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Multiple Risk Markers for Atherogenesis Associated With Chronic Inflammation Are Detectable in Patients With Renal Stones

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Patients with renal stones are known to be at risk of clinical complications such as cardiovascular disease (CVD), nephropathy, and cancer. Recently, it has been realized that almost all risk markers for CVD, nephropathy, etc. are all markers associated with the sequence of reactions of chronic inflammation. It has been reported that chronic inflammation is involved not only in the pathogenesis of nephrolithiasis but also contributes to the development of clinical complications in this condition; therefore, we decided to find out whether these multiple markers are detectable in patients with renal stones so that they can be used to predict the risk of clinical complications in these patients. There were 33 patients with nephrolithiasis included in this study. We found that almost all major markers of chronic inflammation were elevated in patients with renal stones, including proinflammatory cytokine, acute inflammation markers, adhesion molecules, urinary microalbumin (uMA), myeloperoxidase (MPO), 8-hydroxydeoxyguanosine (8-OHdG), 3-nitrotyrosine (3NT), and monocyte chemoattractant protein. It appears that it is possible to assess the risk of clinical complications by monitoring these markers in patients with renal stones. J. Clin. Lab. Anal. 21: 426–431, 2007. © 2007 Wiley-Liss, Inc.

Key words: chronic systemic inflammation; atherogenesis; CVD; renal stones; risk markers; oxidative stress

INTRODUCTION

Development of cardiovascular diseases (CVD) (1), chronic kidney disease (2), and cancer (3,4) have been reported in patients with renal stones. Studies have also shown that development of these clinical complications all shared similar risk factors (1,5,6) and are closely associated with chronic systemic inflammation. In fact, a few markers of chronic inflammation and oxidative stress have been detected in patients with renal stones at early stage (6–8). In addition, markers of oxidative and nitrosative stress such as enzymes involved in lipid peroxidation, peroxynitrite and 3-nitrotyrosine (3NT), have all been detected in the rat kidney due to nephrolithiasis (9,10). Khan (7) has also pointed out from his study that inflammation from renal injury plays a significant role in stone formation, and the stone crystal will further lead to the development of oxidative stress. Apparently, like atherosclerosis (11), both the chronic inflammation and oxidative stress are involved in the pathogenesis and progression of nephrolithiasis.

Individual markers such as interleukin 6 (IL-6) (12), C-reactive protein (CRP) (13), serum amyloid A (SAA)

Abbreviations: hCRP, high sensitive C-reactive protein; CVD, cardiovascular disease; ICAM-1, intercellular adhesion molecule; IL-6, interleukin-6; MPO, myeloperoxidase; ELISA, enzyme linked immunosorbent assay; uMA, urinary microalbumin; MCP-1, monocyte chemoattractant protein; 3NT, 3-nitrotyrosine; 8-OHdG, 8-hydroxydeoxyguanosine; SAA, serum amyloid A; VCAM-1, vascular cell adhesion molecule.

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(14), adhesion molecules (15), urinary microalbumin (uMA) (16), myeloperoxidase (MPO) (17), and 3NT (18) have all been shown independently to be associated with future cardiovascular events such as myocardial infarction (AMI), congestive heart failure, and even stroke. In fact, they have also been shown to be predictors not only for CVD but also other complications such as end-stage renal disease (ESRD). For example, hypertensive or diabetic individuals with microalbuminuria have frequently been found to be at risk for not only the CVDs but also carcinogenesis (19). It was only recently that it was discovered that all these risk markers are also markers associated with the sequence of reactions of chronic inflammation (9,20). Since chronic inflammation plays such an important role in the pathogenesis and disease progression of nephrolithiasis, we decided to find out whether we can detect all the major markers of chronic inflammation and use them for the prediction of clinical complications. In this investigation, we have detected in these patients the elevation of a majority of these markers of chronic inflammation. We have also found that the sensitivity of detection will be improved if multiple risk markers are measured simultaneously.

