform calculi than in the rest (2.874 ± 2.309 μg/l vs. 1.789 ± 1.045 μg/l; p = 0.02). Older patients also had higher figures of β2microglobulin (r = 0.25; p = 0.01) (Fig. 1).

As it occurred with the markers of cell damage, there was a direct correlation between levels of IL-6 in plasma and stone size (r = 0.25; p = 0.01). The association between the volume of the stone and activation of cytokines was observed more intensely in patients with coralliform calculi, in which the TNFα figures (19 ± 12 pg/ml vs. 8 ± 8 pg/ml; p = 0.001), IL-1 (1 ± 0.2 pg/ml vs. 0.25 ± 0.24 pg/ml; p = 0.004), and IL-6 (9 ± 8 pg/ml vs. 4.4 ± 4 pg/ml; p = 0.004) were significantly higher.

State of the oxidative stress markers

The patients with urinary tract lithiasis presented a state of oxidative stress as demonstrated by the increase in LPO
Acute culi such injury, damage. The correlation between the size of the stone and SOD (r = 0.3; p = 0.003).

There were no correlations between the degree of plasma oxidative stress and the cytokines studied. There was only a direct correlation between the levels of LPO and GGT (r = 0.3; p = 0.004).

Discussion

Kidney stones are associated with increased risk of chronic kidney disease; however, the mechanisms by which they are developed are not well defined. The size of the stone may cause further renal cell injury. In the present study, it has been shown that both the patients with larger stones and those with coralliform calculi had a higher renal cell damage expressed by major alterations in β2microglobulin, urinary GGT and LDH.

This increase in β2microglobulin may be due to inability of damaged renal tubules to reabsorb the filtered β2microglobulin. LDH, however, is a high molecular weight protein, so under normal conditions, it is not filtered by the glomerulus, so the increase in urine of LDH levels may also be a test of the dysfunction in the glomerular filtrate. Like the above parameters, the GGT, a membrane enzyme located on the luminal surface of the brush border of the proximal tubule cell, was altered, having proven in previous studies as a very early indicator of kidney damage.9

Among the various pathogenic mechanisms of renal injury, oxidative stress may alter glomerular structure and function mainly due to the effect of ROS on mesangial and endothelial cells.10 The glomerulus is considerably more sensitive to oxidative damage than other segments of the nephron, such as the proximal tubule.11

Table 2 Oxidative stress markers. Comparison study group vs. control group.

<table>
<thead>
<tr>
<th></th>
<th>Control n = 25 Mean ± SD</th>
<th>Study n = 100 Mean ± SD</th>
<th>p</th>
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<tbody>
<tr>
<td>LPO (U/l)</td>
<td>0.10 ± 0.1</td>
<td>0.23 ± 0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>SOD (U/l)</td>
<td>7.9 ± 0.4</td>
<td>4.9 ± 2.9</td>
<td>0.03</td>
</tr>
<tr>
<td>GPx (U/l)</td>
<td>65 ± 39</td>
<td>42 ± 23</td>
<td>0.002</td>
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Figure 2 Box diagrams for LPO values. Group 0: study group; group 1: control group.

Figure 3 Box diagrams for SOD values. Group 0: study group; group 1: control group.
Given the high reactivity of the ROS, living things have developed several efficient mechanisms that enable the stabilization and disposal of them to mitigate their harmful effects. Some of these mechanisms are represented by antioxidant enzymes such as SOD and GPx.

The relation between renal lithiasis and oxidative stress could be corroborated in the patients studied in this work, which had elevated levels of LPO with significant decrease in the activity of the antioxidant enzymes. The activity of the antioxidant enzymes SOD and GPx was significantly higher in the patients with larger stones and coralliform calculi; this could mean an exaggerated antioxidant response to a greater kidney damage.

Apart from oxidative stress, in the glomerular lesions objectified in the patients with lithiasis, inflammatory mediators such as cytokines and chemokines⁵ may also be involved. In fact, in the present study, in addition to significant differences in plasma levels of TNFα, IL-1, and IL-6 between patients and healthy controls, it was shown that they were greater in the patients with larger and coralliform calculi. Several stimuli, including ROS, have been identified as promoters of the inflammatory response that occurs in mesangial cells.¹⁵ The nuclear factor kappaB (NF-κB) is clearly one of the most important regulators of proinflammatory gene expression,¹⁶ and it has also been shown that the ROS can stimulate this activation in mesangial cells,¹⁵ facts that speak for our data.

An example of the existing relation between cytokine and glomerular damage induced by ROS is represented by the TGF-β, which can mediate the increase in glomerular barrier permeability to albumin.¹⁶ Another inflammatory mediator, the IL-1, has shown to have a similar effect in the present study, as there is a relation between the highest levels of IL-1 and the highest microalbuminuria. It has also been documented that the superoxide radical is involved in glomerular cell apoptosis induced by the TNFα.¹⁷ These data suggest that the ROS are involved in the pathophysiology of various types of glomerular lesions, ranging from inflammation to apoptosis and can activate the secretion of proinflammatory molecules and these in turn have harmful effects mediated by these ROS, resulting in a vicious circle that perpetuates the inflammatory response and damage of the renal glomerulus.

In conclusion, the patients with urolithiasis present a state of oxidative stress that is associated with increased proinflammatory cytokines and acute phase markers.

**Funding**

The study has the support of the ‘Pedro Cifuentes Díaz’ prize, awarded in the notification of the Foundation for Research in Urology in 2010.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**