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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% |

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