MATERIALS AND METHODS

Specimens

All specimens, including serum, heparinized plasma, and urine, were collected from 33 patients visiting the emergency room at Chang Gung Memorial Hospital in Taipei, Taiwan with the diagnosis of urolithiasis. There were seven females and 26 males. The average age of these patients was 33 years. All specimens were stored at –70°C and thawed immediately before analyses.

Assays

Most markers were measured with in-house developed enzyme linked immunosorbent assay (ELISA) including CRP with a sensitive assay (21), SAA (22), adhesion molecules (23,24), uMA (16), 8-OHdG (25), MPO (26), 3NT (27), IL-6 (28), and MCP-1 (29). All in-house kits have been compared with commercial kits with good to excellent correlation. Blood creatinine was determined by colorimetric method on a Hitachi 7600 autoanalyzer (Hitachi, Tokyo, Japan).

RESULTS

Acute Inflammatory Markers

To plot all markers of different units side by side on the same scale for easy comparison, all determined values were normalized by dividing by their respective upper cutoffs. Therefore, regardless how different the units are for each marker, the level is elevated when the normalized value is above 1 and is within normal when below 1.

IL-6, CRP, and SAA are all acute inflammation markers, which usually become elevated acutely in response to the insult of inflammatory risk factors (9). As shown in Fig. 1A, highly elevated levels of IL-6, CRP, and SAA (in log scale) were detected in patients with renal stones. Very high elevations were found in both IL-6 and SAA, reaching over 100-fold above their upper cutoffs. CRP was also highly elevated; however, it only reached 50-fold above its upper cutoff.

The percent elevation of various markers is presented in Fig. 1B. Apparently, a considerable percentage of patients with renal stones showed elevated acute inflammatory markers. These results indicate that patients with renal stones are at constant exposure of inflammatory insult, generated by the stone crystal (7). Conceivably, measuring acute inflammation markers may indicate the presence of renal stones and these markers may help assess the success of stone removal.

Adhesion Molecules

Detecting adhesion molecules, including vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule (ICAM-1), and E-selectin, usually follows the appearance of acute inflammatory markers in the coronary artery associated with endothelial dysfunction. In this study, elevation of all three adhesion molecules was detectable in patients with renal stones (Fig. 1). It appears that VCAM-1 had the highest sensitivity in terms of the percent of elevation (36%) in this group of patients. It is interesting to know that Cybulsky et al. (30) have also found that VCAM-1, not ICAM-1, was playing a more dominant role in the initiation of atherosclerosis and is a better marker for early atherosclerosis. Consequently, if cost is a major concern one may only measure VCAM-1 instead of measuring all three adhesion molecules.

uMA

Detection of elevated uMA or microalbuminuria is an indication that the glomerular filtration rate in the kidney is impaired, and it also indicates that there is also a leakage of albumin or other proteins from the blood vessel into urine induced by chronic systemic inflammation (31). In addition, microalbuminuria has been considered as a risk marker for atherogenesis (31). Apparently, microalbuminuria is a downstream event of chronic inflammation and usually takes longer to take place when subjected to chronic inflammation (32). The appearance of elevated uMA is also evidence indicating that inflammation has spread systemically and that renal function was affected. In this study we found a large

