

Original Article



Effects of Nephrolithiasis on Serum DNase (Deoxyribonuclease I and II) Activity and E3 SUMO-Protein Ligase NSE2 (NSMCE2) in Malaysian Individuals*

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Abstract

Objective Nephrolithiasis is one of the most common disorders of the urinary tract. The aim of this study was to examine a possible relationship between DNase I/II activity and E3 SUMO-protein ligase NSE2 in the sera of nephrolithiasis patients to evaluate the possibility of a new biomarker for evaluating kidney damage.

Methods Sixty nephrolithiasis patients and 50 control patients were enrolled in a case-control study. Their blood urea, creatinine, protein levels and DNase I/II activity levels were measured by spectrometry. Serum NSMCE2 levels were measured by ELISA. Blood was collected from patients of the government health clinics in Kuantan-Pahang and fulfilled the inclusion criteria.

Results The result indicated that mean levels of sera NSMCE2 have a significantly increase ($P < 0.01$) in patients compared to control group. Compared with control subjects, activities and specific activities of serum DNase I and II were significantly elevated in nephrolithiasis patients ($P < 0.01$).

Conclusion This study suggests that an increase in serum concentrations of DNase I/II and E3 SUMO-protein ligase NSE2 level can be used as indicators for the diagnosis of kidney injury in patients with nephrolithiasis.

Key words: Nephrolithiasis; DNase I; DNase II; E3 SUMO-protein ligase NSE2; NSMCE2

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INTRODUCTION

Nephroliths are solid pieces of material that form in a kidney when substances normally found in the urine become highly concentrated. Nephrolithiasis is one of the most prevalent conditions of the urinary tract. Most

stones originate within the kidney and proceed distally, creating various degrees of urinary obstruction as they become lodged in narrow areas, including the ureteropelvic junction, pelvic brim, and ureterovesical junction. Location and quality of pain are related to the position of the stone within the urinary tract. Severity of pain is related to the degree

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of obstruction, presence of ureteral spasm, and presence of any associated infections^[1]. The natural history of nephrolithiasis is characterized by recurrence; recurrence rates of 26%-53% after 10 years have been reported^[2].

DNases are commonly divided into two forms, DNase I (EC 3.1.21.1) and DNase II (EC 3.1.22.1), according to their pH optima and metal ion dependencies^[3]. DNase I is a secreted protein detected in serum, saliva, intestinal juice, urine, seminal fluid and lacrimal fluid^[4]. It is a secretory glycoprotein with an endonuclease activity that cleaves double-stranded DNA to yield 5'-phosphorylated polynucleotides. It includes divalent metal ions for catalysis. Distribution studies show increased levels of the enzyme in digestive tissues such as tissues of the parotid, pancreas, submaxillary glands, and the lining of the small intestine. Significant levels of DNase I may be found in the kidney and wherever the enzyme possibly plays a scavenging role^[5].

Deoxyribonuclease II (DNase II, EC 3.1.22.1) is a mammalian endonuclease that catalyzes the hydrolysis of the phosphodiester bonds of DNA to produce 3'-phosphorylated oligonucleotides in acidic conditions without the inclusion of divalent metal ions for catalysis. Activity and mRNA expression of DNase II are observed in numerous tissues^[6]. Huang et al. suggested that DNase II roles as a scavenger. Recent studies have found that DNase II is responsible for DNA degradation within apoptotic cells engulfed by macrophages^[7]. Several studies showed that abnormal DNase levels have been found in many diseases. High serum DNase concentrations were found in patients with renal failure^[8], acute lymphoblastic leukemia^[9], and genitourinary cancer^[10].

Small ubiquitin-like modifier (SUMO) proteins characterize ubiquitin-like proteins^[11]. The SUMOylation process consists of three enzyme reactions^[12]. E3 SUMO-protein ligase NSE2 is a component of the SMC5-SMC6 complex, which is involved in DNA double-strand break repair by homologous recombination. Performances as a E3 ligase mediating SUMO attachment to several proteins for instance SMC6L1 and TRAX, the shelter in complex subunits TERF1, TERF2, TIN2, and TERF2IP, and possibly the cohesion components RAD21 and STAG2. Essential for recruitment of telomeres to PML nuclear bodies. SUMO protein-ligase activity is essential to avoid DNA damage-induced apoptosis by enabling DNA repair, and for

development of APBs in ALT cell lines. Required for parallel chromatid cohesion during prometaphase and mitotic progression^[13-14]. Thus, blood E3 SUMO-protein ligase NSE2 (NSMCE2) and deoxyribonucleases (DNases) may not play solely defensive roles. Rather, these enzymes may also be involved in the pathogenesis of many diseases. In recent scientific literature, there are incomplete and often contradictory data sets on relative activity and content of blood DNases I and II in health and diseases. This possibly reflects insufficient consideration to study of activity of these enzymes and employment of differing methods, which may not always give correct results. This study aimed to assess the correlation of serum DNase I and II activities to NSMCE2 content in patients with nephrolithiasis.

