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Study the effect of kidney stones on serum xanthine oxidase, ecto-5′-nucleotidase activity and E3 SUMO-protein ligase NSE2 (NSMCE2) in Malaysian individuals

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ABSTRACT

Objective: To verify possible relations between 5′-nucleotidase, xanthine oxidase to E3 small ubiquitin-like modifier-protein ligase non structural maintenance of chromosomes elements 2 in sera patients with kidney stones and to evaluate the possibility of a new biomarker for the evaluation of kidney damage.

Methods: A sixty patients with known kidney stones who appeared the government health clinics in Kuantan–Pahang and fifty apparently healthy were taken as control group. The 5′-nucleotidase, xanthine oxidase and other biochemical parameters were measured by colorimetric tests. The serum NSMCE2 were measured by enzyme linked immunosorbent assay.

Results: The mean serum xanthine oxidase [(39.98 ± 19.70) IU/L] and ecto-5′-nucleotidase activity (40.03 ± 9.53 IU/L) were significantly higher than the controls' levels of (18.04 ± 6.26) and (16.06 ± 4.61) IU/L respectively. There were 85.00% and 83.33%, of patients with kidney stones who had abnormal ecto-5′-nucleotidase activity and uric acid respectively while xanthine oxidase activity was less sensitive 58.33%.

Conclusions: The present study suggests that the increase in serum of xanthine oxidase,ecto-5′-nucleotidase activities E3 small ubiquitin-like modifier-protein ligase NSE2 concentration can be used as biomarkers for diagnosis of kidney damage in patients with kidney stone, also in developments of change DNA damage and inflammation disorders in these patients.

1. Introduction

A kidney stone is a small particles, generally made up of calcium crystals, that formed inside the kidney wherever urine collects. The stone commonly reasons different problem till it reach to the ureter, the tube that drains the kidney into the bladder, and reasons an obstacle, decreasing urine from draining out of the kidney and frequently causing severe pain. The stones are made of mineral and acid salts. Frequently, stones form when the urine come to be concentrated, permitting minerals to crystallize and stick together [1].

Small ubiquitin-like modifier (SUMO) proteins are a family of major proteins belongs to the ubiquitin and ubiquitin-like protein family [2]. Similar to ubiquitin, SUMO is produced as soon as the last four amino acids of the C-terminus have been cleaved off to format an isopeptide bond between the C-terminal glycine residue of SUMO and lysine on the target protein. In contrast to ubiquitination, sumoylation does not support the degradation of proteins but instead changes different functional parameters of proteins, depending on the protein substrate in question. These parameters consist of but are not limited to properties such as subcellular localization, protein partnering, and DNA-binding and/or transactivation roles of transcription features [3,4].

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Ecto-5′-nucleotidase (EC 3.1.3.5) catalyzed the hydrolysis of adenosine monophosphate (AMP) to adenosine, and operated as a key to switch on adenosine signaling via the P1 receptors [5]. Serum ecto-5′-nucleotidase has a significant role in regulation of purine nucleotide metabolism, then stimulation of this pathway happens with a depletion of adenosine triphosphate, an inhibitor of this enzyme, and an increase of inosine monophosphate and AMP, substrates for this enzyme. On the other hand, another study indicated that plasma ecto-5′-nucleotidase may not influence intracellular nucleotide degradation [6]. It is an central membrane glycoprotein existing as an ectoenzyme in a different of mammalian cells, hydrolyzes 5′-nucleotides to their corresponding nucleosides [5]. Adenosine has an important role in the kidney, such as preserves the kidney structure and defends from ischemia, arteriolar vasoconstriction in the external cortex and vasodilatation in the cortex and medulla, inhibition of renin release, and important role in electrolyte transport through the proximal tubules [7].

Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and xanthine to uric acid, which performs as a secondary product in a superoxide molecule [8]. The metabolic reactions of xanthine oxidase have a broad effect cellular protection from toxic complexes and also general protection like innate immunity [9]. Uric acid is the finale product of purine metabolism in human. Hyperuricemia can be the result of increased uric acid production or reduced excretion. At all cause for reduction glomerular filtration, tubular excretion or increased reabsorption would result in an elevated serum uric acid [10].