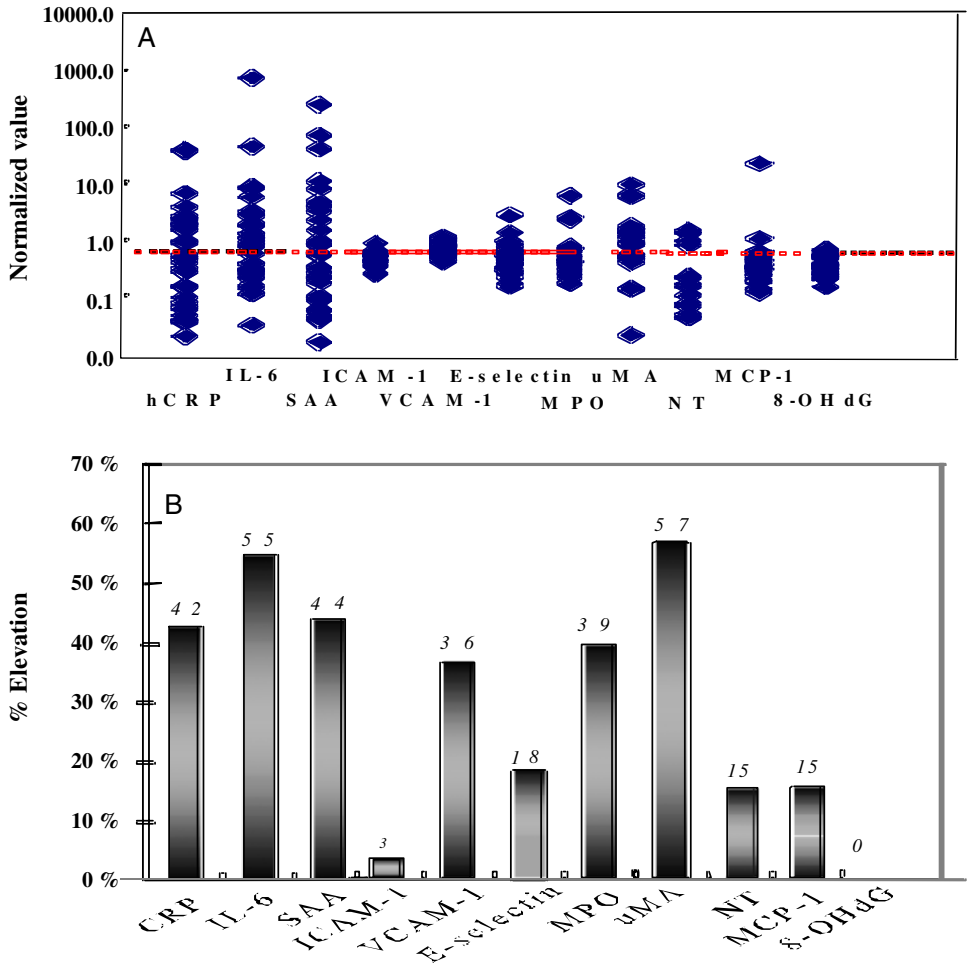

Fig. 1. Elevation of multiple risk markers for atherogenesis associated with chronic inflammation are detectable in patients with renal stones. **A:** Normalized values (determined value/upper cutoff) of all markers measured. The dotted line indicates that normalized value equals to 1. Any value above 1 is elevated (also see Results). **B:** Positive rate in percent elevation of all markers measured. The numeric values of percent elevation are also listed on top of the bar for each marker. Individual upper cutoffs applied in this study: hCRP, 3 mg/L; SAA, 6.8 mg/mL; IL-6, 6.4 pg/mL; ICAM-1, 414 ng/mL; VCAM-1, 769 ng/mL; E-selectin, 91 ng/mL; MCP-1, 480 pg/mL; 3NT, 25.2 nmol/L; MPO, 154 ng/mL; uMA, 19.3 μg/mg creatinine; and 8-OHdG, 58 ng/mg creatinine.

percentage of patients (57%) with renal stones who had microalbuminuria. However, levels of uMA were not highly elevated (Fig. 1A), suggesting that these patients had not developed a severe form of renal dysfunction such as albuminuria (>300 μg/mg creatinine). Since all values of uMA measured in these patients were below 300 μg/mg, apparently these patients were still at the early stage of nephropathy, which is known to be reversible with medications (33). Conceivably, uMA should be monitored in patients with renal stones to prevent progression of microalbuminuria into an advanced stage of renal failure.

