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Serum E3 SUMO-protein ligase NSE2 level and peroxynitrite related to oxidative stress in nephrolithiasis patients

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ABSTRACT

Objective: To prove probable relations between serum E3 SUMO-protein ligase NSE2 (NSMCE2) concentration, peroxynitrite related to oxidative stress in nephrolithiasis patients.

Methods: A total of 60 patients with nephrolithiasis and 50 healthy volunteers were involved in this study. Colorimetric method was used to detect blood urea, creatinine, uric acid, protein, albumin, total antioxidant status, total oxidant status, peroxynitrite, nitric oxide and oxidative stress index. Glutathione, NSMCE2 and superoxide dismutase were measured by ELISA.

Results: A significant increase in level of peroxynitrite, total oxidant status, NSMCE2 and oxidative stress index in patients was observed, while total antioxidant status and glutathione were significantly decreased.

Conclusions: The study concluded that serum NSMCE2 significantly correlated with peroxynitrite and oxidative stress in patients with nephrolithiasis.

1. Introduction

The prevalence of nephrolithiasis is increasing worldwide. It is the main health associated problems of last year [1]. It is the presence of kidney calculi caused by a disorder in the equilibrium between solubility and precipitation of salts in kidneys. The small stone can pass and produce slight pain while bigger stone may block the urinary tract, lead to severe pain and may be flow of blood [1,2].

In kidney tissue, the reactive oxygen species are made by reacting calcium oxalate or calcium phosphate crystals [3,4]. Peroxynitrite is the product of reaction of nitric oxide with superoxide radicals. In cells, peroxynitrite reacts at slow rate despite it is a strong oxidant. Peroxynitrite passes via anion channels in the cell membranes [5]. Nitric oxide is an important controller of kidney hemodynamics and tubular function at renal vasculature level, glomerulus and renal tubules [6]. The E3 SUMO-protein ligase NSE2 is a structural maintenance of chromosomes protein 5 (SMC5)-SMC6 complex component, which is contained a double-strand of DNA break repair by recombination

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of homologous. Performances as a E3 ligase mediating SUMO attachment to several proteins for instance SMC6L1 and TRAX [7,8].

No previous studies have reported on serum peroxynitrite with NSMCE2 in patients with nephrolithiasis. Detection of the correlation of serum peroxynitrite and oxidative stress factors to NSMCE2 in patients with nephrolithiasis was the aim of the present study.

2. Materials and methods

The study protocol was performed according to the Helsinki declaration and approved by Institutional Ethics Committee No. IIUM/305/14/11/2/IIUM Research Ethics Committee (IREC300).

2.1. Patients collection and samples storage

A total of 60 patients with nephrolithiasis and 50 healthy controls were involved in current study. The samples were collected from patients that were hospitalized at government health clinics in Kuantan, Pahang, Malaysia. Patients with diabetes mellitus type 2, diabetic nephropathy, heart disease, history of alcohol intake, taking potent antioxidant, pregnant females and smokers were excluded from the current study. Biochemical factors (blood sugar, urea, albumin, creatinine, protein, sodium, potassium and chloride) and general urine test were used for categorization of cases and control. Serum samples for the measurement of serum peroxynitrite and other biochemical parameters were stored at −20 °C.

2.2. Estimation of biochemical parameters

The serum peroxynitrite was measured according to the method of Vanuffelen *et al.* [9]. Levels of oxidative stress index (OSI), total antioxidant status (TAS) and total oxidant status (TOS) in sera of studied group were measured according to methods developed by Erel [10,11] and Kumari *et al.* [12]. A modified method of Satoh was used to measure malondialdehyde (MDA) [13]. Serum NSMCE2, nitric oxide, superoxide dismutase (SOD) and glutathione (GSH) were measured by ELSIA. Serum sodium, potassium and chloride were measured by Olympus AU2700 analyser. Other clinical parameters including urea, creatinine, protein, albumin and uric acid were conducted using commercial kits.

2.3. Statistical analysis

The data analysis was conducted by using software SPSS 20.0 version. Data were analysed using Pearson's correlation and two-tailed student's *t*-test.