This study aimed to assess the correlation of serum xanthine oxidase and ecto-5′-nucleotidase activities to NSMCE2 patients with kidney stones and assess the possibility of a new biomarker for the evaluation of kidney damage.

2. Materials and methods

2.1. Patients collection and storage of samples

A total of sixty patients with kidney stones and fifty healthy as control were comprised in the study. These patients were hospitalized at government health clinics in Kuantan–Pahang. Five milliliter of blood was collected and it was allowed to clot for 10–15 min. Serum was removed after centrifuged and separated into two parts the first to measure the biochemical parameters and the other part was stored at −20 °C till the time of NSMCE2 assay. The International Islamic University Malaysia, Research Ethics Committee operates in according to Declaration of Helsinki International Conference of Harmonization Good Clinical Practice Guidelines, Malaysia Good Clinical Practice Guidelines and Council for International Organization of Medical Sciences, International Ethical Guidelines, no. IIUM/305/14/11/2/IREC 300 on October 2014.

2.1.1. Exclusion criteria

Patients suffering from major infections like HIV, type 2 diabetes mellitus, diabetic nephropathy, heart disease, history of alcohol intake, taking potent antioxidant, and pregnant females were excluded from the current study.

2.1.2. Inclusion criteria

Patients of kidney stones only attending outpatient department and admitted in the ward of urology, Hospital Tengku Ampuan Afzan, who agreed to participate in the study were included. Medical history, standard physical examination, and test of biochemical parameters (blood sugar, urea, creatinine, protein, albumin, sodium, potassium and chloride) listed in Table 1.

Table 1
Symptoms for and abnormal findings of abdominal ultrasonography (%).

Symptoms	Percent
Kidney stones	100
Abdominal pain	90
General check up	100
Non-specific symptoms	10
Benign prostatic hyperplasia	0

2.2. Determination of serum protein, NSMCE2, ecto-5′-nucleotidase, xanthine oxidase and other biochemical factors

Total protein concentration was determined by Lowry's method [11]. Serum albumin concentration was determined by bromocresol green binding using Randox kit. Serum globulin was measured mathematically from the subtraction albumin concentration from that of total protein. The concentration of albumin divided by the concentration of globulin was expressed as albumin to globulin ratio. Ecto-5′-nucleotidase activity was measured in serum according to Wood and Williams's method [12]. Xanthine oxidase activity was determined by the method of Ackermann method [13]. The serum uric acid and levels were measured by spectrophotometric methods. The serum NSMCE2 were measured by enzyme linked immunosorbent assay (Cusabio Biotech Com.).

2.3. Statistical analysis

Data were analyzed by using a statistics software package (SPSS for Windows v.21.0). All groups showed normal distribution, so that parametric statistical methods were used to analysis the data. One-way ANOVA test was performed. Results are presented as means ± SD.

3. Results

3.1. Comparison of demographic, hematological data and biochemical parameters in studied groups

Two groups were included in this study: Group 1 consisted of sixty men suffering from kidney stones (The mean age of the

Table 2
Demographic, hematological data and biochemical parameters in patients group and control.

Characteristics	Patients group (n = 60)	Control group (n = 50)
Age (year)	52.42 ± 10.19	50.27 ± 8.21
Hb (g/dL)	12.05 ± 1.40	12.88 ± 0.69
PCV (%)	37.45 ± 4.92	38.88 ± 3.00
WBC (1×10^3 cell/mL)	6.88 ± 0.34	6.17 ± 0.44
Serum urea (mg/dL)	40.25 ± 7.57	37.89 ± 8.36
Serum protein (g/dL)	7.65 ± 0.43	7.68 ± 0.32
Serum uric acid (mg/dL)	6.90 ± 0.94*	5.32 ± 0.33
Serum albumin (g/dL)	4.39 ± 0.58	4.61 ± 0.57
Serum globulin (g/dL)	3.26 ± 0.77	3.07 ± 0.67
Albumin/globulin ratio	1.47 ± 0.55	1.62 ± 0.59

Table 3
The mean and standard deviation of serum ecto-5′-nucleotidase, xanthine oxidase and NSMCE2 in patients (n = 60) and control (n = 50) groups.