Markers of Oxidative and Nitrosative Stress

Chronic inflammation invariably leads to oxidative and nitrosative stress if not treated. MPO and reactive oxygen species released from recruited leukocytes, plus the abundant nitric oxide (NO) generated at the site of inflammation (23), will generate peroxynitrite and promote oxidative and nitrosative stress. Markers such as MPO (26), 8-OHdG (25), and 3NT (27) are all useful to reflect the severity of oxidative stress. In this study, elevated MPO (39%) and elevated 3NT (15%) were all detectable in patients with renal stones. It is not clear why we did not detect any elevation of the marker of oxidized DNA, namely the 8-OHdG. It could be because that all patients with renal stones were not exposed to severe oxidative and nitrosative stress.

Chemokine

MCP-1, a chemokine, is known to be produced in increased concentration by renal epithelial cells

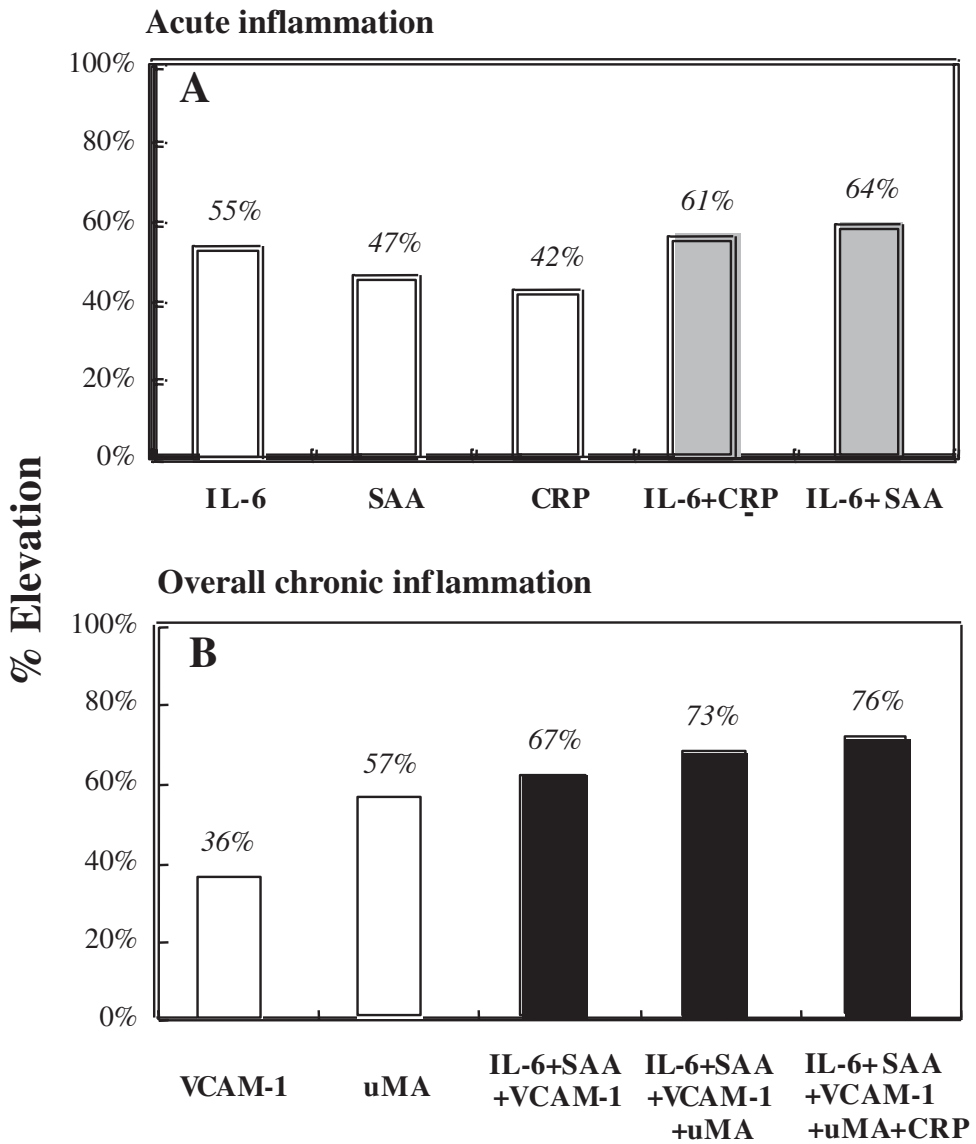

Fig. 2. Improved sensitivity with the measurement of multiple markers. **A:** This figure indicates that sensitivity of detecting acute inflammatory response will be increased by measuring both IL-6+SAA or IL-6+CRP simultaneously. **B:** For overall chronic inflammation, the highest sensitivity can be provided by measuring IL6+SAA+CRP+VCAM-1+uMA.