METHODS

Materials

Sixty patients with nephrolithiasis and fifty healthy patients as controls were enrolled in the study. The present study was performed with support from the International Islamic University Malaysia under the Research Management Center grant, project NO. IUM/504/5/29/1. The IUM Research Ethics Committee (IREC) operates in according to Declaration of Helsinki International Conference of Harmonization Good Clinical Practice Guidelines (ICH-GCP), Malaysia Good Clinical Practice Guidelines, and Council for International Organization of Medical Sciences (CIOMS) International Ethical Guidelines, NO. IUM/305/14/11/2/IREC 300 in October 2014. Five milliliters of blood was collected from patients hospitalized at government health clinics in Kuantan-Pahang. Patients' medical histories were recorded and a physical examination was given; tests of biochemical parameters (urea, creatinine, protein) and general urine tests were used for selection and categorization of nephrolithiasis patients and control subjects. Blood samples were allowed to clot for at least 10-15 min. After centrifugation, the serum was divided into two parts; the first part was used to measure the biochemical parameters and the second part was stored at -20 °C until NSMCE2 assay was performed.

Determination of DNase I, II Activities and Total Protein

The activity of serum DNase I was measured by

using a method proposed by Kunitz^[15]. The rate of increase in the absorbance of the sample solution was recorded at 260 nm and 25 °C after 1.5 min. The serum DNase II activity was determined by using a method proposed by Kunitz^[16]. Total protein concentration was determined by Lowry's method^[17].

Determination of Serum Urea, Creatinine, and NSMCE2

The serum urea and creatinine levels were measured using Randox kits. The serum NSMCE2 was measured by ELISA (CUSABIO Life).

Statistical Analysis

In order to determine statistical significance, one-way ANOVA was performed using SPSS version 21.0 for Windows Statistical Package for Social Science, Inc. All groups showed no deviation from the normal distribution, so parametric statistical methods were used to analyze the data. Our results are presented as means±SD. All *P* values <0.05 were regarded as statistically significant.

RESULTS

Two groups were included in this study: Group

1 consisted of sixty patients suffering from nephrolithiasis (mean age of nephrolithiasis patients was 52.42±10.19 years), and Group 2 was the control group (mean age of control subjects was 50.27±8.21 years). All patients underwent X-ray examination in addition to kidney ultrasound screening by an ultra-sonographer.

There were non-significant differences (*P*>0.05) in the mean values of hemoglobin, serum urea, creatinine, and protein levels of the nephrolithiasis patients as compared to control subjects (Table 1).

The present study showed that mean levels of sera NSMCE2 are elevated (*P*<0.01) in patients when compared to control subjects (Table 1).

The general urine tests listed in Table 2 were used for selection and categorization of patients and control subjects. The results showed that 38.33% of nephrolithiasis patients had lowered specific gravity urine. When compared to control subjects, approximately 50.00% and 66.67% of nephrolithiasis patients were found to have elevated leukocyte and erythrocyte levels respectively, while 36.68% had protein in their urine.

The results in Table 3 showed a significant increase in DNase I and II activities and specific activities in sera of the nephrolithiasis patients when compared to control subjects (*P*<0.01).

Table 1. Hematological Data and Biochemical Parameters of Experimental and Control Groups

Characteristics	Patients Group (n=60)	Control Group (n=50)	<i>P</i> Value
Age (y)	52.42±10.19	50.27±8.21	>0.05
Hb (g/dL)	12.05±1.40	12.88±0.69	>0.05
B. urea (mg/dL)	40.25±7.57	37.89±8.36	>0.05
S. Creatinine (mg/dL)	1.16±0.42	1.10±0.24	>0.05
S. Protein (g/dL)	7.65±0.43	7.68±0.32	>0.05
S. NSMCE2 (pg/mL)	80.41±8.45	74.63±4.00	<0.01

Table 2. The Mean and Standard Deviation of Microbiology in Urine of Patients Group

Characteristics	Patients Group with the Normal Range	Patients Group with Up or Less Normal Range	Percent % Up or Less Normal Value
Specific gravity	1.018±0.003	1.008±0.003	38.33%
Leukocytes (cells/UL)	6.60±1.41	229.95±130.21	50.0 %
Erythrocytes/blood	4.10±1.01	112.13±57.13	66.67%
Protein (g/L)	0.08±0.02	2.17±1.11	36.68%

Statistically significant correlations between DNase I and E3 SUMO-protein ligase NSE2 (NSMCE2) levels and between DNase II and NSE2 levels in patients with nephrolithiasis were detected. There were no significant correlations between DNase I or DNase II and age in the patient or control groups (Tables 4 and 5). There were no significant correlations between E3 SUMO-protein ligase NSE2 (NSMCE2) with age in patients and the control group.