Group	Ecto-5′-nucleotidase activities (IU/L)		Specific activities (IU/mg)		Xanthine oxidase activities (IU/L)		Specific activities (IU/mg)		Serum NSMCE2 (pg/mL)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Patients group	40.03 ± 9.53*	25.33–59.54	0.53 ± 0.13*	0.30–0.79	39.98 ± 19.70*	12.91–80.56	0.52 ± 0.26*	0.17–1.16	80.41 ± 8.45†	70.04–107.81
Control group	16.06 ± 4.61	5.55–29.77	0.21 ± 0.07	0.07–0.39	18.04 ± 6.26	6.45–30.00	0.24 ± 0.11	0.05–0.44	74.63 ± 4.00	63.80–82.80

*: $P < 0.001$; †: $P < 0.01$.

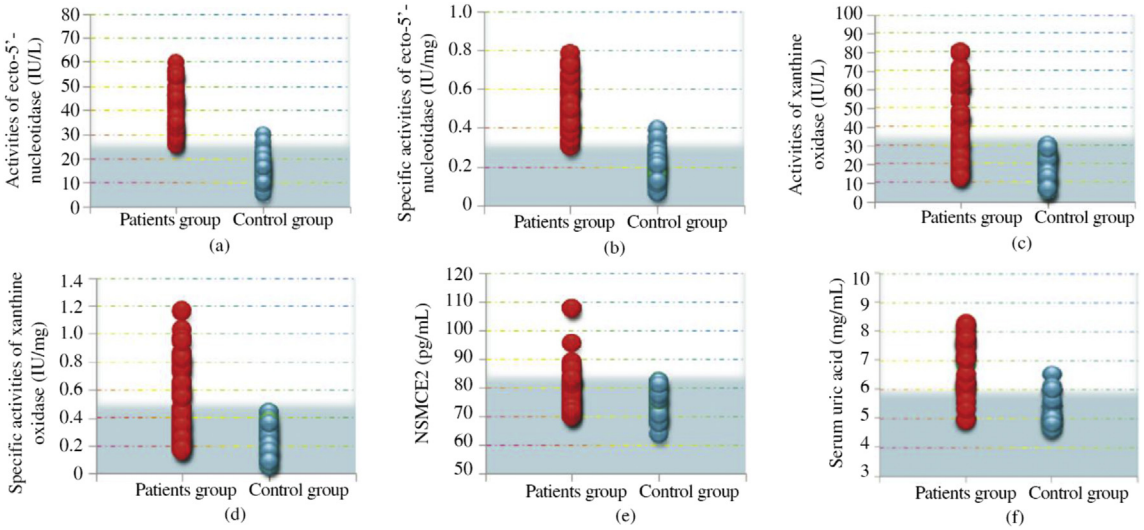

Figure 1. Values of ecto-5′-nucleotidase, xanthine oxidase activities, specific activities (a–d) and NSMCE2, uric acid (e, f) in patients and control group.

Table 4
The sensitivity of the biochemical test for patients with kidney stones.

Test		Sensitivity (%)
Ecto-5′-nucleotidase activity	≥ 25.28 IU/L	85.00
Ecto-5′-nucleotidase specific activities	≥ 0.35 IU/mg	88.33
Xanthine oxidase activity	≥ 30.56 IU/L	58.33
Xanthine oxidase specific activities	≥ 0.46 IU/mg	55.00
Serum uric acid	≥ 5.98 mg/dL	83.33
NSMCE2	≥ 82.63 pg/mL	41.67

Table 5
Correlation between E3 SUMO-protein ligase NSE2 (NSMCE2) with several biochemical parameters in patients and control groups (pg/mL).