responding to local inflammation. Injury caused by crystal stimulated inflammation, which serves to attract monocytes and macrophages to the sites of crystal deposition (32). Several earlier studies correlating renal stones with inflammation have detected elevated MCP-1 in patients with renal stones (6,34). In our study, we also found elevated MCP-1 in our group of patients. However, the percent elevation was very low (15%). It appears that MCP-1 has less sensitivity than that of other inflammation markers that we have measured in this study. The appearance of MCP-1 could also be a later event in the inflammation reaction.

Improved Sensitivity With Multiple Markers

For reasons not clear at the present time, very few markers corresponding to various sequential events of overall chronic inflammation had the same sensitivity. None of these individual markers exhibited sufficient sensitivity. We have found in this study that measuring multiple markers simultaneously, such as IL-6+CRP or Il-6+SAA, would increase the percent elevation (Fig. 2A) compared to that of the single marker.

To reflect the overall chronic inflammation we choose to add adhesion molecule VCAM-1 to the panel

of multiple markers. We also found that adding uMA to the panel would further improve the sensitivity of detection (Fig. 2B). Our study indicated that measuring all these five markers simultaneously provided the highest sensitivity (Fig. 2B).

As mentioned above, chronic inflammation will lead eventually to oxidative and nitrosative stress if the inflammation reaction continues to exist. We believe that measurement of MPO, 8-OHdG, and 3NT all at the same time will give the best indication regarding the overall extent of oxidative and nitrosative stress. However, only the group of MPO+3NT needs to be monitored for patients with renal stones because no elevation of 8-OHdG was found in these patients. 3NT is a marker of oxidative stress but is also associated with nitrosative stress (27). Since we did not find any correlation between them, we therefore recommend that MPO and 3NT should be measured at the same time to maximize the sensitivity of detection of oxidative and nitrosative stress.

DISCUSSION

It is interesting to note that almost all risk markers found earlier as independent risk markers for atherogenesis and other degenerative diseases are associated with chronic inflammation and are actually markers associated with the sequence of events of chronic inflammation. Conceivably, chronic inflammation is a major risk factor for CVD and other degenerative diseases (11). Because patients with renal stones were known to be at risk for CVD, etc., and inflammation is also found (6) to play an important role in the pathogenesis and disease progression of renal stones, it was not really surprising to detect these marker of chronic inflammation elevated in renal stone patients. Conceivably, measurement of these markers of chronic inflammation may be useful for predicting the risk of clinical complications in patients with renal stones.

Our results also indicate that chronic inflammation is systematic and plays an important role not only in CVD and type 2 diabetes, but also in renal stones. We believe that these multiple markers are potentially useful for assessing the success of treatment such as stone removal, monitoring the progression of the disease, and predicting their risk not only for CVD but also for other inflammatory diseases such as nephropathy, etc. We need to find out these potential applications in future investigations.

Detection of elevated uMA in 57% of these patients (Fig. 1B) indicates that many patients with renal stones also had impaired renal function. Even though these patients apparently were still at the early and reversible stage of nephropathy, as shown in their low level of uMA, uMA should routinely be monitored to prevent further progression to albuminuria and to end-stage renal disease.

Apparently, reducing inflammation in general is important to prevent not only the development of renal stones but also these additional clinical complications for patients with renal stones. In fact, it has been reported that suppressing inflammation was also beneficial to patients with calculi; antiinflammatory medication (aspirin) (26), vitamin E, and green tea have all been found capable of reducing urinary oxalate excretion and calcium oxalate deposit formation (35–37).

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