DISCUSSION

The present study showed a non-significant increase in serum urea and creatinine in the nephrolithiasis patient group relative to levels of control subjects (Table 1). If the functional capacity of the kidneys was slightly damaged by long-term urinary obstruction and urinary reflux, creatinine levels might

be elevated. However, the likelihood of forming stones or the frequency of stone formation would be due to other elements in the bloodstream and in urine, and would be unrelated to creatinine^[18-20].

Several studies have focused on ROS-mediated SUMOylation, one of the post-translational modifications implicated in vascular inflammation^[21-22]. The SUMO modification of proteins has been suggested to regulate numerous biological processes, such as stress responses, transcriptional regulation, and protein localization. We have previously reported that nephrolithiasis patients have a higher level of sera NSE2 than control subjects^[23]. We have also previously reported that the reduction of ADA and AMP-aminohydrolase activities could cause immunosuppression. Additionally, the increase in NSMCE2 may play a role in responding to developing DNA damage and inflammation in patients with nephrolithiasis.

Table 3. Activities and Specific Activities of Sera DNase I of the Patients and Control Groups

Group	DNase I		DNase II	
	Activity×10 ³ (U/L)	Specific Activity (U/mg)	Activity×10 ³ (U/L)	Specific Activity (U/mg)
Patients group (n=60)	23.20±4.99*	0.30±0.06*	69.60±15.00*	0.91±0.18*
Control group (n=50)	10.80±1.59	0.14±0.02	32.37±4.77	0.42±0.06

Note. *, $P < 0.01$ compared to control group.

Table 4. Correlation between DNase I with E3 SUMO-protein Ligase NSE2 (NSMCE2) (pg/ml) and Age in the Patients and Control Groups

Characteristics	DNase I Activity (U/L) (patients group)		DNase I Activity (U/L) (control group)	
	Pearson Correlation	Sig. (2 tailed)	Pearson Correlation	Sig. (2-tailed)
NSMCE2 (pg/mL)	0.78	0.01	0.20	N.S
Age (y)	0.19	N.S	0.22	N.S

Table 5. Correlation between DNase II with E3 SUMO-protein Ligase NSE2 (NSMCE2) (pg/mL) and Age in the Patients and Control Groups

Characteristics	DNase II Activity (U/L) (patients group)		DNase II Activity (U/L) (control group)	
	Pearson Correlation	Sig. (2 tailed)	Pearson Correlation	Sig. (2 tailed)
NSMCE2 (pg/mL)	0.77	0.01	0.22	N.S
Age (y)	0.17	N.S	0.19	N.S

Our results shown in Table 3 showed a significant increase ($P < 0.01$) in DNase I and II activities in sera of patients than in sera of the control subjects; the explanation for this may be increased cellular death and subsequent release of intracellular DNases into the plasma due to disease progression^[24]. During programmed cell death, DNase plays important roles in DNA fragmentation and degradation^[25]. DNase I has been assumed to be responsible for internucleosomal DNA degradation by apoptosis^[26]. In apoptosis, cells shrink, lose microvilli and cell junctions, and break up into a number of membrane-bound condensed apoptotic bodies^[27]. Programmed cell death, which is critical in development and maintenance of homeostasis, is known to involve apoptosis and has been shown to play roles in many diseases^[28]. Cells undergoing necrosis exhibit neither an increase in phosphatidylserine on the cell surface nor compression of chromatin and DNA fragmentation into specifically sized fragments. Finally, cells undergoing apoptosis separate from the basement membrane and break apart into smaller bodies that neighboring cells phagocytose, while necrotic cells rupture and release their contents into the nearby area. As a result, cell necrosis can incite an inflammatory response, though cell death by apoptosis has a reduced probability of doing so^[29]. Apoptosis plays an essential physiological role in the elimination of embryonal cells not required in later development, cells that have differentiated improperly, senescent cells, and cells that have been damaged^[30-31]. Apoptosis has important roles in embryogenesis and normal tissue turnover, which seems reasonable as apoptosis is a non-inflammatory process^[32]. In fact, during embryogenesis, events of this type take place at such predictable time points that they are frequently referred to as programmed cell deaths, a term that is often erroneously used interchangeably with apoptosis^[27]. SUMO protein-ligase activity is necessary for the prevention of DNA damage-induced apoptosis by facilitating DNA repair^[33].

The increase NSMCE2 may be due to the active role of this enzyme in the repair of DNA damage. The NSMCE2 is essential for viability, is required for several aspects of DNA metabolism, including combinational repair and maintenance of the DNA damage checkpoint. The increase in NSMCE2, and DNase I and II activities could be as a suitable marker for complication due to nephrolithiasis. As detailed above, it appears that the process of renal tubular

cell injury is of key importance in nephrolithiasis formation. Though many aspects of the mechanism of renal stone formation remain unclear, it is certain that renal tubular cell injury is a very important part of it. At present, though few useful substances for the prevention of nephrolithiasis are available, the development of medications that prevent renal tubular cell injury will provide a novel strategy for preventing and treatment of this disease.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest with regard to this study.

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