Characteristics	Patients group		Control group	
	Pearson correlation	Sig. (2-tailed)	Pearson correlation	Sig. (2-tailed)
Ecto-5′-nucleotidaseactivity (IU/L)	0.71	0.01	0.23	N.S
Xanthine oxidase activity (IU/L)	0.74	0.01	0.19	N.S
Uric acid (mg/dL)	0.62	0.01	0.10	N.S
Globulin (g/dL)	0.70	0.01	0.39	N.S

patients was 52.42 ± 10.19 years), and the second group was the control men with mean age (50.27 ± 8.21) years. All patients underwent kidney ultrasound screening through ultrasonographer. The abdominal ultrasound investigation was done for several reasons (Table 1).

Hematological parameters: Hb, packed-cell volume (PCV) and white blood cell (WBC) count in two groups included in this study according to age were evaluated (Table 2). As the results in Table 2 showed there was a non-significant different ($P > 0.05$) in the mean value of erythrocytes hemoglobin, PCV and WBC account between patients group in comparison with control group. Meanwhile, serum urea level, protein, albumin, globulin and albumin/globulin ratio non-significantly changed ($P > 0.05$) in patients group, when they were compared with control group (Table 2).

3.2. Serum, ecto-5′-nucleotidase, xanthine oxidase, NSMCE2 and other biochemical factors

The activates and specific activities of serum ecto-5′-nucleotidase and xanthine oxidase showed a highly significant increase ($P < 0.001$) in patients group compared to control group, (Table 3 and Figure 1). The present study showed that mean levels of sera NSMCE2 have a significantly increase ($P < 0.01$) in patients group compared to control group as shown in Table 3. Serum NSMCE2 showed increase in 41.67% of patients with kidney stones according to the cut-off value 82.63 pg/mL (Table 4, Figure 1). There were 85.00% of patients with kidney

stones who had abnormal ecto-5′-nucleotidase activity values according to the cut-off value 25.28 IU/L [Cut-off values: values above or below the mean ± 2SD], (Table 4, Figure 1). Also in same table and figure. Xanthine oxidase activity 30.56 IU/L (cut-off value) was less sensitive 58.33%, while serum uric acid 5.98 mg/dL was more sensitive 83.33%.

There were a significant different correlations between NSMCE2 with ecto-5′-nucleotidasea, xanthine oxidase, uric acid and globulin in patients with kidney stones compared to control group (Table 5).

4. Discussion

In the clinic, over 50% of all urological patients are stone patients. Up to the present time, it is unknown how exactly stones form in the kidney system in addition how they can develop to a clinically significant size. Stones may possibly be clinically noiseless for a long time. Though, when they develop beyond a size of spontaneous clearance through the urinary tract, they possibly will cause infection, obstruction, permanent kidney damage, and finally loss of the kidney [14]. There was a significantly increased in serum uric acid level when comparing patients group with control group (Table 2). Numerous works showed the effects of uric acid in kidney cells and hyperuricemic animal models. Firstly, hyperuricemia induces endothelial dysfunction and inflammation [15]. Also, uric acid can induce the contraction [16] and reactive oxygen species produced in mesangial cells [17].

As our knowledge this is the first time the increase in ecto-5′-nucleotidase and xanthine oxidase activities in patient with kidney stones have been described. Another study showed that ecto-5′-nucleotidase is expressed in glomeruli together with mesangial cells, and it can contribute to the generation of adenosine [18]. In the cortical pars recta of the proximal tubule, ecto-5′-nucleotidase staining is little but increases somewhat towards the medullary pars recta of the proximal tubule. In the initially loops of the proximal convoluted tubule, prominent staining of ecto-5′-nucleotidase is obvious in the luminal brush border membrane [19]. The luminal localization of ecto-5′-nucleotidase was difficult in the purine salvage pathway of the filtered or luminal nucleotides releasing that after dephosphorylation may affect luminal adenosine concentrations or by nucleoside carriers taken up in the brush-border membrane [20]. On the other hand, luminal ecto-5′-nucleotidase activities yield variations in adenosine concentrations at the basolateral sites of the tubular epithelium and in the interstitium [21]. The fibroblasts in the interstitium of kidney produce contact with tubular cells, also form a sheath around afferent and efferent arterioles of the glomerulus. Under normoxic conditions the ecto-5′-nucleotidase-positive cells are exclusively located in the cortex and cannot be demonstrated in the medulla. The concentration of fibroblast staining in the cortex alterations in parallel with increased production of erythropoietin. Under normal conditions, the staining is located predominantly in the deep cortex. The staining increases, however, throughout the whole cortex under challenges of hypobaric oxygen breathing or anemia [22]. There were 85.00% of patients with kidney stones who had abnormal ecto-5′-nucleotidase activity values, this finding might be due to a physiologic effects of stones formation on glomeruli and mesangial cells, and it can contribute to the generation of adenosine which may be lead to increase in ecto-5′-nucleotidase activity.