3. Results

Age and biochemical parameters for patients and control were shown in Table 1. No significant difference was found in sera of urea, creatinine, protein, albumin, sodium, potassium and chloride in patient with nephrolithiasis in comparison to control group.

Table 2 showed that 38.33% of patients had urine specific gravity less than normal value, while there were 50.00% and 66.67% increase in leukocytes and erythrocytes respectively, and 36.68% had protein in their urine.

A significant increase of serum peroxynitrite has been showed in patients with nephrolithiasis in comparison to control group (Table 3). No significant difference was found in nitric oxide level between studied groups (Table 3).

Serum TAS was significantly decreased in patients (Table 3). Serum OSI, TOS, MDA and NSMCE2 were significantly increased in patients with nephrolithiasis compared to control (Table 3) while GSH showed significant decreased in patients ($P < 0.01$). Serum SOD and uric were similar to controls (Table 3).

Correlative values of serum NSMCE2 with other biochemical parameters were presented in Table 4. A significant

Table 1
Age and biochemical parameters for patients with nephrolithiasis and healthy controls.

Parameters	Age (year)	Urea (mg/dL)	Creatinine (mg/dL)	Protein (g/dL)	Albumin (g/dL)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Patients (n = 60)	52.42 ± 10.19	40.25 ± 7.54	1.16 ± 0.42	7.65 ± 0.43	4.39 ± 0.58	138.76 ± 2.25	4.19 ± 0.33	102.94 ± 2.11
Control (n = 50)	50.27 ± 8.21	37.89 ± 8.36	1.10 ± 0.24	7.68 ± 0.32	4.61 ± 0.57	140.26 ± 1.31	3.92 ± 0.21	101.22 ± 1.23
P value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

Table 2
Urine microbiology of patients group.

Characteristics	Specific gravity	pH	Leukocytes (cells/μL)	Erythrocytes /blood	Protein (g/L)	Glucose	Ketones	Urobilinogen	Bilirubin	Nitrite
Patients group with the normal range	1.018 ± 0.003	6.18 ± 0.95	6.60 ± 1.41	4.10 ± 1.01	0.08 ± 0.02	0.48 ± 0.10	Negative	Negative	Negative	Negative
Patients group with up or less normal range	1.008 ± 0.003	6.35 ± 0.85	229.95 ± 130.21	112.13 ± 57.13	2.17 ± 1.11	0.45 ± 0.12	Negative	Negative	Negative	Negative
Percent % up or less normal value	38.33%	0.00%	50.00%	66.67%	36.68%	0.00%	0.00%	0.00%	0.00%	0.00%

Table 3
Biochemical parameters in patients and control groups.

Groups	Characteristic									
	Peroxynitrite (μmol/L)	Nitric oxide (μmol/L)	TOS (μmol H_2O_2 Eq/L)	TAS (mmol trolox Eq/L)	OSI (arbitrary unit)	MDA (nmol/mL)	GSH (μg/mL)	SOD (pg/mL)	Serum NSMCE2 (pg/mL)	Uric acid (mg/dL)
Patients	7.34 ± 0.78	13.11 ± 2.37	18.98 ± 3.01	1.68 ± 0.42	10.81 ± 3.90	3.78 ± 0.53	1.59 ± 0.21	17.50 ± 1.90	80.41 ± 8.45	5.90 ± 0.94
Control	3.22 ± 0.49	12.82 ± 1.97	13.60 ± 1.71	2.03 ± 0.49	6.18 ± 2.01	1.23 ± 0.25	1.83 ± 0.19	17.20 ± 0.34	74.63 ± 4.00	5.32 ± 0.53
P value	< 0.01	> 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.05	> 0.05	< 0.01	> 0.05

Table 4
Pearson's correlation values in patients and control groups.

Groups	Correlative variables							
	NSMCE2 *vs.* Peroxynitrite	NSMCE2 *vs.* Nitric oxide	NSMCE2 *vs.* TOS	NSMCE2 *vs.* TAS	NSMCE2 *vs.* OSI	NSMCE2 *vs.* MDA	NSMCE2 *vs.* GSH	NSMCE2 *vs.* SOD
Patients group	0.78**	0.59*	0.69**	−0.72**	0.73**	0.68**	−0.61*	0.17
Control group	0.11	0.09	0.10	−0.11	0.06	0.09	0.05	0.06

*: $P < 0.01$; **: $P < 0.001$.

correlation was detected between NSMCE2 with peroxynitrite, nitric oxide, TOS, TAS, OSI, MDA and GSH in patients group, while no significant correlation was observed in control (Table 4).