Xanthine oxidase is a ubiquitous complex cytosolic molybdo-flavoprotein which controls the rate limiting step of purine catabolism by converting xanthine to uric acid [21]. Serum xanthine oxidase levels are increased in several pathological states: inflammation, ischemia-reperfusion, aging and atherosclerosis [23]. The reactive oxygen species are involved in oxidative damage. The inhibition of this enzymatic pathway might be beneficial. Excess of uric acid, the metabolic product of xanthine oxidase, can lead to gout [24]. Increased in xanthine oxidase may be leads to increase formation of uric acid and decrease of xanthine thus is present at low concentration in the ureters and urine, and because of its poor solubility precipitates out readily, especially in hot climates where urine volumes are small and urine very concentrated which leads to increased uric acid in blood. We describe the increase in NSMCE2 in sera of patients with kidney stones compared to control group in previous study [25].We found in previous study that the reduction of adenosine aminohydrolase and AMP-aminohydrolase activities could cause a state of immune suppression, also the increase in NSMCE2 may play a role in developments of change DNA damage and inflammation disorders in the patients with kidney stones. In the recent studies indicate a role for sumoylation in the regulation of inflammation. Inflammation is initiated in response to kidney tissue damage. Inflammatory responses must be regulated properly, and unrestricted inflammation can lead to inflammatory disorders in patients with kidney stones. In conclusions the increase in serum of NSMCE2 level, xanthine oxidase and ecto-5′-nucleotidase activities can be used as biomarkers for diagnosis of kidney damage in patients with kidney stone also to evaluate the increase in damage made in the DNA and because of the active role of NSMCE2 in the treatment of damage made in the DNA.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Scales CD Jr, Smith AC, Hanley JM, Saigal CS, Urologic Diseases in America Project. Prevalence of kidney stones in the United States. *Eur Urol* 2012; **62**: 160-5.

[2] Alegre KO. *Structural and functional characterization of the SUMO proteases SENP6 and SENP7* [dissertation]. Barcelona: Universitat Autònoma de Barcelona; 2013.

[3] Gao C, Xiao G, Hu J. Regulation of Wnt/beta-catenin signaling by posttranslational modifications. *Cell Biosci* 2014; **4**(1): 13-8.

[4] Le NT, Corsetti JP, Dehoff-Sparks JL, Sparks CE, Fujiwara K, Abe J. Reactive oxygen species, sumoylation, and endothelial inflammation. *Int J Inflam* 2012; http://dx.doi.org/10.1155/2012/678190.

[5] Zimmermann H, Zebisch M, Strater N. Cellular function and molecular structure of ecto-nucleotidases. *Purinergic Signal* 2012; **8**: 437-502.
[6] Edwards NL, Recker D, Manfredi J, Rembecki R, Fox IH. Regulation of purine metabolism by plasma membrane and cytoplasmic 5′-nucleotidase. *Am J Physiol* 1982; **243**: C270-7.
[7] Grenz A, Osswald H, Eckle T, Yang D, Zhang H, Tran ZV, et al. The reno-vascular A2B adenosine receptor protects the kidney from ischemia. *PLoS Med* 2008; **5**: e137.
[8] Sowndhararajan K, Joseph JM, Rajendrakumaran D. *In vitro* xanthine oxidase inhibitory activity of methanol extracts of *Erythrina indica* Lam. leaves and stem bark. *Asian Pac J Trop Biomed* 2012; **2**: S1415-7.
[9] Shah A, Khand F, Khand T. Effect of smoking on serum xanthine oxidase, malondialdehyde, ascorbic acid and α-tocopherol levels in healthy male subjects. *Pak J Med Sci* 2015; **31**(1): 146-9.
[10] Poude B, Yadav BK, Kumar A, Jha B, Raut KB. Serum uric acid level in newly diagnosed essential hypertension in a Nepalese population: a hospital based cross sectional study. *Asian Pac J Trop Biomed* 2014; **4**(1): 59-64.
[11] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; **193**: 265-75.
[12] Wood RJ, Williams DG. Colorimetric determination of serum 5-nucleotidase without deproteinization. *Clin Chem* 1981; **27**: 464-5.
[13] Ackermann E, Brill A. Xanthine oxidase activity. In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. 2nd ed. U.S.A: Academic Press, Inc.; 1974, p. 521-2.
[14] Buchholz NP, Abbas F, Afzal M, Khan R, Rizvi I, Tlati J. The incidence of silent kidney stones-an ultrasonographic screening study. *J Pak Med Assoc* 2003; **53**(1): 24-5.
[15] Yu MA, Sánchez-Lozada LG, Johnson RJ, Kang DH. Oxidative stress with an activation of the renin-angiotensin system in human vascular endothelial cells as a novel mechanism of uric acid-induced endothelial dysfunction. *J Hypertens* 2010; **28**(6): 1234-42.
[16] Albertoni G, Maquigussa E, Pessoa E, Barreto JA, Borges F, Schor N. Soluble uric acid increases intracellular calcium through an angiotensin II-dependent mechanism in immortalized human mesangial cells. *Exp Biol Med (Maywood)* 2010; **235**(7): 825-32.
[17] Convento MS, Pessoa E, Dalboni MA, Borges FT, Schor N. Pro-inflammatory and oxidative effects of noncrystalline uric acid in human mesangial cells: contribution to hyperuricemic glomerular damage. *Urol Res* 2010; **39**(1): 21-7.
[18] Burke M, Pabbidi MR, Farley J, Roman RJ. Molecular mechanisms of renal blood flow autoregulation. *Curr Vasc Pharmacol* 2014; **12**: 845-58.
[19] Gandhi R, Le Hir M, Kaissling B. Immunolocalization of ecto-5-nucleotidase in the kidney by a monoclonal antibody. *Histochemistry* 1990; **95**: 165-74.
[20] Angielski S, Le Hir M, Dubach UC. Transport of adenosine by renal brush border membranes. *Pflugers Arch* 1983; **397**: 75-7.
[21] Rodriguez-Mulero S, Errasti-Murugarren E, Ballarin J, Felipe A, Doucet A, Casado F, et al. Expression of concentrative nucleoside transporters SLC28 (CNT1, CNT2, and CNT3) along the rat nephron: effect of diabetes. *Kidney Int* 2005; **68**: 665-72.
[22] Agarwal A, Banerjee A, Banerjee UC. Xanthine oxidase: a journey from purine metabolism to cardiovascular excitation-contraction coupling. *Crit Rev Biotechnol* 2011; **31**(3): 264-80.
[23] Jasim NA, Mehdi WA. Correlation between E3 SUMO-protein ligase NSE2 (NSMCE2) with 5′-nucleotidase, XOR, uric acid and total protein in patients with atherosclerosis. *Adv Nat Appl Sci* 2015; **9**(2): 67-75.
[24] Harrison R. Structure and function of xanthine oxidoreductase: where are we now. *Free Radic Biol Med* 2002; **33**: 1913-26.
[25] Yusof F, Mehde AA, Mehdi WA, Ghazali H, Abd Rahman A. The effect of renal stones on serum adenosine aminohydrolase and AMP-aminohydrolase in Malaysia. *Asian Pac J Trop Biomed* 2015; **5**(6): 478-84.