4. Discussion

In this study, 27 people of patients were females and 33 were males. Several studies showed that nephrolithiasis was common in men compared to in women [14,15]. Governmental data exhibited change in the female-to-male percentage in ureteral or kidney stone diagnosis from 1:1.7 in 1997 to 1:1.3 in 2002 [14,15]. Obesity may be one of the reasons which cause the rise in stone disease in women [16].

Serum peroxynitrite, nitric oxide, OSI, TAS, TOS and MDA are known as oxidative stress markers. The result showed high levels of peroxynitrite, MDA, OSI and TOS, also decreased TAS and GSH in patients with nephrolithiasis than healthy group, which is due to disparity between pro-oxidants and antioxidants [17]. Patients with chronic kidney diseases have increased oxidative stress as well as the development of disease [3,18]. Another study showed the association of kidney stones with free radicals [19]. The study of Ozbek showed increase oxidative stress condition with stone forming in human sera and cultures [20].

The reaction between superoxide anions and nitric oxide led to formation of peroxynitrite, which produce lipid peroxidation, base modification, cysteine oxidization and dityrosyl-bridges formation. Through a series of reaction, breakdown of peroxynitrite leads to generation of peroxynitrous acid. Nitric oxide can decompose ONOOH. Through these mechanisms, nitric oxide works to abate the oxidation chemistry of reactive nitrogen oxygen species [21]. Nitric oxide was reported to inhibit cell proliferation and induce differentiation. In addition, nitric oxide is a reactive compound and can react with superoxide and may cause the production of the additional damaging compound like peroxynitrite. Nitric oxide can also be a very effective antioxidant to the reactive oxygen species [21]. Several studies have shown that nitric oxide is physiological modulators of peroxynitrite reactivity, thus it confirms the effects of nitric oxide on the inflammation and reperfusion injury in animal models [22,23]. Peroxynitrite is a result of enzymatic reaction of superoxide anion and nitric oxide, which clarified the significant correlation between nitric oxide and peroxynitrite [23].

The present result agrees with other study which hypothesized that nephrolithiasis was related to the increase in uric acid concentration in the blood [24,25]. However, the increase in uric acid in the present study is not significant.

The significant increase in MDA and low level of GSH are coherent with previous findings [19,26]. Another study demonstrated that the increase in peroxidation and reduction of thiol concentration lead to the increase in the activity of oxalate binding, which increases the accumulation of stone parts [26]. A non-significant difference in serum SOD in patients compared to control is similar with other researches observation [26]. We describe here the increase in NSMCE2 in patients with nephrolithiasis compared to control group which is similar with previous study [27]. We found in previous study that the reduction of adenosine deaminase and AMP-aminohydrolase activities could cause a state of immune suppression, and also the increase in NSMCE2 may possibly play a role in developments of alteration of DNA damage and inflammation disorders in the patients with nephrolithiasis [28]. Our findings suggest that NSMCE2 is necessary for the inhibition of DNA damage which induced apoptosis through enabling DNA repair in cells.

In this study, there is a significant correlation between peroxynitrite with TOS, TAS, OSI, MDA and GSH in patients with nephrolithiasis, while non-significant correlation between peroxynitrite with TOS, TAS, OSI, MDA, SOD and GSH in control (Table 4). The data in Table 4 showed the relationship between oxidative markers with NSMCE2, which assessed to act as an indication in the prediction of kidney injury in patients with nephrolithiasis.

Peroxynitrite destructs DNA via removing hydrogen atom from the deoxyribose in the sugar phosphate backbone which causes an opening of the sugar ring that leads to DNA strand breaks [29].

The present study showed that serum NSMCE2 were associated with oxidative stress markers and serum peroxynitrite, nitric oxide in nephrolithiasis patients. This might reflect

increased antioxidant reaction through stones formation consequently increased oxidative stress.

Conflict of interest statement

We declare that we have no conflict of interest.

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